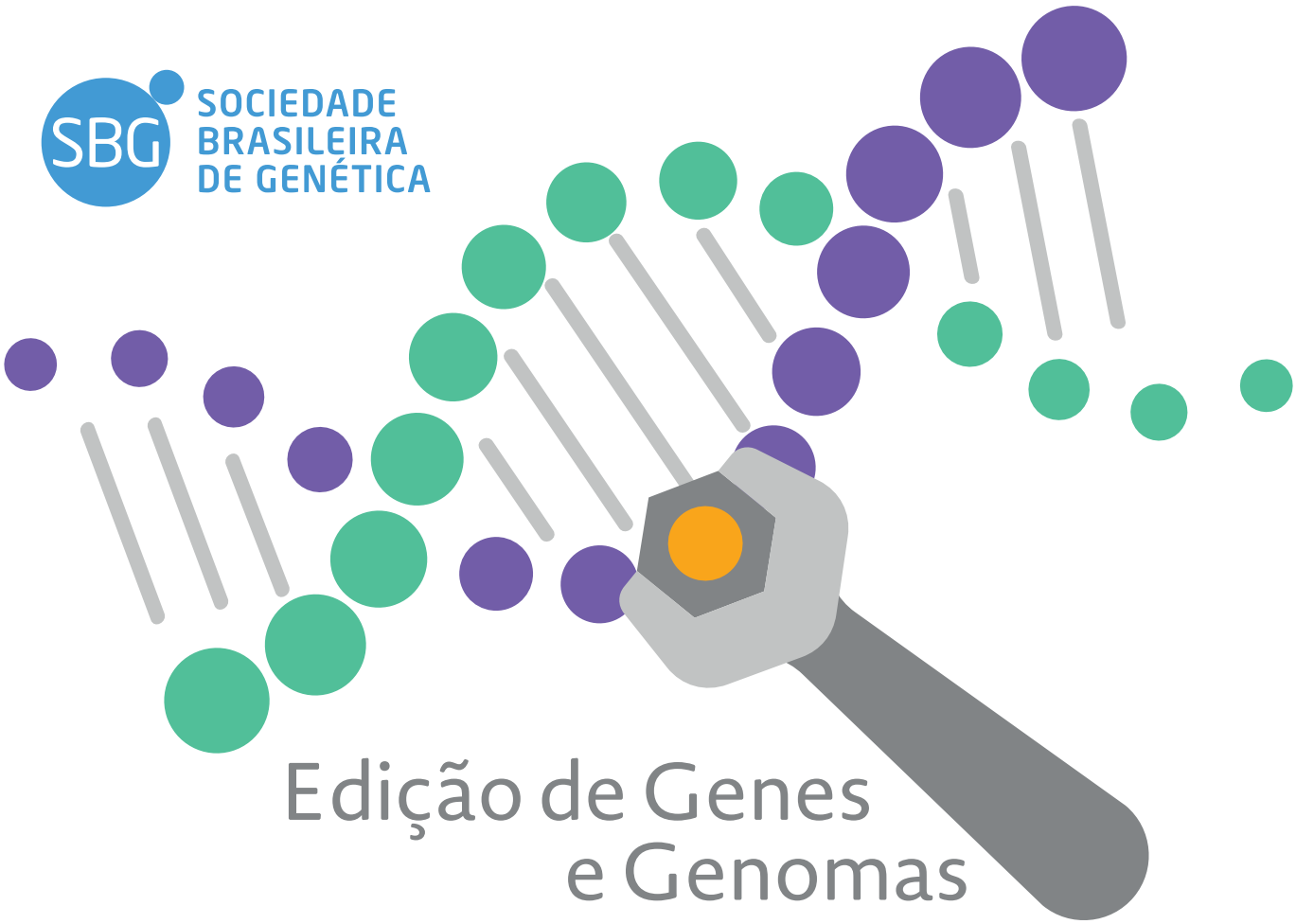
GENÉTICA 2019

17 a 20 de setembro de 2019
Hotel Monte Real Resort - Águas de Lindóia - SP

65th Brazilian Congress of Genetics

Homenageado
Prof. Pedro Manoel Galetti Junior

SBG SOCIEDADE BRASILEIRA DE GENÉTICA



Edição de Genes e Genomas

CAPES FAPESP CNPq
Conselho Nacional de Desenvolvimento Científico e Tecnológico

E-Book

Sumário

Apresentação

Caro(a) colega,

A Sociedade Brasileira de Genética (SBG) realizou o **GENÉTICA 2019**, seu 65º Congresso Brasileiro de Genética, em Águas de Lindóia, SP, no período de 17 a 20 de setembro de 2019.

O tema central foi "Edição de Genes e Genomas". Trata-se de um assunto dos mais relevantes da atualidade, com implicações em todas as áreas das Ciências da Vida. Foram abordados conceitos não apenas técnico-científicos, mas também éticos e regulatórios. Associados a esse tema, participaram palestrantes internacionais e nacionais de renome, dentro de um programa científico atual, vibrante e que cobriu os mais diversos temas relacionados à Genética.

Além da programação científica, homenageamos o Prof. Pedro Manoel Galetti Junior por suas relevantes contribuições científicas e pela formação de recursos humanos na área da Genética, bem como por seu papel à frente da SBG.

Agradecemos por sua importante participação no nosso evento.

Marcio de Castro Silva Filho
Presidente da SBG

Comissão organizadora

Diretoria 2018/2020

Márcio de Castro Silva Filho
ESALQ/USP, Piracicaba/SP

Mara Helena Hutz
UFRGS, Porto Alegre/RS

Igor Schneider
UFPA, Belém/PA

Antonio Mateo Sole Cava
UFRJ, Rio de Janeiro/RJ

Maria Helena de Souza Goldman
FFCLRP/USP, Ribeirão Preto/SP

Célia Maria de Almeida Soares
UFG, Goiânia/GO

Comissão Científica

Genética Humana

Angela Maria Vianna Morgante
IB/USP, São Paulo, SP

Ester Silveira Ramos
FMRP/USP, Ribeirão Preto, SP

Maria Cátira Bortolini
UFRGS, Porto Alegre, RS

Maria Luiza Petzl-Erler
UFPR, Curitiba, PR

Regina Célia Mingroni-Neto
IB/USP, São Paulo, SP

Sergio Danilo Junho Pena
UFMG, Belo Horizonte, MG

Genética Animal

Claudio de Oliveira
UNESP, Botucatu, SP

Fabrício Rodrigues dos Santos
UFMG, Belo Horizonte, MG

Eduardo Eizirik
PUCRS, Porto Alegre, RS

Maria Iracilda da Cunha Sampaio
UFPA, Belém, PA

Klaus Hartmann Hartfelder
FMRP/USP, Ribeirão Preto, SP

Genética de Micro-organismos

Augusto Schrank
UFRGS, Porto Alegre, RS

Maristela Pereira
UFG, Goiânia, GO

Samuel Goldenberg
Fiocruz, Curitiba, PR

Genética Vegetal

Ana Maria Benko Issepon
UFPE, Recife, PE

Elizabeth Pacheco Batista Fontes
UFV, Viçosa, MG

Marcia Maria A. N. P. Margis
UFRGS, Porto Alegre, RS

Maria Lúcia Carneiro Vieira
ESALQ/USP, Piracicaba, SP

Mutagênese

Carlos Frederico Martins Menck
USP, São Paulo, SP

Carlos Renato Machado
UFMG, Belo Horizonte, MG

Catarina Satie Takahashi
FFCLRP/USP, Ribeirão Preto, SP

Elza Tiemi Sakamoto Hojo
FFCLRP/USP, Ribeirão Preto, SP

Genômica e Bioinformática

Ana Tereza Ribeiro Vasconcelos
LNCC, Petrópolis, RJ

Glória Regina Franco
UFMG, Belo Horizonte, MG

Lucymara Fassarella Agnez Lima
UFRN, Natal, RN

Ensino/Genética na Praça

Eliana Maria Beluzzo Dessen
IB/USP, São Paulo, SP

Adlane Vilas-Boas Ferreira
UFMG, Belo Horizonte, MG

Secretaria Executiva

Agnes Pierri Portella

Eveli Alexandre

Prêmio Jovem Geneticista Francisco Mauro Salzano

Selecionados

Mir-450a acts as a tumor suppressor in ovarian cancer by regulating energy metabolism
Bruna Rodrigues Muys, *FMRP/USP, Ribeirão Preto, SP*

Incomplete lineage sorting, introgression and hybridization had a strong impact on the phylogenetic relations in the new world primates
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Whole-exome sequencing reveals the impact of UVA light mutagenesis in xeroderma pigmentosum variant human cells
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Prêmios orais

Prêmio Newton Freire-Maia - Genética Humana

Patrocínio: Profa. Dra. Eleidi Alice Chautard Freire Maia

Rare genetic variants in a patient with autistic spectrum disorder reveal interconnection between intracellular signaling pathways in an oligogenic pattern of inheritance
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In vivo gene editing improves cardiovascular alterations in mucopolysaccharidosis i mice
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Prêmio Darcy Fontoura de Almeida - Molecular Biology/Genomics/Bioinformatics

Patrocínio: Fundação Danilo Pena (FUNDAPE)

Functional annotation of SNPS associated with gdf15 levels in a putative enhancer region activated by metformin
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Bioprospecting for potential bifunctional α-larabinofuranosidase/xylobiohydrolase of *Trichoderma harzianum*
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Relative contribution of environmental and spatial variables to the genetic diversity of *Avicennia germinans* and *Avicennia schaueriana*
Michele Fernandes Da Silva, *UNICAMP/Instituto de Biologia, Campinas, SP*

Runx2 tandem repeat associated to mandibular shape in Sigmodontine rodents (Rodentia, Cricetidae)
Rafael de Albuquerque Carvalho, *Universidade Federal de Pernambuco, Recife, PE*

Programa

SEPTEMBER 17, 2019 – TUESDAY

14:00 – 18:00 **REGISTRATION**

14:00 – 18:00 **EXHIBITION AREA OPENING**

14:00 – 17:00 **INTENSIVE COURSES**

Introdução à Bioinformática: Uso da linguagem R para análise de RNA-Seq — **Sala Rubi**
Jessica Rodrigues Plaça, *LGMB/USP, Ribeirão Preto, SP* e Wilson da Araújo Silva Junior, *FMRP/USP, Ribeirão Preto*

Atividades experimentais "*hands on*" para ensino de Biologia Molecular — **Sala Safira**
Elgion Lucio da Silva Loreto, *Universidade Federal de Santa Maria, RS*

Introdução a CRISPR — **Sala Esmeralda**
Tiago Campos Pereira, *FFCLRP/USP, Ribeirão Preto, SP*

Proteômica e sistemas biológicos no estudo de doenças humanas — **Sala Topázio**
Alessandra Vidotto, *FAMERP, São José do Rio Preto, SP*

Métodos de análise para identificação de susceptibilidade genética de doenças complexas usando dados de GWAS — **Sala 8**
Luciana Tovo Rodrigues, *UFPel, Pelotas, RS*

Sinalização Celular: principais vias de transdução e mecanismos de regulação — **Sala 7**
Ricardo Garcia Correa, *NCI-Designated Cancer Center/SBP Medical Discovery Institute, La Jolla, CA, EUA*

Genética e a conservação da Natureza — **Sala 6**
Antonio Mateo Solé Cava, *UFRJ, Rio de janeiro, RJ*

Origem e evolução do cromossomo eucariota — **Sala 5**
Marcelo dos Santos Guerra Filho, *UFPE, Recife, PE*

18:30 – 19:30 **CEREMONY**

Opening Ceremony — **Salão Real**
Genetics 2019 Honoree: Prof. Dr. Pedro Manoel Galetti Junior

19:30 – 20:30 **OPENING LECTURE**

Expanding the CRISPR-Cas Genome Editing Toolbox — **Salão Real**
Lucas B. Harrington, *University of California, Berkeley, California, USA*

SEPTEMBER 18, 2019 – WEDNESDAY

9:00 – 10:00 FULL LECTURES

18 years fighting the HIV/AIDS epidemics in Africa — Salão Real
Amilcar Tanuri, *IB/UFRJ, Rio de Janeiro, RJ*

Implementing personalized and genomic medicine in the context of precision medicine: present and future directions — Sala Rubi
Iscia Lopes Cendes, *UNICAMP, Campinas, SP*

Magnetic 3D Bioprinting, from Spheroids to Fingerprinting Cells in 3D Using a 2D Workflow — Sala Esmeralda
Glauco R. Souza, *University of Texas Health Science Center at Houston, TX USA/Greiner Bio-One*

10:00 – 10:30 COFFEE BREAK

10:30 – 12:00 SYMPOSIA

Novel signaling routes and molecular signatures in cancer development — Salão Real
Chair: Carlos Frederico Martins Menck, *ICB/USP, São Paulo, SP*

NOD-like receptors: an interface between innate immunity and cancer progression
Ricardo Garcia Correa, *NCI-Designated Cancer Center/SBP Medical Discovery Institute, La Jolla, CA, EUA*

Novel telomerase inhibitors
Vinay Tergaonkar, *Institute of Molecular and Cell Biology (IMCB)/National University of Singapore, Singapore*

Prediction of poor response to neoadjuvant chemoradiation in rectal cancer patients using a DNA repair deregulation score
Anamaria Aranha Camargo, *Hospital Sírio Libanês, São Paulo, SP*

Plant responses against biotic and abiotic stresses — Sala Rubi
Chair: Ana Maria Benko-Iseppon, *UFPE, Recife, PE*

Cowpea defense against virus – specific and crosstalk transcriptomic responses
Ana Maria Benko-Iseppon, *UFPE, Recife, PE*

Understanding how plants express against stress: using 'Micro-Tom' to unveil responses to pathogens
Antonio Vargas de Oliveira Figueira, *Cena/USP, Piracicaba, SP*

Dynamic transcriptome response to cold in *Arabidopsis*
Cristiane Paula Gomes Calixto, *Universidade de Dundee, Escócia, UK*

Evolutionary genetics of Neotropical primates – a symposium in honor of Horácio Schneider — Sala Esmeralda
Chair: Maria Iracilda da Cunha Sampaio, *UFPA, Belém, PA*

The legacy of Horácio Schneider to primatology
Maria Iracilda da Cunha Sampaio, *UFPA, Belém, PA*

Genetic and genomic analyses of a neotropical primate hybrid zone: a window to understand reproductive isolation and adaptive introgression
Liliana Cortés-Ortiz, *University of Michigan, Ann Arbor, USA*

Biogeography of the *Saguinus midas* clade and the rise and fall of *Saguinus bicolor*
Tomas Hrbek, *UFAM, Manaus, AM*

The Hologenomic Theory and its evolutionary implications — Sala Safira
Chair: Elgion Lucio da Silva Loreto, *UFSM, Santa Maria, RS*

Hologenomics and the concept of the individual in the context of Biological Evolution
Karen Luisa Haag, *UFRGS, Porto Alegre, RS*

The microbiont can express genes from its micro biome or from the DNA it ingests
Elgion Lucio da Silva Loreto, *UFSM, Santa Maria, RS*

Horizontal transfer: Co-option of microbiome genes by their host
Gabriel da Luz Wallau, *FIOCRUZ/PE, Recife, PE*

Evolutionary Genomics — Sala Topázio

Chair: Eduardo Eizirik, *PUCRS, Porto Alegre, RS*

Evolutionary and conservation genomics of wild carnivores
Eduardo Eizirik, *PUCRS, Porto Alegre, RS*

Deciphering Adaptive Evolution with Genomics: case studies
Agostinho Antunes, *University of Porto, Porto, Portugal*

The Genetic Bases of Adaptive Convergence in Cichlid fishes
Frederico Henning, *UFRJ, Rio de Janeiro, RJ*

Conferência Técnica BD — Sala 8

Flow Cytometry providing new insights to single cell analysis: From profiling to sorting
Iris Arantes de Castro, *BD*

Conferência Técnica Uniscience — Sala 7

A bioimpressão 3D como ferramenta prática para extrair mais dados de sua investigação tecidual
Paulo Madeira, *PhD – Uniscience*

12:00 – 14:00 MEETING

SBG Executive Council meeting/Lunch — Restaurante Hotel Monte Real

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12:00 – 14:00 LUNCH

14:00 – 15:00 FULL LECTURES

Applications of stem cells: From cosmetics to psychedelics — Salão Real

Stevens Rehen, *ICB/UFRJ, Rio de Janeiro, RJ*

Development of gene editing technologies for crop improvement — Sala Rubi

Yunde Zhao, *University of California, San Diego, USA*

Molecular basis of focal disorders of cortical development — Sala Esmeralda

Joseph Gleeson, *University of California San Diego, USA*

15:00 – 15:30 COFFEE BREAK

15:30 – 17:00 ORAL COMMUNICATION SESSIONS

Prêmio Jovem Geneticista – Francisco Mauro Salzano — Salão Real

Mir-450a acts as a tumor suppressor in ovarian cancer by regulating energy metabolism
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Prêmio Paulo Sodero Martins - Evolução — Sala 6

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Gabrielle Azevedo Rizzato, *Instituto de Biociências da Universidade de São Paulo, São Paulo, SP*

Relative contribution of environmental and spatial variables to the genetic diversity of *Avicennia germinans* and *Avicennia schaueriana*
Michele Fernandes Da Silva, *UNICAMP/Instituto de Biologia, Campinas, SP*

Runx2 tandem repeat associated to mandibular shape in Sigmodontine rodents (Rodentia, Cricetidae)
Rafael de Albuquerque Carvalho, *Universidade Federal de Pernambuco, Recife, PE*

17:00 – 19:00 POSTER SESSION

Os pôsteres com numeração **PAR** deverão ser fixados no dia **18/09 (quarta-feira) a partir das 10 horas** e retirados impreterivelmente até as **10 horas da manhã do dia 19/09 (quinta-feira)**.

17:00 – 19:00 **OFICINAS DE GENÉTICA NA PRAÇA**

1. **Compactando o DNA por meio de um modelo didático tridimensional de baixo custo**
   Tiago Maretti Gonçalves, *Universidade Federal de São Carlos, SP* (tiagobio1@hotmail.com)
2. **Síntese de proteínas**
   Carlos Fernando Campos, *Universidade Federal de Uberlândia* (carllosfernando20@hotmail.com)
3. **BioGenGeogo: O Jogo da Genética Biogeográfica**
   Luís Gustavo da Conceição Galego, *Universidade Federal do Triângulo Mineiro, MG* (luis.galego@uftm.edu.br)
4. **Kit Educativo - Cariótipo Humano e Alterações Cromossômicas**
   Lígia Souza Lima Silveira da Mota e Adriane Pinto Wasko, *Universidade Estadual Paulista - UNESP* (ligia.mota@unesp.br)
5. **A genética da Tulipa Negra**
   Alexandre de Sá Freire, *SEEDUC-RJ* (alefreire2001@yahoo.com.br)
6. **Desenhando a Genética**
   Alessandra Vidotto, *Faculdade de Medicina de São José do Rio Preto - FAMERP* (alessandravidotto@yahoo.com.br)
7. **Doenças Genéticas em protagonistas da História - um jogo de carta**
   Angelica Aparecida dos Santos; Gabriel Jordão Batista de Souza; Milena Gemelgo de Morais; Nathalia Santos Cardoso; Rafael César Bolleli Faria, *IFSULDEMINAS* (rafael.bolleli@ifsuldeminas.edu.br)
8. **Caçadores de mitos da Genética**
   Adriele Garcia Costa; Jéssica Aparecida Pereira; Michaela Vilas Boas Guimarães; Valdir Bronzato Coimbra; Rafael César Bolleli Faria, *IFSULDEMINAS* (rafael.bolleli@ifsuldeminas.edu.br)
9. **Experimentos remotos para o ensino de Citogenética**
   Carla Leandra Silva Godoi; Janneffer Kathleen Costa Silva; Jeniffer Gabrieli dos Santos; Lidiane Augusta Junqueira; Thalia Aparecida de Lima; Eduardo Galembeck; Rafael César Bolleli Faria, *IFSULDEMINAS* (rafael.bolleli@ifsuldeminas.edu.br)
10. **Utilizando softwares de genética em sala de aula**
    Lucas Ramos Vieira; Luis Gustavo Talarico Rubim; Thiago Henrique dos Reis Pádua; Sabrina Larissa de Miranda Lopes; Rafael César Bolleli Faria, *IFSULDEMINAS* (rafael.bolleli@ifsuldeminas.edu.br)

20:00 – 22:00 **MEETING**

Reunião dos Editores da Revista Genetics and Molecular Biology **Sala Topázio**

20:00 – 22:00 **GET TOGETHER DINNER**

Get Together Dinner **Restaurante Hotel Monte Real**

Os alunos devem estar no restaurante às 19:45 horas

SEPTEMBER 19, 2019 – THURSDAY

9:00 – 10:00 FULL LECTURES

Translational control of gene expression and its role in the molecular responses of a fungal pathogen — Salão Real
Matthew S. Sachs, *Texas A&M University, Texas, EUA*

Genomics methods and their potential applications in neotropical wildlife conservation — Sala Rubi
Camila Mazzoni, *Berlin Center for Genomics in Biodiversity Research (BeGenDiv), Berlin, Germany*

Noncoding RNAs in human diseases — Sala Esmeralda
Ândrea Ribeiro-dos-Santos, *UFPA, Belém, PA*

10:00 – 10:30 COFFEE BREAK

10:30 – 12:00 SYMPOSIA

Gene Editing — Salão Real
Chair: Guilherme Marcello Queiroga Cruz, *Bayer Crop Science*

Genome Edition Regulatory framework: Biosafety and Scientific Development advancing together
Maria Sueli Soares Felipe, *UCB/CNBIO, Brasília, DF*

Scientific and regulatory advances of genome editing in Brazil and World - cases discussion
Hugo Bruno Correa Molinari, *Embrapa, Brasília, DF*

Gene Editing: Engage in the conversation
Guilherme Marcello Queiroga Cruz, *Bayer Crop Science*

Genome editing in biomedical sciences — Sala Rubi
Chair: Geraldo Aleixo Passos, *FORP/FMRP/USP, Ribeirão Preto, SP*

Genome Editing in Trypanosomatides
Santuza Maria Ribeiro Teixeira, *ICB/UFMG, Belo Horizonte, MG*

Applications of CRISPR in the immune system
Martin Herman Bonamino, *INCA, Rio de Janeiro, RJ*

Gene therapy of rare diseases using CRISPR
Guilherme Baldo, *HCPA/UFRGS, Porto Alegre, RS*

The peopling of Americas revisited — Sala Esmeralda
Chair: Fabricio Rodrigues do Santos, *UFMG, Belo Horizonte, MG*

Archaeogenetics of pre-colonial Brazil
André Menezes Strauss, *MAE/USP, São Paulo, SP*

Towards a consensus between historical disciplines on the pre-Columbian settlement of Americas
Fabricio Rodrigues do Santos, *UFMG, Belo Horizonte, MG*

Reconstructing the history of the native american populations from brazilian coast
Tábita Hünemeier, *IB/USP, São Paulo, SP*

Environmental mutagenesis — Sala Safira
Chair: Catarina Satie Takahashi, *FFCLRP/USP, Ribeirão Preto, SP*

How and why chronic studies represent the best scenario for environmental assessment of genotoxicants
Cesar Koppe Grisolia, *UnB, Brasília, DF*

Effects of mineral coal on human health
Juliana da Silva, *ULBRA, Canoas, RS*

Oxidative stress, mutagenic effects, and cell death induced by retene
Silvia Regina Batistuzzo de Medeiros, *UFRN, Natal, RN*

Sex and B chromosome enigmas — **Sala Topázio**

Chair: César Martins, *IBB/UNESP, Botucatu, SP*

Evolution of neo-sex chromosomes: insigths from grasshoppers
Diogo C Cabral-de-Mello, *IB/UNESP, Rio Claro, SP*

Genome organization of the W chromosome in the Parodontidae fish
Marcelo Ricardo Vicari, *UFPG, Ponta Grossa, PR*

B chromosome, a new player in the genome evolution
César Martins, *IBB/UNESP, Botucatu, SP*

Conferência Técnica Illumina — **Sala 7**

Learn how the latest technologies from Illumina are being used to change the face of genomics-based research and applications
Marcia Dellamano, *Sr Field Applications Scientist*

10:30 – 12:00 — **FORUM**

Fórum da Pós-Graduação — **Sala 8**

12:00 – 14:00 — **FORUM**

Fórum dos Ex-Presidentes/Lunch — **Restaurante Hotel Monte Real**

12:00 – 14:00 — **LUNCH**

14:00 – 15:00 — **FULL LECTURES**

Uncovering the evolutionary paths to virulence for a rational design of an effective and safer live-attenuated poliovirus vaccine — **Salão Real**
Raul Andino, *University of California, San Francisco, EUA*

3D Cell Culture Systems – Moving from 2D to 3D cell culture — **Sala Rubi**
Teddy Lin, *Biology Technical Marketing, Merckgroup*

The Genetic Complexity of Diseases — **Sala Esmeralda**
Maria Luiza Petzl-Erler, *UFPR, Curitiba, PR*

15:00 – 15:30 — **COFFEE BREAK**

15:30 – 17:30 — **ASSEMBLEIA**

Assembleia Ordinária da SBG — **Salão Real**

17:30 – 19:30 — **POSTER SESSION**

Os pôsteres com numeração **ÍMPAR** deverão ser fixados no dia **19/09 (quarta-feira) a partir das 10 horas** e retirados impreterivelmente até as **10 horas da manhã do dia 20/09 (sexta-feira)**.

SEPTEMBER 20, 2019 – FRIDAY

09:00 – 10:00 FULL LECTURES

Uncovering the Earth's Virome: Discovery and applications — Salão Real
David Paez Espino, *Joint Genome Institute, California, EUA*

Regulation of DNA repair pathway choice by EXO1 and its implications for glioblastoma therapy — Sala Rubi
Sandeep Burma, *UT Southwestern Medical Center, Dallas, USA*

10:00 – 10:30 COFFEE BREAK

10:30 – 12:00 SYMPOSIA

Plant-Pathogen interactions — Salão Real

Chair: Elizabeth Pacheco Batista Fontes, *UFV, Viçosa, MG*

Inverse modulation of antiviral and antibacterial immunity by transmembrane immune complexes
Elisabeth Pacheco Batista Fontes, *UFV, Viçosa, MG*

Suppression of the plant immune system by the root microbiota
Paulo José Pereira Lima Teixeira, *ESALQ/USP, Piracicaba, SP*

Leveraging genetic and genomic tools to dissect the molecular interaction between soybean and *Phakopsora pachyrhizi*
Sergio Herminio Brommonschenkel, *UFV, Viçosa, MG*

Plant-pathogen interaction: how can plants win the battle?
Angela Mehta, *Embrapa/Cenargen, Brasília, DF*

Genomic editing in humans — Sala Rubi

Chair: Maria Rita dos Santos e Passos Bueno, *IB/USP, São Paulo, SP*

Patients' cells or CRISPR-Cas9 edited cells for modeling human diseases?
Maria Rita dos Santos e Passos Bueno, *IB/USP, São Paulo, SP*

CRISPR/Cas9 for sickle cell disease treatment
Priscila Keiko Matsumoto Martin, *UNICAMP, Campinas, SP*

The generation of mosaic trisomy 21 model cells using patient cells with full trisomy 21 by trisomy rescue during cell reprogramming and their modification with fluorescent nuclear markers by genome editing technique
Silvia Natsuko Akutsu, *Hiroshima University, Hiroshima, Japão*

Genomics, Evolution and Conservation of fish — Sala Esmeralda

Chair: Pedro Manoel Galleti Junior, *UFSCar, São Carlos, SP, Brazil*

Genome bases for seasonal reproductive migration in freshwater fish: where are we?
Pedro Manoel Galleti Junior, *UFSCar, São Carlos, SP, Brazil*

Understanding geodispersal process among coastal basins using large-scale genomic data
Jorge Luis Ramirez Malaver, *Universidad Nacional Mayor de San Marcos, Lima, Peru*

Genomics of adaptation in fish: relevance in evolution and conservation
Agostinho Antunes, *Universidade do Porto, Porto, Portugal*

DNA repair and mutagenesis — Sala Safira

Chair: Carlos Renato Machado, *UFMG, Belo Horizonte, MG*

DNA repair and mutagenesis in the oportunistic pathogen *Pseudomonas aeruginosa*
Rodrigo da Silva Galhardo, *ICB/USP, São Paulo, SP*

Effects of multiple environmental stressors on genetic stability of amphibians
Andre Passaglia Schuch, *UFSM, Santa Maria, RS*

Protein ADP-ribosylation, genome stability and human genetic disorders
Nicolas Carlos Hoch, *IQ/USP, São Paulo, SP*

Bioinformatics — Sala Topázio

Chair: Sandro José de Souza, *UFRN, Natal, RN*

Network Medicine
Helder Takashi Imoto Nakaya, *FCF/USP, São Paulo, SP*

Combined analysis of multiple proteomics datasets
Gustavo Antonio de Souza, *UFRN, Natal, RN*

Computer simulations of evolutionary scenarios: Testing the Extended Fitness Hypothesis
Sandro José de Souza, *UFRN, Natal, RN*

12:00 – 14:00 **LUNCH**

14:00 – 15:30 **SYMPOSIA**

Symposium in memory of Prof. Dr. Warwick Estevam Kerr — Salão Real

Chair: Zilá Luz Paulino Simões, *FFCLRP/USP, Ribeirão Preto, SP*

Insights into the evolutionary dynamics of sex determining genes - Lessons from Hymenoptera species
Martin Hasselmann, *Institute of Animal Science, University of Hohenheim, Stuttgart, Germany*

Eusocial behavior in Hymenoptera, a remarkable achievement and model for advanced studies in biology
Zilá Luz Paulino Simões, *FFCLRP/USP, Ribeirão Preto, SP, Brazil*

Use and conservation of genetic resources of vegetable species by traditional farmers in Amazonia
(Uso e conservação de recursos genéticos de espécies vegetais por agricultores tradicionais na Amazônia)
Hiroshi Noda, *INPA/UFAM, Manaus, AM, Brazil*

Evolution and pathogenicity of microorganisms — Sala Rubi

Chair: Maristela Pereira, UFG, Goiânia, GO

Adaptive immunity in mosquitoes and the acquisition of endogenous viral elements
Raul Andino, *University of California, San Francisco, EUA*

A complex regulatory network connects the circadian clock and the reserve carbohydrate metabolism glycogen and trehalose in *Neurospora crassa*
Maria Célia Bertolini, *IQ/UNESP, Araraquara, SP*

Bacterial chemical signaling: are these bacteria really "speaking"?
Cristiano Gallina Moreira, *UNESP, Araraquara, SP*

The obligate intracellular bacterium *Rickettsia rickettsii* exert different effects on its natural tick vectors *Amblyomma sculptum* and *Amblyomma aureolatum*
Andréa Cristina Fogaça, *ICB/USP, São Paulo, SP*

Evo-Devo — Sala Esmeralda

Chair: Tiana Kohlsdorf, *FFCLRP/USP, Ribeirão Preto, SP*

Evolution of gene regulatory networks in insects
Rodrigo Nunes da Fonseca, *NUPEM/UFRJ, Macaé, RJ*

Molecular evolution behind EvoDevo: the first necessary step towards genetic manipulation
Mariana Freitas Nery, *UNICAMP, Campinas, SP*

Developmental rates and the evolution of the skeleton of birds
João Francisco Botelho, *Facultad de Ciencias Biológicas/PUC-Chile, Santiago, Chile*

University and Basic Education Intercommunication - Science Divulgation, Research, Teaching and Innovation — Sala Safira

Chair: Adriane Pinto Wasko, *IBB/UNESP, Botucatu, SP*

How to spread ideas with a different approach - Meet AgDC initiative
Adriane Pinto Wasko, *IBB/UNESP, Botucatu, SP*

CEPIDs and the induction to the practices of education and science outreach in SP: the CBME and CIBFar experience
Leila Maria Beltramini, *IF/USP, São Carlos, SP*

Successful training and social insertion experiences: vaccation courses and teaching/learning materials
Lígia Souza Lima Silveira da Mota, *IBB/UNESP, Botucatu, SP*

Plant genome editing

Sala Topázio

Chair: Marcio Alves Ferreira, *UFRJ, Rio de janeiro, RJ*

Genome editing in the model C4 Grass *Setaria viridis*
Marcio Alves Ferreira, *UFRJ, Rio de janeiro, RJ*

Genome editing in monocots: Sugarcane as a model
Hugo Bruno Correa Molinari, *Embrapa, Brasília, DF*

Genome editing in crops: new tools in an old toolbox
Agustin Zsogon, *UFV, Viçosa, MG*

15:45 **CEREMONY**

Closing Ceremony

Salão Real

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Resumos

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OFF-TARGETS ANALYSIS IN SILICO TO MUCOPOLYSACCHARIDOSIS TYPE I BY USING THE CRISPR/CAS9 SYSTEM

Paola Barcelos Carneiro[1]; Martiela Vaz de Freitas[2]; Ursula da Silveira Matte[3]

[1]Graduate Program in Biology, Porto Alegre, Brazil. [2] Post-Graduate Program in Genetics and Molecular Biology, UFRGS, Porto Alegre, Brazil. [3] Post-Graduate Program in Genetics and Molecular Biology, UFRGS, Porto Alegre, Brazil.

pa0labarcellosca@gmail.com

Key words: Mucopolysaccharidosis, CRISPR, off-targets.

Mucopolysaccharidosis type I (MPS I) is caused by alpha-L-iduronidase deficiency (EC.3.2.1.76) coded by *IDUA* gene. The enzymatic deficiency leads to accumulation of heparan and dermatan sulfate (GAGs) into lysosomes triggering a multisystemic disease. CRISPR/Cas9 is a prokaryote's immune mechanism that promotes the cleavage of specific regions guided by a 20 nucleotide RNA. Therapy with CRSIRP/Cas9 has been developed to treat MPS I, however a detailed investigation on off-target effects must be performed before it reaches clinical application. The aim of this study is to evaluate possible off-target regions for the most predominant variant found in MPS I patients (p.Trp402*). In silico predictors CHOPCHOP, COSMID, Cas OFFinder, CCTOP and CRISPOR were used. A total of 63 off-targets were obtained for this variant. The choice of sequences is based in off-targets up to 6 bases pairs different and with or without up to 2 indels that are similar to the on-target region. The sequences with conservation of 5 nucleotides upstream PAM were prioritized, since it is associated with Cas9 activity in the cleavage site. The evaluation of these off-target sequences will be performed by a next generation gene panel in gene edited human fibroblasts in vitro.

Finance agency: CNPq, UFRGS, HCPA

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Correlations among plasma visfatin/NAMPT, nitrite and sFLT-1 levels, and gene interactions in the visfatin/NAMPT pathway: role on pharmacogenetics of Preeclampsia

Lídia L. F. Coura[1]; Daniela A. Pereira[2]; Valéria C. Sandrim[3]; Ana C. T. Palei[4]; José E. Tanus-Santos[5]; Vanessa A. Belo[2]; Ricardo C. Cavalli[6]; Marcelo R. Luizon[1,2].

1Department of Genetics, Ecology and Evolution, Institute of Biological Sciences, Federal University of Minas Gerais, Belo Horizonte, Minas Gerais, Brazil. 2Graduate Program in Genetics, Institute of Biological Sciences, Federal University of Minas Gerais (UFMG), Belo Horizonte, MG, Brazil. 3Department of Pharmacology, Institute of Biosciences, Universidade Estadual Paulista (UNESP), Botucatu, SP, Brazil. 4Department of Physiology and Biophysics, University of Mississippi Medical Center, Jackson, MS, USA. 5Department of Pharmacology, Ribeirao Preto Medical School, University of Sao Paulo, Ribeirao Preto, SP, Brazil. 6 Department of Gynecology and Obstetrics, Ribeirao Preto Medical School, University of Sao Paulo, Ribeirao Preto, SP, Brazil. daniela.biomed1@gmail.com

Keywords: Endothelial disfunction, Nitric Oxide (NO), Visfatin/NAMPT

Introduction: Preeclampsia (PE) is a major cause of maternal and perinatal morbidity/mortality, and the diagnosis includes pregnancy-induced hypertension and other clinical criteria, which may be proteinuria. Antihypertensive therapy prolongs gestation and decreases adverse outcomes. However, 40% of pregnant are nonresponsive to antihypertensive therapy and associated with adverse outcomes. Abnormal placentation and placental ischemia occur in PE, followed by the release of soluble tyrosine kinase-1 (sFLT-1) and soluble endoglin, which leads to lower nitric oxide (NO) bioavailability and results in endothelial dysfunction. Vifastin/Nicotinamide phosphoribosyltransferase (NAMPT) favors endothelial NO production. However, visfatin/NAMPT has been shown to impair endothelium-dependent relaxation by activation of NAPH oxidase and arises as a potential biomarker of endothelial dysfunction. Objective: We examined correlations between plasma visfatin/NAMPT, nitrite (marker of endogenous NO formation), sFLT-1 levels, and characterized the interactions among polymorphisms in candidate genes of the pathway induced by visfatin/NAMPT in normotensive pregnant, in pregnant with PE and in the subgroups of that are responsive and nonresponsive to antihypertensive therapy. We further examined whether *NAMPT* and *VEGF* polymorphisms affect plasma nitrite levels. Methods: Genotypes were determined by Taqman allelic discrimination assay. Plasma nitrite levels were measured by ozone-based chemiluminescence assay and the visfatin/NAMPT concentrations by ELISA, and were analyzed by Spearman's correlation tests. Gene-gene interaction analyzes were performed using the Multifactor Dimensionality Reduction. Results: In patients with PE, visfatin/NAMPT levels were negatively correlated to plasma nitrite levels (r=-0.257, 95% CI=-0.477/-0.007, P=0.038) and positively correlated to sFLT-1 levels (r=0.326, 95% CI=-0.084/0.532, P=0.007). Conversely, in normotensive pregnant, visfatin/NAMPT levels were positively correlated to plasma nitrite levels (r=0.317, 95% CI=0.110/0.497, P=0.002) and negatively correlated to sFLT-1 levels (r=-0.340, 95% CI=-0.592/-0.027, P=0.029). In the subgroup of patients with PE who were nonresponsive to antihypertensive therapy, we found a negative correlation between visfatin/NAMPT and plasma nitrite levels (r=-0.376, 95% CI=-0.643/-0.026, P=0.031). Moreover, we found lower plasma nitrite levels in patients nonresponsive to antihypertensive therapy carrying the TC+CC genotypes of *NAMPT* polymorphism rs1319501 compared to the TT genotype. We observed interactions between the genotypes for the *NAMPT* rs1319501 T>C and *VEGF* rs2010963 G>C polymorphisms in normotensive pregnant and patients with PE (both P=0.001-0.002) and genotypes of the polymorphisms VNTR 27pb of NOS3 and rs2010963 G>C of *VEGF* (P=0.013) in the comparison between subgroups of PE patients responsive and nonresponsive to antihypertensive therapy. Discussion and Conclusions: Our findings suggest that high levels of visfatin/NAMPT do not favor nitric oxide production in the subgroup of patients with PE who were nonresponsive to antihypertensive therapy, and that visfatin/NAMPT has a distinct relationship with plasma nitrite and sFLT-1 in the groups, suggesting an inhibitory effect on NO formation in PE.

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IDENTIFICATION OF AN UNKNOWN PEQUIRA FISH SPECIES: GENETIC, MORPHOLOGICAL AND CONSERVATIONIST ASPECTS

Larissa Arruda Mantuaneli[1*]; Joyce Fico Ramalhães de Souza[1]; Beatriz Queiroz[1]; Giulianna Rondineli Carmassi[1]; Alexandra Sanches[1]

[1]UFSCar – Universidade Federal de São Carlos, Centro de Ciências da Natureza, Campus Lagoa do Sino.

*E-mail: la.mantuaneli@gmail.com

Key-words: Characidae; Hidden diversity; mtDNA.

The eating of pequira fish (Pisces: Osteichthyes) by tourists at southwestern region of São Paulo, is an important source of income for local residents. The literature presents several species with the same popular name. Therefore, the main purpose of this study was perfom a genetic and morphological identification of fish species unknown and treated with "pequira" in the Paranapanema River Basin. For this, two lots of 1 kilo of the fish were purchased at a commercial establishment in Salto, during the dry and full season. Some individuals were selected, grouped in relation to their morphotypes, and had a piece of tissue extracted. All animals were fixed in formaldehyde (10%). The 16S mitochondrial marker was used for the genetic identification of each morphotypes. The purified amplicons were sent to sequence in outsourced company and results were analyzed in BLAST program of NCBI. Through morphological analysis, 14 morphotypes were identified from the lots and 4 species were identified genetically from the 14 previously identified morphotypes. One 1stwas identified as *Bryconamericus stramineus* (99,8-98,12% of identity for diferent samples) or *Piabarchus analis* (99,45- 99,19% of identity for diferent samples), that is the most prevalent species of the portions purchased. The 2ndspecie was identified as *Astyanax fasciatus/A. scabripinnis* (both with 99,19% of identity). The 3rdwas *Acinocheirodon melanogramma/Odontostilbe stenodon* with 98,4-98,99% of identity for diferent samples. Due to evolutionary proximaty of these pairs of species, the mitochondrial marker COI will be sequenced from these individuals for the confirmation of the species. The 4thwas *Serrapinnus notomelas* (100% of identity). The specimens were sent for morphological identification by a specialist. Analyzes of the gonadal development of these fish have shown an additional worrying result: the vast majority of individuals from each lot purchased were composed of immature individuals. Genetic identification is an effective tool in science for conservation and can work together with the morphological identification. However, some genes (most conserved) did not had differentiation in some animals. Thus, some mitochondrial segments may not present sufficient resolution for the problem to be solved. In the case of this study we identified different species composing the lots sold of pequira fish. The identification of the species becomes fundamental for the knowledge of biodiversity and as an aid in future research on the ichthyofauna of the southwestern region of SãoPaulo.

Funding Agency: FAPESP.

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CLINICAL AND CYTOGENETIC INVESTIGATION OF 30 BRAZILIAN PATIENTS OF RUBINSTEIN-TAYBI SYNDROME

Sofia Mizuho Miura Sugayama[1], Marcos Ely Senhorini Escobar[1], Gabriela de Souza Barbosa[1], Vicente Odone Filho

[1]Ambulatório de Investigação e Aconselhamento Genético de Doenças Genéticas com Tumores Pediátricos-Juvenis, e Doenças Raras do ITACI (Instituto de Tratamento de Câncer Infantil) - Serviço de Oncologia Pediátrica-Depto de Pediatria-Hospital das Clínicas da FMUSP.

*sofia.sugayama@hc.fm.usp.br

Key words: Rubinstein-Taybi syndrome; mental retardation; karyotyping.

Introduction: Rubinstein-Taybi syndrome (RTS) is a rare autosomal dominant disease characterized by distinct craniofacial dysmorphisms, broad thumbs and toes, mental and growth deficiency. The prevalence has been estimated to be 1:125.000 to 1:330.000 liveborns. The diagnosis is primarily based on clinical features. RTS has been associated with mutations in CREBBP gene (55%) and EP300 gene (8-16%). Objectives: Clinical and cytogenetic assays of 30 Brazilian RTS patients. Methods: Retrospective and prospective study and metanalysis. Chromosomal analysis was performed by GTG- banding. Results and Discussion: Most patients were female (60%).The mean age of diagnosis was 3y8months all made by pediatric geneticist1.Delayed psychomotor development, typical facies, grimacing smile, broad thumbs, finger pads and feeding problems during childhood were present in 100% of the patients. Short stature and microcephaly were present in 80% and 76% of the patients,, respectively. Main craniofacial characterisitics are frontal bossing (86%), wide nasal bridge (60%), ocular hypertelorism (60%), high arched eyebrows (96%), long eyelashes (93%), epicanthal folds (76%), down slanting palpebral fissures (76%), small opening of the mouth (93%), retrognathism (76%), grimacing smile (100%), high arched palate (93%), and dental anomalies (83%). Other findings included strabism, refractive error, lacrimal obstruction, external ears anomalies (rotation, implantation and morphology),congenital heart defects, hypotonia, stiff gait, undescendent testis, hemangioma, wide angulated thumbs, broad distal falanges of the other fingers, clinodactily, angulated halux, crowded toes, hypertrichosis,and hemangioma. Some children has presented repeated respiratory infections during infancy and childhood. One female patient has presented reciprocal de novo translocation: 46, XX, t(2;16)(q36.3;p13.3). The following features analysed together with Hennekam et al. (1990)'s largest clinical sample (n=571) were in confidence interval (95%): short stature, developmental delay, retrognathism, high arched palate, broad thumbs and halux, clinodactily, and stiff gait. Conclusions: The rarity of RTS and the wide spectrum of clinical findings usually delay the clinical diagnosis of RTS. All children with RTS should be evaluated by a pediatric geneticist, pediatric neurologist, cardiologist, opthalmologist and dentist.

Alternative splicing in ABC transporter as a mechanism associated with development in *Trichophyton rubrum*

Marcos Eduardo Ramos Lopes; Tamires Aparecida Bitencourt; Antônio Rossi; Nilce M. Martinez-Rossi

Department of Genetics, Ribeirão Preto Medical School, University of São Paulo, Brazil

Keywords: MDRs, intron retention, dermatophytes.

Dermatophytes are pathogenic fungi that colonize keratinized tissues, affecting skin, hair, and nails of animals and humans. The dermatophytes are classified into three groups according to their primary association niche: anthropophilic, zoophilic, and geophilic. Among these organisms, at least 31 are known to be human pathogens as *Trichophyton rubrum*, which is a cosmopolitan specie, being the etiologic agent of the most common clinic cases of superficial mycosis in worldwide. The clinical treatment of fungal infections is challenger because the fungi can develop resistance to antifungal drugs commonly used in therapy. The Multidrug resistance (MDR) is a common fungal defense mechanism, which is related to the efflux of drugs by increasing the expression of drug transport proteins, such as the ABC type (*A*TP-*B*inding Cassette). The ABC transporters are transmembrane proteins found in all living organisms. Beyond that, the dermatophytes can change their genetic machinery in response to antifungal therapies through alternative *splicing* events, which result in isoforms of mRNAs from a single gene. The intron retention is the predominant alternative *splicing* event that may occur in *Ascomycetes*. To detect alternative *splicing* events, the RNA-seq methodology is a powerful tool. The objective of this work was to assess the occurrence of alternative *splicing* in encoding genes of drug transporters using available high throughput data from *T. rubrum* exposed to antifungals. A screening of RNA-seq data from *T. rubrum* exposed to undecanoic acid (UDA) highlighted TERG_04309, which encodes an ABC transporter with retention for intron-4 after 3h and 12h of UDA exposure. To validate the occurrence of intron-4 retention, and also to assess the extension of this fungal response, we pre- cultivated *T. rubrum* conidia for 96h (control-0h), and then the resulting mycelia were transferred to fresh RPMI medium in the presence and absence of antifungals, which are represented by Itraconazole (ITZ), caspofungin (CASP), terbinafine (TRB), and UDA for 1h and 12h. After that, an RT_PCR, using surrounding primers for intron-4, was carried out. The results showed the occurrence of intron-4 retention in both times (1h and 12h), in the presence or absence of drugs, with the exception for 0h time point, where just conventional isoform was detected. Furthermore, *in silico* prediction of the resulting protein from intron-4 retention showed a truncated protein with 451 amino acids long, in which it was observed a loss in a transmembrane domain (TMD) and part of the ATP- Binding Domain (NBD). In contrast, the resulting protein from conventional splicing has 726 amino acids long and shows two transmembrane domains and an ATP binding domain. These data suggest the alternative *splicing* of this ABC transporter as a mechanism associated with fungal development.

ANALYSIS OF MATERNAL POLYMORPHISMS OF THE *GCK*, *TCF7L2* AND *LEPR* GENES AND THE INFLUENCE IN BIRTH WEIGHT: A CLINICAL AND MOLECULAR CORRELATION

Lyvia Neves Rebello Alves[1]; Jéssica Aflávio dos Santos[1]; Eldamária de Vargas Wolfgramm dos Santos[1]; Fernanda Mariano Garcia de Souza Rodrigues[1]; Gisele Queiroz de Carvalho[2]; Djanilson Barbosa dos Santos[3]; Iúri Drumond Louro[1].

[1]Núcleo de Genética Humana e Molecular, Departamento de Ciências Biológicas – Universidade Federal do Espírito Santo, Vitória, Espírito Santo, Brazil. [2]Universidade Federal de Juiz de Fora, Governador Valadares, Minas Gerais, Brazil. [3]Universidade Federal do Recôncavo da Bahia, Santo Antônio de Jesus, Bahia, Brazil.

lyviarebello@gmail.com

Key words: birth weight; glucose metabolism; pregnancy outcome.

Birth weight is the main cause of neonatal mortality and morbidity and has long been used as an important public health indicator. Furthermore, birth weight can have long- term health consequences once it is closely related to the development of chronic diseases in adult life. Due to the significant role of maternal glucose concentration as a determinant factor of offspring birth weight, genes that alter glucose homeostasis are good candidates to have an impact on fetal growth, and thus birth weight. To evaluate the influence of maternal genetic variants in the offspring birth weight, three polymorphisms related to glucose metabolism were analyzed (*GCK* rs1799884, *TCF7L2* rs7903146 e *LEPR* rs1137101) in 250 pregnant women who participate of a prospective cohort of Santo Antônio de Jesus – BA, Brazil. Genotyping was performed through TaqMan® assays and the Real Time Polymerase Chain Reaction (qPCR) technic. Maternal characteristics as well as newborn's data were obtained through standardized questionnaires. Samples genotypes were correlated with obstetrical results and clinical, anthropometrics and life habits data of the mother. No significant direct association was found between maternal polymorphisms and the offspring birth weight. This result may be due to sample particularities, especially in relation to ethnicity, since 84% of the analyzed samples are black or brown. It was possible to verify a significant association ($p<0.05$) between the birth weight and the variables baby gender, gestational length and maternal prepregnancy BMI for all the three polymorphisms. Moreover, associations among the *LEPR* rs1137101 maternal genotypes with gestational length ($p=0.037$) and drinking alcohol ($p=0.04$) were found. These results suggest that other factors, whether environmental or genetic, seem to be more related with offspring birth weight than maternal genetic variants that are associated with the glucosemetabolism.

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FUNCTIONAL CHARACTERIZATION OF THE HOXB2 GENE IN GLIOBLASTOMA CELL LINES

Natália Volgarine Scaraboto[12*]; Cibele Cardoso[12]; Rodolfo Bortolozo Serafim[12]; Jessica Rodrigues Plaça[2]; Valeria Valente[3]; Wilson Araújo Silva Jr[12]

[1]Department of Genetics at the Ribeirão Preto Medical School, University of São Paulo, Ribeirão Preto, São Paulo, Brazil. [2]Center for Cell Therapy(CEPID/FAPESP);National Institute of Science and Technology in Stem Cell and Cell Therapy (INCTC/CNPq), Hemocentro Foundation of Ribeirão Preto, Ribeirão Preto,Brazil. [3]Faculty of Pharmaceutical Sciences,Paulista State University"Júlio de Mesquita Filho", Araraquara,Brazil.

*natalia.scaraboto@usp.br.

Keywords: Targets; transcriptome; *HOXB2*.

HOX genes are a subgroup of the Homeobox family characterized by a high degree of conservation among fungi, plants, and animals. In mammals, there are 39 HOX genes distributed in four clusters: HOXA, HOXB, HOXC, and HOXD, located on chromosomes 2, 7, 17 and 12, respectively. HOX genes are transcription factors that act during embryonic development, regulating fundamental biological processes such as proliferation, differentiation, migration, angiogenesis, and apoptosis. Recent studies have indicated a tissue-specific expression profile of HOX genes in different tumor types, suggesting an important role in tumorigenesis. Previous results carried out by our group, we demonstrated that 85% of the HOX genes are overexpressed in glioblastoma (GBM). We also have shown that the high expression of *HOXB2* is correlated with low GBM survival. We planned to use some functional assays to assess whether *HOXB2* regulates some of the biological processes in the GBM. The preliminary results have demonstrated that the silencing of *HOXB2* promotes significant changes in the rate of proliferation, apoptosis, senescence, and cell cycle, in GBM cell lines. In a comprehensive in *silico* analysis, we identified a total of 295 HOXB2 targets in eight cancer types, which are related to biological process such as migration, cell morphogenesis and development, signaling, proliferation and apoptosis. The next step will be to validate these targets using ChiP-Seq combined with RNA-Seq. We believe that our study could provide information to elucidate the functional role of the *HOXB2* gene in glioblastoma. Further, it also could identify new targets for cancer therapies.

Financial support: FAPESP, CAPES and CNPq.

EVIDENCE OF KARYOTIPIC EVOLUTION IN *Astyanax rivularis*: SYMPATRY OF DIFFERENT KARYOMORPHS AND PRESENCE OF B CHROMOSOMES

Igor Henrique Rodrigues Oliveira[1,2]; Karine Frehner Kavalco[2]; Rubens Pazza[2]

[1]Programa de pós graduação em Manejo e Conservação de Ecossistemas Naturais e Agrários, Universidade Federal de Viçosa,*Campus* Florestal, Minas Gerais,Brazil. [2]Laboratório de Genética Ecológica e Evolutiva, Universidade Federal de Viçosa, Campus Rio Paranaíba, Minas Gerais, Brazil.

*igorbiooliveira@gmail.com.

Key-words: speciation; rDNA; FISH

Astyanax is a genus of fish from neotropical region, popularly known as tetras. The group presents many cryptic species and complex taxonomy. *A. rivularis* is an endemic species from the upper São Francisco river basin, underexplored by genetics and populational studies. In this group, the cytotypes range from 46 to 50 chromosomes. The purpose of this study is to characterize two different cytotypes of *A. rivularis* living in sympatry and based on cytogenetic markers, discuss ideas about the evolutionary relationships between them. For this work, we had collected the specimens at Lage stream (Arapuá, Minas Gerais State, Brazil). We euthanized the specimens with 1% eugenol and extracted the renal tissue to obtaining the mitotic chromosomes. We acquired patterns of heterochromatic distribution and the nucleolar organizer regions (NORs) applying C-banding and Ag-NORs techniques, respectively. To physical gene mapping of the rDNA 18S, we performed Fluorescent in situ hybridizations (FISH). There are no macrokaryotipical differences between males and females. Eleven individuals presented karyotype composed by 2n=46+B (6m+24sm+10st+6a+1a) and three composed by 2n=48 (6m+22sm+10st+10a). Both cytotypes showed heterochromatic bands in the terminal region of the long arm of four subtelocentric plus three acrocentric chromosome pairs. The 2n=46 cytotype also showed C+ marking on its entire B chromosome. The Ag-NORs were located in three chromosomes (one submetacentric and two subtelocentric). Unlike the uniformity shown using classic markers, we observed two patterns in the rDNA 18S gene distribution. In the cytotype with 2n=46+B, we observed ten terminal signals and in the cytotype with 2n=48, eight signals were obtained. Both karyotypes are very similar, excepting by the presence of an extra submetacentric pair in the 2n=46 karyomorph and two extra acrocentric pairs in the 2n=48 karyomorph. It seems that these chromosomes are involved in the differentiation of the two cytotypes. As the 2n=46 cytotype is widely found in rivers of the region, it is possible that it has originated the cytotype of 2n=48 by a centric fission process of one of its submetacentric chromosomes pairs. The similarity found in the macromarkers (C-band and Ag-NOR) suggests that the separation between the two cytotypes is recent since the differentiation observed in the karyotypes is incipient. However, the analysis using in situ hybridization to locate the 18S ribosomal gene, plus the presence of B chromosomes involving only the 2n=46 cytotype, and the differences in diploid number could demonstrate a tendency of fixation of these karyomorphs in sympatry. It is necessary to apply more specific markers to confirm the possible fission event that led to the main differentiation of the two cytotypes, but the approach used in this work was efficient to demonstrate differences between the two karyomorphs and the great local diversity within the genus *Astyanax*.

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WHAT DO YOU REALLY KNOW ABOUT GENETICS? AN INSIGHT INTO THE EDUCATIONAL DEMANDS OF HIGH SCHOOL STUDENTS

Marielly de Campos[1]; Cassiane M. Barbosa[1], Adriane P. Wasko[1]

[1]São Paulo State University (Unesp), Institute of Biosciences of Botucatu, Botucatu, SP.

Kew words: Teaching; High School; Genetics.

The involvement of students in learning approaches, especially in High School, is a huge challenge. This fact can be related to several circumstances, as no innovative and practical classes, inadequate school infrastructure, excessive number of students per class, and even problems on teachers´ content update. Since science education and science outreach activities should not be assumed only by basic school, the goal of this work was to develop a survey among public High School students of the Regional Teaching Area of Botucatu city (SP) in order to highlight curricular contents of Genetics that are more difficult to learn and that evoke extra curiosity. Questionnaires were distributed to 220 students, containing different topics that are part of the Biology curricular content. It was possible to collect data from 128 students (91 females and 37 males, mean age of 16 years) enrolled in the 1st, 2nd, and 3rd High School years of 30 schools. Quantitative analyzes of theoretical or practical class activities and of the learning level of different topics were carried out with the objective of evaluating which contents students considered that were effectively given and for which there was a real learning. Topics as 'Alleles", "Allele and Gene Interactions" and "Chromosome" were highlighted as subjects that were not addressed or highly misunderstood throughout High School. Moreover, it was particularly noted that the students has just a partial understanding of the meaning of chromosome function and structure due to the complexity of this theme. However, a learning progress was evidenced for topics as DNA structure and mitosis and meiosis. Although most of the Genetics issues were explored by theoretical classes, it was possible to verify that practical lessons lead to a better learning level. The data was useful not only to identify problems in the teaching/learning Brazilian High School system but also to subsidize future editions of vacation courses that are developed in UNESP every year in order to teach Genetics to low income High School students throughout experimental and playful activities. The proposed actions subsidize the university consolidation as a teaching and research institution and resolution of social and inequality problems, and are in conformity to national politics for basic education, in order to transpose Science and Technology data to basic school.

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Variation in aggressiveness of *Sporisorium scitamineum* haplotypes, the sugarcane smut pathogen

Lâina da Silva de Oliveira[1]; Gustavo Schiavone Crestana[2]; Juliana Benevenuto[3]; Claudia Barros Monteiro-Vitorello[4]

[1,2,4]Department of genetics, "Luiz de Queiroz" College of Agriculture, University of São Paulo, Piracicaba – Brazil; [3]Horticultural Science Department, University of Florida, Gainesville – United States

laina.oliveira@usp.br

Keywords: sugarcane smut; whip; isolates.

Sporisorium scitamineum is a fungal pathogen causing sugarcane smut, one of the most important diseases of the crop. The characteristic symptom of infected plants is the emission of a black whip-like structure, where fungal sporogenesis occurs through the formation of billions of teliospores. These spores spread easily by wind or rain. The objective of this work consisted in comparing aggressiveness between two isolates from distinct haplotypes. The haplotypes defined previously were also further analyzed using the gene g1052_chr02 encoding a candidate effector protein. Twenty five isolates were sequenced and a protein-based dendrogram obtained by the UPGMA method, using the MEGA5 software. The evolutionary distances were computed using the Poisson method with the bootstrap test (10,000 replicates). Buds of plants moderately resistant to smut were inoculated with representants of the two haplotypes (isolates SSC04, collected in Bahia and SSC39, collected in São Paulo). Inoculation was performed by perforation at the base of buds with a solution of teliospores or deionized water for control plants. The experiment lasted 125 days and every 30 days were evaluated quantitative components such as height, number of shoots, stem diameter and number of whips emitted. Plants inoculated with the SSC39 isolate developed the first whip 103 DAI, and during the experiment 6.67% of the plants emitted only one whip per plant. Plants inoculated with SSC04 presented their first whip 68 days DAI, and 28% of the plants developed whips, some of them presenting more than one whip. The evaluations regarding the height, diameter and number of shoots did not show significant changes based on Student´s t- test between treatments SSC39 and SSC04. However, considering the time of development and number of whips, SSC04 isolate was more aggressive than the SSC39. The causes leading to changes in aggressiveness of this isolate are not yet known and should be further investigated. However, the understanding of the molecular components involved in sporogenesis leading to whip emission may help to understand the pathosystem and prevent the maintenance of the pathogen in the environment.

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POPULATION GENETIC DIVERSITY OF *Epinephelus marginatus*: IMPLICATIONS FOR THE MANAGEMENT OFTHAT THREATENED GENETICRESOURCE

Jussara Oliveira Vaini[1*]; Kenneth Gabriel Mota[1]; Rodrigo Rodrigues Domingues[2]; Alexandre Wagner Silva Hilsdorf[1]

[1]University of Mogi das Cruzes, UMC, Mogi das Cruzes, SP. [2]University Federal of São Paulo, UNIFESP, São Paulo, SP.

*jussaravaini@hotmail.com.

Keywords: Grouper; Microsatellite; Conservation

The species *Epinephelus marginatus* (Lowe, 1834) is a marine fish monandric protogynic hermaphrodite and considered vulnerable by International Union for Conservation of Nature and by the Ministry of the Environment in Brazil. This way, the aim of the present study was to describe the patterns of genetic diversity within and between populations of *E. marginatus* using species-specific microsatellites, to test the hypothesis of panmixia in sampled populations in the Atlantic coast of South America and characterize genetic resources of this species as subsidy for its sustainable use in Atlantic coast of South America. Therefore, 351 individuals of *E. marginatus* sampled in Azores Archilpelago, Spain, Greece, Algeria, Pondoland, Argentina and Brazil (São Paulo/São Sebastião, Paraná's coast, Santa Catarina's coast, Rio Grande do Sul/Rio Grande) were genotyped with 15 species-specific microsatellite loci. Analysis between populations, considering all the samples, revealed 8 genetic groups (DAPC), ranged between moderated and high genetic differentiation (D_{EST}= 0,331; p<0,05) and AMOVA showed greatest difference within populations (85,50%; Φ_{ST} 0,15) than between groups (2,79%; Φ_{ST} 0,02). In populations of *E. marginatus* from Algeria, Spain, Greece and Paraná it was verified a sign of bottleneck effect. Samples of Atlantic coast of South America also showed strong population structuring, and population from Rio Grande do Sul was the most different genetically. This study was the first to use a panel of species-specific microsatellite markers for *E. marginatus* in order to verify the genetic diversity of wild populations of dusky grouper in Atlantic coast of South America, suggesting that in Rio Grande's region of Rio Grande do Sul the populations of grouper must be managed and protected as an Independent Management Unit. Rio Grande do Sul the populations of grouper must be managed and protected as an Independent Management Unit.

Financial Support: CAPES, FAPESP and FAEP

INTRA-ARTICULAR GENE THERAPY WITH THE CRISPR/CAS9 SYSTEM IN MUCOPOLYSACCHARIDOSIS TYPE I: SHORT TERM EVALUATION

Hallana Souza Santos[1]; Luisa Natalia Pimentel Vera[2], Mirian Farinon[3], Roberto Giugliani [4]; Roselena Silvestri Schuh[5], Guilherme Baldo[6]

[1]Centro de Terapia Gênica do Hospital de Clinicas de Porto Alegre-Programa de Pós- Graduação em Genética e Biologia Molecular da Universidade Federal do Rio Grande doSul.[2]CentrodeTerapiaGênicadoHospitaldeClinicasdePortoAlegre-Programade Pós-Graduação em Genética e Biologia Molecular da Universidade Federal do Rio Grande do Sul. [3]Laboratório de Doenças Autoimunes do Hospital de Clínicas de Porto Alegre- Programa de Pós-Graduação em Medicina: Ciências Médicas da Universidade Federal do Rio Grande do Sul.[4] Centro de Terapia Gênica do Hospital de Clinicas de Porto Alegre-Programa de Pós-Graduação em Genética e Biologia Molecular da Universidade Federal do Rio Grande do Sul.[5] Centro de Terapia Gênica do Hospital de Clinicas de Porto Alegre-Faculdade de Farmácia da Universidade Federal do Rio Grande do Sul.[6] Centro de Terapia Gênica do Hospital de Clinicas de Porto Alegre- Programa de Pós-Graduação em Genética e Biologia Molecular e Programa de Pós- Graduação em Ciências Biológicas: Fisiologia da Universidade Federal do Rio Grande doSul.

hallanasantos@hcpa.edu.br

Key-words: CRISPR/Cas9**;** Genome editing**;** Mucopolysaccharidosis.

Mucopolysaccharidosis type I (MPS I) is caused by IDUA deficiency and lysosomal accumulation of glycosaminoglycans (GAGs). In this disease, the orthopedic complications reduce mobility. Current treatments do not correct bone/joint condition, probably due to poor penetration of the enzyme in chondrocytes and inability to correct or replace osteocytes. Therefore, it is necessary to look for new therapies. An alternative is gene therapy using the CRISPR\Cas9 system. This work aims to evaluate the efficiency of intra-articular administration of cationic liposomes carrying plasmids with the CRISPR\Cas9 system plus a donor vector to replace IDUA in vivo in MPS I mice. Treated group (n=3) received a intra-articular injection containing the gene editing system. In the control group (n=3), MPS I mice received intra-articular injection of PBS. IDUA results were calculated in U/mg and shown as percentage of a normal mice (wild type C57/BL6). After 7 days the animals were euthanized and the IDUA enzyme activity was assessed in tissues (joints, synovial fluid, serum, liver and lung). The injection significantly increased the activity of IDUA in the joints (12.4 ± 2.6%, while untreated mice was 5.5 ± 2.6%, p=0.007). The enzyme was not increased in serum, synovial fluid, liver or kidney (p>0.05). These results suggest that the administration of the CRISPR\Cas9 system in the joints promotes the increase of the enzymatic activity in situ, without spreading of the enzyme to serum or other organs. This adds important information regarding safety and feasibility of such treatment for MPS, as well as other joint diseases. Long term efficacy is currently being evaluated, but current results hold promises for gene editing as a potential new treatment for MPS.

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Inhibition of the *NTRK2* protein involved in the epithelial mesenchymal transition for the treatment of head andneck cancer: a computationalanalysis

Lennon Pereira Caires[1], Vilson Serafim Junior[1], Eny MariaGoloni-Bertollo[1], Tiago Henrique[2], Érika Cristina Pavarino[1].

1. Genetics and Molecular Biology Research Unit - UPGEM, FAMERP- São José do Rio Preto Medical School – Brazil. 2. Laboratory of Molecular Markers and Bioinformatics, FAMERP - São José do Rio Preto Medical School – Brazil.

Key word: Head and neck cancer, Molecular docking and Epithelial-mesenchymal transition.

The low survival rate in squamous cell carcinoma of the head and neck (HNSCC) may be associated with the epithelial-mesenchymal transition (EMT) process. In consequence, the cell to acquires invasive and migration characteristics and high resistance to apoptosis. Through the *in silico* analysis and molecular docking, it is possible to develop new drugs and optimization of existing drugs, enabling an increase in the survival rate of the patients with cancer, including HNSCC. The objective of this study was to inhibit the neurotrophic receptor tyrosine kinase 2 (*NTRK2*) protein, that promotes EMT, using the natural compound Luteolin as possible therapeutic strategy for HNSCC. In addition, we tested the monoclonal antibodies Larotrectinib and Gefitinib as control. The three-dimensional structure of the *NTRK2* protein was obtained on Protein Data Bank (PDB ID: 1WWB) and used as receptor for docking simulation. After, this three-dimensional structure was prepared using the software *Autodock* Tools. The atomic coordinates of the target molecule (*NTRK2* protein) and the three ligands (Luteolin, Larotrectinib and Gefitinib) were used as input data for the molecular docking. The interaction of these ligands with the target was performed using the *AutoDock* Vina program and validated by redocking. Molecular docking analysis showed a binding energy of -6.9 kcal/mol for Gefitinib, -7.6 kcal/mol for Luteolin and - 8,2 Kcal/Mol for Larotrectinib. The lowest energy, which represents the best efficiency of the ligand to the target, was observed for Larotrectinib. This monoclonal antibody has inhibitory action on the *NTRK2* protein and has been used currently as an antineoplastic agent. Gefitinib that is a known inhibitor of genes involved in the EMT process and that has as a possible off-target the *NTRK2* gene showed the highest energy. Luteolin is a flavonoid that induces apoptosis and cell cycle arrest and exhibited lower docking energy value than Gefitinib. In conclusion, our results showed that the natural compound Luteolin has potential to inhibit *NTRK2* and may to blockthe process of EMT in HNSCC, decreasing the migratory and invasive capacity. Therefore, further studies in vitro are necessary to validate our results.

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CYTOKINES IFN-γ AND TNF-α INDUCE MITOCHONDRIAL DYSFUNCTION IN HUMAN CARDIOMYOCYTES: IMPLICATIONS FOR CHAGAS DISEASE CARDIOMYOPATHY

João Paulo Silva Nunes[1,2,3]; Karla Alcântara[1,2,3]Christophe Chevillard[4], Jorge Kalil[1,2,3], Rafael R Almeida[1,2,3], Edecio Cunha-Neto[1,2,3*]

[1]Laboratory of Immunology. Heart Institute (InCor) University of São Paulo School of Medicine, São Paulo, Brazil. [2]Division of Clinical Immunology and Allergy. University of São Paulo School of Medicine, Brazil. [3]Institute for Investigation in Immunology (iii), INCT, São Paulo, Brazil. [4]INSERM UMR_1090, Aix-Marseille Université, France

*edecunha@gmail.com, jpaulo.nunes@hotmail.com

Key words: mitochondrial dysfunction; Chagas disease;IFN-γ/TNF-α;

Chagas disease cardiomyopathy (CCC), an inflammatory dilated cardiomyopathy, occurs in 30% of patients chronically infected by *Trypanosoma cruzi*. Myocardial tissue from CCC patients is an inflammatory-rich environment with augmented expression of IFN-γ and TNF-α. Increasing evidence link mitochondrial dysfunction and HF severity, and suggest that host genetic factors contribute to disease progression. Mitochondrial damage seems to be especially severe in CCC. Our group found that rare heterozygous pathogenic variants in mitochondrial genes were associated exclusively with CCC and absent in asymptomatic (ASY) siblings, in multiple Chagas families. Our working hypothesis is that persistent production of inflammatory cytokines in CCC damage cardiomyocytes by inducing mitochondrial dysfunction. The objective of this study was to evaluate mitochondrial dysfunction in response to IFN-γ and TNF-α stimulus in the human cardiomyocyte cell line AC-16. AC-16 cells were stimulated with IFN-γ (10 ng/ml), TNF-α (5 ng/ml) and the combination of both for 24 or 48h. We evaluated, by High-Content System, mitochondrial membrane potential (ΔΨm) using TMRE, mitochondrial superoxide (mtROS) production with MitoSox and MitoTracker Green probes and cell viability using propidium iodide (PI) stain. We also measured cell metabolism by MTT and changes in expression of oxidative stress- and mitochondrial- related genes by RT-qPCR. Our data show that IFN–γ impairs ΔΨm in a time- dependent manner (24h: average±SD -23.37±7.8%; 48h: -30.24±13.64%; $p < 0.0001$; n=8) and its effect is enhanced when combined with TNF-α (24h: -32.4±4.78%; 48h: - 42.46±10.9%; $p < 0.0001$; n=6) with no impact on cell viability. We also found that TNF- α alone increased mtROS 48h after stimulation (+23.95±3.6% $p < 0.001$ n=3), compared to control. The MTT assay indicated NAD(P)H-dependent cell metabolism was modulated by IFN-γ and TNF-α and IFN-γ+TNF-α after 24 and 48h of treatment. These results indicate that IFN-γ/TNF-α have a direct effect on mitochondrial function of cardiomyocytes. This may provide a link between inflammation and mitochondrial dysfunction observed in CCC. We will next assess the effect of cytokines on cardiomyocytes derived from induced pluripotente stem cells from CCC patients bearing mitochondrial allowing a better understanding between of interaction of mitochondria and inflammatory cytokines in the pathogenesis of CCC.

INFECTION OF TOMATO "MICRO-TOM" SHOOTS BY *Moniliophthora perniciosa*, THE CAUSAL AGENT OF CACAO WITCHES´ BROOM DISASE, INDUCES ROOT MALFORMATION

Daniele Paschoal[2]; Eder Marques da Silva[2]; Juliana Aparecida Aricette[3], Piotr Mieczkowski[4], Paulo José Pereira Lima Teixeira[5]; Antonio Figueira[2]

[1]Departamento de Genética e Melhoramento de Plantas, Escola de Superior de Agricultura "Luiz de Queiroz", Universidade de São Paulo.[2]Laboratório de Melhoramento de Plantas, Centro de Energia Nuclear na Agricultura, Universidade de São Paulo.[3]Laboratório Nacional de Ciência e Tecnologia do Bioetanol.[4]University of North Carolina at Chapel Hill, Chapel Hill, NC, USA.[5]Departamento de Ciências Biológicas, Escola de Superior de Agricultura "Luiz de Queiroz", Universidade de São Paulo.

danielepaschoal@usp.br

Key words: RNA-seq; root; pathogen

Witches' broom, caused by *Moniliophthora perniciosa*, is a devastating cacao disease. The S-biotype that infects Solanaceae enabled the use of the tomato 'Micro-Tom' (MT) as a model to investigate the pathogenesis. We determined that MT plants infected at the shoot apex exhibit a reduced root biomass, but not root length. We hypothesized that the limited roots may substantially reduce water and nutrient uptake. To understand the physiological reasons for the effect of infection on roots, we analyzed roots from inoculated MT plants by RNA-seq and GC-TOF-MS. At 5 days after inoculation (dai), root biomass from inoculated plants was not altered; however, RNA-seq analysis revealed the up-regulation of genes associated with responses to biotic stress, including *CC–NBS–LRR, THAUMATIN-LIKE PROTEIN*, and related to the phenylpropanoid metabolism. After 10 dai, inoculated plants showed significantly reduced root biomass. The level of sugars in roots from infected plants decreased at 10 and 20 dai. Accordingly, at 10 dai, the most repressed gene in roots from inoculated plants was *SWEET,* involved in sugar transport, and at 20 dai, glucosyl transferases and the *GLUCOSE TRANSPORTER 8* gene were down-regulated. At 10 and 20 dai, there was an accumulation of trehalose, which corroborated the up-regulation of *TREHALOSE 6 PHOSPHATE SYNTHASE* gene, and the increase in fucose. Members from dehydration-responsive gene families, *ABSCISIC ACID INSENSITIVE 3* and ABA receptors were up-regulated at 30 dai, corroborating the water stressed phenotype from reduced roots. Also, the accumulation of galactonic acid and overexpression of glutaredoxins suggested a stress response. The levels of some amino acids decreased at 20 and 30 dai, possibly as an attempt to recover energy by oxidation to supply carbon and energy. The decrease in asparagine and glutamine, plus the up-regulation of *GLUTAMATE SYNTHETASE* and genes related with nitrate and ammonium metabolism suggested an increase in N remobilization. The induction of auxin responsive genes, together with the repression of ethylene response factors since 5 dai, may indicate an effort to promote lateral root growth, unsuccessful due to restriction of energy supply. Genes associated with jasmonate signaling were upregulated at 10 and 30 dai, while those related to cytokinin metabolism were induced at 20 and 30 dai. Cytokinin may accumulate in roots after exposure to growth-limiting conditions to favor resource reallocation to roots. Overall, our results indicate a shift in root metabolism that may explain the reduction in root biomass of infected MT plants because of sugar deprivation due to the formation of a strong sink in infected stems. We consider that the impaired root system may lead to a negative impact in water and, possibly, nutrient uptake in infected plants directly affecting yield.

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Transposable elements characterization and horizontal transfer analysis among Culicidae species

Elverson Soares de Melo[1]; Gabriel da Luz Wallau[1,*]

[1] Department of Entomology, Aggeu Magalhães Institute, Oswaldo Cruz Foundation (Fiocruz) Pernambuco, Professor Moraes Rego Avenue, s/n - Campus da UFPE - Cidade Universitária, Recife/PE – Brazil, 50740-465.

* gabriel.wallau@cpqam.fiocruz.br

Palavras-chave: Transposable elements; Horizontal Transfer; Mosquitoes

Transposable elements (TEs) are DNA sequences that can move between different genomic loci and even between genomes. There are several types of TEs, but most of them are classified as ClassI and ClassII TEs based on its transposition mechanism. The main mechanism by which the TEs are inherited is through vertical transfer, however, mounting evidence are showing that they can also transpose the species barrier and invade another species genome through horizontal transfer (HT). The transposable element content is barely described in Culicidae family genomes. This family comprises mosquitoes that are vectors of many humans pathogens and due to the importance of TEs to genome structure and host fitness the main focus of this study was characterize all the families of TEs present in 24 mosquito species from the genus: *Aedes*, Anopheles and *Culex* by a computational approach and test these TEs inheritance mode. To achieve these goals, we download the mosquitoes genomes from NCBI and Vectorbase and characterized TE content by a de novo approach using TEdenovo software and by homology with TEs present in Repbase and TEfam databases. After that, we build clusters of TEs respecting the 80-80 rule and analyzed potential HTs using VHICA R package. The proportion of genome composed by TEs varies substantially from high proportion in *Ae. aegypti* (60.5%) followed by *Ae. albopictus* (44%) to species with very few TEs like *An. koliensis* (0.13%). Studding this variation, we found a positive correlation between genome size and TE content. The majority of these TEs was acquired by vertical transmission, but we found 301 potential horizontal transfer events ($p<0.01$). Sixty-seven are ClassII TEs, 119 are non-LTR TEs and 115 are LTR TEs. The LTRs and non-LTRs Hts occurred only between species form the same genus, on the other hand ClassII TEs, composed in majority by Tc1-Mariner superfamily, were transferred between species from *Aedes* and *Anopheles genus*. The lasrgest part of HT events occurred between *Ae. aegypti* and *Ae. albopictus*, we estimate that around 6% of the genome of these species are derived from horizontally transferred TEs. On the other extreme there are species not involved in HT events, like *Cu. quinquefasciatus*, *An. darlingi, An. punctulatus* and *An. koliensis*. The absence of HT involving these 3 species of Anopheles is probably associated with their very small TE content, by contrast *Cu. quinquefasciatus* has a large TE content and the absence of HT detection is probably related with the small number of disponible genome sequence of other Culicinae subfamily mosquitoes. This work show the large diversity of TEs in the Culicidae family and that HT events occurs more frequently than expected.

MOLECULAR IDENTIFICATION AND AUTHENTICITY OF SEAFOOD COMMERCIALIZED AS FLOUNDER IN RIO DE JANEIRO

Daniela Souza[1*]; Rafaela Soares[1]; Marcela Simões [1]; Frederico Hening[1]; Antonio Sole-Cava[1]

[1]Universidade Federal do Rio de Janeiro, Departamento de Gnética, Instituto de Biologia, UFRJ.

*danielafdsds@gmail.com

Palavras Chave: Seafood Mislabeling, Authentication, Flounder

Global seafood trade has grown for the past decades, and flounders are among the most produced and expensive products, for being considerate a noble fish, due to the excellent quality of their meet. A large over fishing and decreasing flounder production, while prices remain high, has led to increases in mislabeling cases. In Brazil, the fish legally treated as "Flounder" correspond to species of the family Paralichthyidae, genera *Syacium* and *Paralichthys*. On the national and international trade, species of lower commercial value, such as *Atheresthes stomias*, *Pangasionodon hypophthalmus* and *Pleuronectes platessa* are often illegally labeled and sold as flounder. This mislabeling generates several consequences for consumers, that range from economic losses until the damages to the public health, due to some of this species may contain allergenic or contaminant agents. As an authenticator, identification of fishes and fish products, especially processed, are necessary to detect and prevent such mislabeling. Molecular techniques such PCR based, FINS (forensically important nucleotide substitution) has been presented to be an effective tool, enabling an identification of fishes even after a high level processing. In this panorama, this project objective has been identified and made a mislabeling prevail estimative on the flounder market chain in Rio de Janeiro. The aim is establish and compare suitable authentication methods based on PCR multiplex, PCR RFLP and sequencing as viability, coast and efficiency. A large-scale of 200 samples collection has been performed in restaurants markets, supermarkets and fishmongers. A total of 182 samples have already been collected, had your DNA extracted and 104 of these were sequenced for two mitochondrial genes (cytochrome b and cytochrome oxidase I), using capillary system (ABI3500). For the new applied tools, PCR multiplex and PCR RFLP, yardsticks have been bilding, based on diagnostic standarts, produced respectively by amplification and fragmentation of different sizes amplicons, and until this moment PCR multiplex showed batter results. FINS analysis has shown a 63% of mislabeling on sample collections, distributed in eight species, some commonly found and others without previous records, such as *Micropogonias furnieri* and *Merluccius hubbsi*. Among all, however, the Panga fish (*Pangasionodon hypophthalmus*) was the most found, used 56% as a substitute of flounder, this fish is observed in other substitutions reported on literature, assnappers,"cacao", Cod and Nile perch. All these reports are frequently associated with low cost and easier acquisition of Panga on your account you crop origin, reaching to cost four times less than some replaced. Moreover, on the flounder market chain, restaurants appeared with the biggest mislabeling incidence, in 95% of the cases. This result is especially interesting if we consider that restaurants are not focus on seafood inspections, therefore, the efforts authentication public or private, must be redirected.

ANALYSIS OF CHR9P22.1-P21.3 LOCUS POINTS TO GENES OF POSSIBLE PROGNOSTIC VALUE IN SEVERAL TYPES OF CANCER

Paola Gyuliane Gonçalves[1]; Rui Manuel Reis[1]; Lucas Tadeu Bidinotto[1,2*]

[1]Molecular Oncology Research Center - Barretos Cancer Hospital, Barretos, Brazil. [2]Barretos School of Health Sciences, Dr Paulo Prata – FACISB, Barretos, Brazil.

*lucasbidinotto@gmail.com

Key words: Cancer; *In silico* analysis; TCGA

The LOH of genes on chr9p22.1-p21.3 region has been described as an early event in the development of cancers, and several studies point to the presence of important tumor suppressor genes, besides CDKN2A and CDKN2B. Our aim was to identify genes in 31 types of cancer from the TCGA (The Cancer Genome Atlas) studies which may be subject of further studies, using in silico tools. Genomics and clinical data from different types of cancer were imported from TCGA database using the package TCGA2STAT, implemented in R, and further processed. CGH microarray data was downloaded to analyze the copy number variation of 24 genes present in the locus (SLC24A2, MLLT3, FOCAD, PTPLAD2, IFNB1, IFNW1, IFNA21, IFNA7, IFNA10, IFNA16, IFNA17, IFNA14, IFNA5, KLHL9, IFNA6, IFNA2, IFNA8, IFNA1, MTAP, C9orf53, CDKN2A, CDKN2B, DMRTA1, ELAVL2) in 8499 samples across 31 datasets. Then, quantity of RNA sequencing reads (RSEM normalized) from these genes was imported from the same samples. In order to analyze if the ploidy would be the main responsible for RNA expression, Pearson's correlation of matched aCGH and RNASeq data was performed in the patients for each dataset. Finally, the patients were categorized into high- and low- expression based on the median expression for each dataset, and Kaplan-Meier plots were created for all genes in the 31 datasets. $P<0.05$ in log rank tests was considered as statistically significant. Glioblastoma, bladder urothelial carcinoma and mesothelioma datasets presented the higher number of genes with strong ($|0.8|>R>|0.6|$) or very strong ($R>|0.8|$) correlation of copy number alterations and expression. When considering each gene along all the datasets, besides CDKN2Aand CDKN2B, the genes that presented strong/very strong correlation of CNA and expression in a higher number of datasets were KLHL9, MTAP and FOCAD. Overall survival of patients, considering the expression of these genes, showed mesothelioma, kidney renal clear cell carcinoma and brain lower grade glioma datasets with more genes which differential expression was correlated to survival. Furthermore, when considering each gene along all the datasets, we found potential prognostic role of CDKN2A, MLLT3, FOCAD and PTPLAD2 in a higher number of datasets. In conclusion, besides CDKN2A and CDKN2B, there are several other genes in chr9p22.1-21.3 that are possibly related to cancer development, namely MLLT3, PTPLAD2, FOCAD, KLHL9 and MTAP. They may be related to cell cycle, differentiation and metabolism, and might be important of future studies, especially in glioma, bladder urothelial carcinoma, kidney renal clear cell carcinoma and mesothelioma. Further studies must be performed in the aforementioned genes and tumor types which results might contribute to the development of new therapies targeting specific molecules in those cancers.

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miR156 overexpression represses the immune response against *Moniliophthora perniciosa* in tomato

Rafael Monteiro do Carmo[1]; Bruna Marques de Queiroz[1]; Daniele Paschoal[1]; Antonio Vargas de Oliveira Figueira[1]; Eder Marques Silva[1]*

[1]Centro de Energia Nuclear na Agricultura, Universidade de São Paulo, Piracicaba.

*edermarques.silva@usp.br

Key words: SPL/SBP box transcription factor, witches' broom disease, *Theobroma cacao*

The hemibiotrophic basidiomycete *Moniliophthora perniciosa* causes the witches' broom disease (WBD) in Theobroma cacao, the chocolate tree. Biological characteristics of the host, such as growth rate and plant size, limit the investigation of the *M. perniciosa* x *T. cacao* interaction and its molecular aspects. Thus, the existence of *M. perniciosa* isolates pathogenic to Solanaceae (S-biotype) enabled the use of tomato cultivar Micro Tom (MT) as a model system. Previous data from our group showed that the infection of MT with S- biotype led to stem swelling and axillary shoot growth to form broom-like symptoms similar to the biotrophic phase in cacao. Here, we present data that suggest that the module microRNA156/SlSBP box transcription factor may be involved with some symptoms induced by *M. perniciosa* infection. Non-inoculated MT transgenic plants overexpressing the microRNA156 (OEmiR156) displayed a phenotype similar to the symptoms of infected MT plants. When OEmiR156 was infected with S-biotype spores, the plant presented more pronounced symptoms than inoculated MT plants. RNAseq data from inoculated MT plants show that some SPL/SBP box transcription factor genes, that are regulated by miR156, are repressed during the infection. These results led us to think that the repression of SPL/SBP box transcription factors may facilitate the colonization by *M. perniciosa*. To date, there is no information regarding small RNAs control pathways (e.g. microRNAs) been implicated with *M. perniciosa* symptoms. Dissecting this mechanism during *M. perniciosa* x host interaction may be an important step toward understanding the symptoms progression and assist with effective strategies to control the disease.

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DELIVERY METHOD OF THE SILENCERS RNA BY IRON OXIDE NANOPARTICLES FOR THE GLIOBLASTOMA MULTIFORM TREATMENT

Cyro von Zuben V. Negrão [1]; Valker Feitosa[2], Patrícia Leo[2], Natalia N. P. Cerize[2]; Henrique Marques-Souza[1]

[1]Biology's Institute; University of Campinas. [2]Bionanomanufacturing Centre; Institute for Technological Research

cyrozvn@ipt.br

Key-words: RNAi; GBM; iron oxide nanoparticles.

Glioblastoma multiform (GBM) has been reported as the most severe and deadly brain cancer, with prognosis of only 14 months. The most common first line treatment is realized by extraction of the tumor, followed by radiotherapy and chemotherapy. Nowadays, brain chemotherapy has encountered major obstacles to become effective due to two factors: drug resistance by patients and difficulty of drug molecules to cross the blood-brain barrier. Cancer biology has revealed a viarety of genes tha promote growth, survival and spread. Gene silencing via RNA interference (RNAi) represents a great promise to target these genes, but a method for delivering small interfering RNA (siRNA) specifically and efficiently to cancer cells have been a major challenge. Iron oxide nanoparticles (IONP) has been used in several medical applications and could be used as a vehicle to drive siRNA into specific cells and tissues. The aim of this work is to develop a delivery method of siRNA using IONP for the treatment of GBM. To achieve this, specific peptides will be used to functionalize the surface of IONP to enrich targeting to tumor cells and funcionalized IONP will be complexed with siRNA molecules targeting genes that cause tumor maintenance and resistance to Temozolomide, the most important drug used today in GBM chemotherapy. By combining RNAi and nanoparticles we intend to develop a method for tumor-specific gene silencing that could complement current methods to promote less invasive and toxic therapies to fightcancer.

Funding Agency: CAPES; FIPT.

CYTOTOXICITY, GENOTOXICITY AND APOPTOSIS OF HUMAN GINGIVAL FIBROBLAST TREATED WITH EXTRACTS OF ENDODONTIC SEALERS INCORPORATED WITH SILVER VANADATE

Ana Beatriz Vilela Teixeira[1]; Natália Chermont dos Santos Moreira[2]; Catarina Satie Takahashi[3]; Andréa Cândido dos Reis[1*]

[1]Departamento de Materiais Dentários e Prótese, Faculdade de Odontologia de Ribeirão Preto/ Universidade de São Paulo. [2]Departamento de Genética, Faculdade de Medicina de Ribeirão Preto/ Universidade de São Paulo [3]Departamento de Biologia, Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto/ Universidade de São Paulo.

*andreare73@yahoo.com.br.

Palavras-chave: Endodontic Sealer; Nanoparticles; Mutagenicity Tests

This study incorporated the nanostructured silver vanadate decorated with silver nanoparticles ($AgVO_3$) into endodontic sealers and evaluated the cytotoxicity, genotoxicity and apoptosis of human gingival fibroblast (HGF). The $AgVO_3$ was incorporated at sealers AH Plus and Sealer 26 at concentrations 0%, 2.5%, 5% and 10%, and after setting of sealers, extracts of the specimens were obtained in culture medium DMEM e HAM F10 by 24 h and 7 days. FGH were grown, the cytotoxicity was evaluated by XTT assay, the genotoxicity by the comet assay, and apoptosis, by the cell death assay. Cells were treated with culture medium (negative control), 300 and 500 µM methylmethanesulfonate (positive control - comet and cytotoxicity, apoptosis respectively) and extracts from the specimens by 24 h (n=3). After the treatment, the cells were collected and submitted to the comet assay and analyzed in the software Comet Assay IV. For cytotoxicity, after treatment and 24 h in the culture medium, the cells were placed in contact with the XTT kit for 3 and a half hours, and cell viability was quantified in microplate reader. For apoptosis, after treatment and 96 h in the culture medium, the cells were collected and the Guava Nexin reagent was added for 30 min. Viable, apoptotic and necrotic cells were evaluated on flow cytometry. Data were analyzed by ANOVA and Tukey (α=0.05). The cell viability of AH Plus groups showed no statistical difference of the negative control ($p > 0.05$), except for 10% (7- days extract - $p = 0.038$). The groups of Sealer 26 had a reduction in the cell viability of FGH in relation to the negative control ($p < 0.05$), independent of the incorporation of $AgVO_3$. Cytotoxicity may be inherent to the sealers components and it is not possible to evaluate the influence of $AgVO_3$. The comet and cell death assay showed that there was no statistical difference between the negative control and the experimental groups ($p<0.05$), except for AH Plus 10% (7-days extract), which presented greater induction of apoptotic cells in relation to the negative control ($p<0.05$). It was concluded that the incorporation of $AgVO_3$ into the AH Plus did not influence FGH cell viability (except AH Plus 10% 7-days extract), and it was not possible to verify its influence on Sealer 26 cytotoxicity. The incorporation of $AgVO_3$ into endodontic sealers does not induce DNA damage and FGH apoptosis, except for AH Plus 10% (7-daysextract).

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SEARCHING FOR A DIFFERENTIAL PATTERN OF GENOMIC DNA METHYLATION IN MESIAL TEMPORAL LOBE EPILEPSY ASSOCIATED WITH HIPPOCAMPAL SCLEROSIS

J. C. Geraldis[1], D. B. Dogini[1], A.M. Canto[1], S.H. Avansini[1], M. Alvin[2], F. Rogerio[3], C.L. Yasuda[2], B. S. Carvalho[4], F. Cendes[2], I. Lopes-Cendes[1]

[1]Department of Medical Genetics and Genomic Medicine, [2]Department of Neurology, [3]Department of Anatomical Pathology; School of Medical Sciences, (FCM), [4]Department of Statistics, Institute of Mathematics, Statistics and Scientific Computing (IMECC), University of Campinas (UNICAMP), Campinas, SP, Brazil; and the Brazilian Institute of Neuroscience and Neurotechnology (BRAINN).

Palavras-Chave: Epilepsy; methylome; brain tissue

Introduction: Mesial temporal lobe epilepsy (MTLE) associated with hippocampal sclerosis is one of the most common and severe types of epilepsy since many patients are refractory to antiepileptic drug treatment. In these patients, surgical treatment may be a therapeutic alternative, which includes the surgical resection of brain tissue, presenting epileptic activity and histopathological changes. DNA methylation is the most studied epigenetic mechanism; it acts on gene regulation and may be reversible. Our main hypothesis is that the differences in gene expression identified in surgical specimens of patients with MTLE may be caused, at least in part, by differentially methylated regions in the human genome. In addition, to better understand the complex biological mechanisms underlying MTLE, we aim to integrate the methylome data with transcriptome and proteomics obtained from the same tissue. Materials and Methods: We selected tissue from ten patients with MTLE and five controls from an autopsy. The patients are organized into two groups: one with less than 20 years of disease (n = 5); another with more than 20 years (n = 5). Tissue (*hippocampal formation*) from patients and autopsy were cut in a cryostat, and we obtained eight blades with four slices (60 µm each slice). Laser microdissection was performed in these slices, and the dentate gyrus was separate from the rest of the tissue for further experiments. DNA was extracted using phenol-chloroform protocol and quantified with *Qubit High Sensitivity* (*Thermo Fisher*). We performed the bisulfite conversion and the library construction using the *Pico Methyl-Seq Library Kit* (*Zymo Research*) for further sequencing (*NovaSeq Illumina Platform*). Results: All the tissues from patients and controls were successfully microdissected. The phenol-chloroform protocol produced DNA with good quality but with low quantity, mainly due to the low input DNA obtained after laser microdissection. However, after several optimization steps, we were able to achieve a satisfactory DNA concentration to attain ideal library preparation for NGS sequencing. Discussion/Conclusions: We believe that by integrating multiple omics modalities, we will achieve a better understanding of the complex molecular mechanism involved in MTLE and the characteristic histopathological lesion occurring in hippocampal sclerosis.

Mobilome of *Ustilago hordei*: Insights into transposable elements and their association with effector candidate genes

Clesivan Pereira dos Santos, Juliana Benevenuto, Hugo Rody Vianna Silva, Claudia Barros Monteiro-Vitorello.

Microbial Genetics Laboratory, Department of Genetics, University of São Paulo/Luiz de Queiroz College of Agriculture (USP/ESALQ), Piracicaba, Brazil.

clesivanpq@usp.br

Key-words: mobile elements, effectors, covered smut.

The biotrophic fungus *Ustilago hordei* (covered smut) comprises isolates specialized in the infection of barley and oat plants. Effectors are proteins secreted by pathogens to overcome plant defenses, and are potentially determinants of host specialization. In several smut fungi, genes encoding effectors are found commonly located in repetitive regions of the genomes, rich in transposable elements (TE). Previously, we have predicted a notable repertoire of effector genes, and identified a high proportion of TEs in the genomes of two *U. hordei* strains. Here, we hypothesize that the proximity of TEs to effectors are associated with the rapid evolution of these genes in pathogen populations. Hence, we aimed to advance on understanding of how TEs are leading effectors diversification in *forma especialis* differentiation of *U. hordei* isolates. Genome sequences of three *U. hordei* strains: Uh4857-4 and Uh364 isolated from barley, and Uh01 from oat were retrieved from NCBI. *De novo* identification of repeats was performed by RepeatModeler v1.0.11. Annotation of repeats and genome masking were obtained with RepeatMasker v4.0.7. Alternatively, Tephra v0.12.3 was also used for *de novo* identification and classification of TEs. Prediction, annotation and orthology of effector candidates genes were accessed in Benevenuto et al. (2018). Furthermore, BLAST tool was employed to associate TEs with 10 predicted effector families (*Cmu1, Stp1, ApB73, Pep1, Pit2, See, Avr1, Mig1, Mig2 and Eff1*), well characterized in *U. maydis*. The TE contents ranged from 5.45 and 11.73% (Uh4857-4) to 21.90 and 26.46% (Uh364) strains according to Tephra and RepeatModeler analyses, respectively, indicating that a larger genome size assembled covered a higher TE rate. The majority of TEs classified was assigned to retrotransposons (class I), LTR order and subfamilies Copia and Gypsy. DNA transposons (class II) represented a lower rate of total TEs, and comprised by TIR (Tc1/Mariner and CACTA superfamilies) and Helitron orders. Particularly, TEs were near to two *Cmu* identical copies located in different scaffolds in Uh364. Conversely, the other strains exhibited only one copy of the gene with no apparent TE association, indicating that a recent transposition event duplicated *Cmu* gene in Uh364. *Avr1* and 2 were distributed in tandem and immersed in TE-rich region in both Uh4847-4 and Uh01 strains. Interestingly, only Uh01 exhibited an insertion of 7kb TE between *Avr* paralogous, suggesting a possible regulatory role for the element. Thereafter, two *Mig* genes of the families 1 and 2 were detected proximal to TEs in all strains. Curiously, a mobile element was linked to a *Mig* (Uh01), undetected in the genomes of both strains infecting barley. In addition, *ApB73* (Uh364), *Eff1* and *1a* (Uh4857-4), *Stp1* and *Eff1* (Uh01) were also linked to TEs. Overall, such a peculiar TE-effector genes signatures in each strain suggests its potential role in shaping (a) virulence and host specialization of *U. hordei* isolates.

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VIRAL PERCEPTION AT THE CELL SURFACE BY THE NIK1 IMMUNE RECEPTOR

Ruan Maloni Teixeira[1,2]; Marco Aurélio Ferreira[1,2], Gabriel Ângelo Saraiva Raimundo[1,3], Virgílio Adriano Pereira Loriato[1,2], Caio Campos Araújo Pádua[1,2], Pedro Augusto Braga dos Reis[1,2] and Elizabeth Pacheco Batista Fontes[1,2]

[1]National Institute Of Science And Technology In Plant–Pest Interactions, Bioagro, Universidade Federal De Viçosa,Viçosa, Minas Gerais 36570-000,Brazil.[2]Departament Of Biochemistry and Molecular Biology,Universidade Federal De Viçosa,Viçosa,Minas Gerais 36570-000, Brazil. [3]Agronomy Institute, Universidade Federal De Viçosa, Campus Florestal, Florestal, Minas Gerais 35690-000, Brazil.

ruanmaloni@gmail.com

Keywords: *Molecular Biology; plant immunity; viral PAMPs*

Recognition of viral components or effectors activates immune responses by host receptors. This activation can occur through two layers of defense, immunity triggered by pathogen-associated molecular pattern (PTI) and effector-triggered immunity (ETI). Although the ETI has been long demonstrated as an efficient defense against virus, the concept of PTI has been only recently linked to host-virus interaction models, such as RNA silencing processes activated by double stranded RNA molecules (dsRNA) produced during infection. Studies in Arabidopsis thaliana of virus-related defenses have identified a novel antiviral defense pathway by the interaction of the NSP (Nuclear shuttle protein) of Begomovirus, a genus of the family *Geminiviridae*, with the protein NIK1 (NSP-interacting kinase), a receptor-like kinase. On the cytoplasmic side of the cell membrane, the binding of the viral NSP protein to the NIK activation loop inhibits its kinase activity, creating a more favorable environment for virus infection. NIK1 has been shown to autophosphorylate at the threonine residue 474 for activation of the kinase domain. The activation of NIK1 leads to the phosphorylation of the ribosomal protein (RP) RPL10 and its subsequent translocation to the nucleus, where interacts with LIMYB (L10-interacting MYB domain containing protein) to fully down-regulate components of the translational machinery. This leads to repression of the global translation impairing host and viral mRNA translation. Transcriptome studies by overexpressing LIMYB in Arabidopsis also demonstrated that LIMYB represses the network of ribosomal genes, and components of the photosynthetic apparatus. However, the viral activator particles of the NIK1 pathway remain unknown. In this study, we presented evidence that viral nucleic acids are recognized as PAMPs that trigger the NIK1 immune responses. Furthermore, we showed that LIMYB may couple global translation repression and the inhibition of photosynthesis, a possible link between begomovirus resistance and adaptive responses. We showed that treatment of plants with DNA and RNA from begomovirus-infected plants activates the NIK1-mediated antiviral pathway, which was monitored by analyzing the nucleic acid-induced repression of ribosomal protein genes and phosphorylation of RPL10. Our results indicate that begomoviral RNA and DNA function as PAMPs to activate the NIK1-mediated antiviral signaling pathway. A more direct link between translational repression and photosynthesis inhibition is also demonstrated as the viral PAMPs also induce repression of the photosynthetic apparatus components.

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SOYBEAN GENOME EDITING BY CRISPR-CASFOR ANTINUTRITIONAL FACTORSSILENCING

Alessandra Koltun[1]; Liliane Márcia Mertz-Henning[2*]; Alexandre Lima Nepomuceno[2]; Leandro Simões AzeredoGonçalves[3].

[1]Universidade Estadual de Maringá, Maringá, Paraná, Brazil. [2]Embrapa Soja, Londrina, Paraná, Brazil. [3]Universidade Estadual de Londrina, Londrina, Paraná, Brazil.

*liliane.henning@embrapa.br

Key words: Kunitz trypsin inhibitor; lectin; non-transgenic.

Brazil is among the top producers and exporters of meat and products derived from poultry and swine. Thus, the animal feed industry is highly relevant in Brazilian agriculture and livestock economic activities, in which the soy complex is the major item in the country's trade balance. Although this legume is the main protein source for animal feeding, it contains compounds that prevent the full use of its nutritional potential, so- called antinutritional factors (ANFs), leading to low feed conversion and hampering the sector profitability. The most severe ANFs found in soybeans are Kunitz trypsin inhibitors (KTI) and lectins (LEC), which seriously interfere with digestion and absorption of nutrients from feed, reduce the growth and development of animals, and decrease their performance, affecting mainly monogastrics. Elimination of these compounds by heat treatment represents a high cost for the industry and inadequate heating may leave residues of ANFs (sub-processing) or decrease grain quality due to degradation of amino acids (super-processing). Moreover, albeit natural variation is a relevant source for developing superior cultivars, conventional breeding is a laborious and time-consuming process. CRISPR-Cas is a powerful tool that enables site-directed genome modifications and can be applied for genetic silencing of the main soybean ANFs, representing a faster and cheaper strategy to generate cultivars that improves zootechnical performance. Therefore, the objective of this study was to silence KTI and LEC genes in soybean by the CRISPR-CAS technique. The genes expressed specifically in soybean seeds were identified for each protein family (KTI3 and LEC1) and guide RNAs (sgRNAs) were designed with a high probability of genome edition and low chances of editing off-targets. Vectors were constructed with essential regulatory elements for the expression cassette containing the Cas9 enzyme and the specific sgRNAs. The soybean genotypes BRS 283 and BR14-3465 were transformed by the half-seed method using *Agrobacterium tumefaciens*. BRS 283 showed higher multiple shooting and recovery rates. The transformation method was optimized with 50 mg L^{-1} of Meropenem in order to successfully contain contamination of culture media, 50 mg L^{-1} of L-asparagine to facilitate DNA transfection, and 3 mg L^{-1} of ammonium glufosinate to select for transformants. The insertion of the CRISPR-Cas9 system in the plants will be confirmed through PCR, and genome editing will be verified by gene sequencing. Then, subsequent generations will allow segregation of the transgene in order to obtain edited and non- transgenic soybean plants.

Mg-Al-LDH-BASED NANOSYSTEM FOR FUNCTIONAL INTERFERENCE OF microRNAS AS A POTENTIAL NEW THERAPEUTIC METHOD IN EPILEPSY

Mykaella Andrade de Araújo[1]; Bruna Priscila dos Santos[1]; Ygor Daniel Ramos[1]; Erivaldo Davi Júnior[1]; Pedro Augusto Tibúrcio Paulino[1]; Thayuanne Silva de Melo[1]; Ênio José Bassi[2]; Camila Braga Dornelas[3]; Daniel Leite Góes Gitaí[1]

[1]Department of Cellular and Molecular Biology, Institute of Biological Sciences and Health, Federal University of Alagoas (UFAL), Maceió-AL, Brazil;[2]Laboratory of Research in Virology and Imunology, Institute of Biological Sciences and Health Federal University of Alagoas (UFAL), Maceió-AL, Brazil;[3]Quality Control Laboratory of Drugs and Medicines, Postgraduate Program in Pharmaceutical Sciences, School of Nursing and Pharmacy, Federal University ofAlagoas (UFAL), Maceió-AL, Brazil.

danielgitai@gmail.com

Keywords: Mg-Al-LDH, microRNAs, gene expression.

Differential gene expression studies have shown that microRNAs (miRs) are critical in epileptogenesis, which makes them highly attractive targets for developing therapeutic drugs. Recently, by using microarray coupled RT-qPCR approach, we identified that miR-196b and miR-352 are up-regulated in post *Status Epilepticus* rat hippocampus. Functional studies based on the depleting of these miRs through inhibitor molecules are crucial for a better understanding of their role in epileptogenesis. However, there are still some challenges in the use of miRs inhibitors (AMOs) in vitro assay, since they suffer from problems such as biodegradability and poor cell uptake. In the current study, we addressed this shortfall by development of an inorganic nanoparticle Mg-Al-Layered Double Hydroxide (Mg-Al-LDH) loaded with AMOs against miR-196b-5p. LDHs were synthesized by a co-precipitation method, and they were characterized as hydrodynamic size and zeta potential by DLS (Nanosizer Nano ZS instrument). The LDH morphology was evaluated by electronic transmission microscopy (TEM). The chemical group's composition was assessed by Fourier transform infrared spectroscopy (FTIR) SSU-8000 (Shimadzu). X-ray diffraction (XRD) patterns were recorded by XRD-7000 (Shimadzu). Quantification of LDHs was measured by inductively coupled plasma atomic emission spectroscopy (ICP-OES) and energy dispersive X-ray (EDX). Nanosystem was formed by vigorous agitation of Mg-Al-LDH and oligonucleotides (AMO or Alexa-fluor 555 labeled dsDNAs) and characterized as described for Mg-Al-LDH alone. For the in vitro assays, the thymic endothelioma cells (tEnd.1) were chosen due its high levels of miR- 196b by RT-qPCR. After the treatment with the nanosystem, we assessed the cell viability by MTT and trypan blue exclusion assays, transfection efficiency by flow cytometry, and the miR-196b-5p depletion by RT-qPCR. LDH nanoparticles are stable in aqueous solutions, presenting zeta potential of +45mV, 0.15 of PDI, size mean of 90nm and a physical-chemical pattern of the Mg-Al-LDH group. TEM images revealed LDHs with a regular hexagonal shape. No discernible cytotoxic effect on tEnd.1cells was observed after LDH administration at concentrations up to 100 µg/ml. HDL loaded with Alexa-fluor 555 labeled dsDNA at concentration of 310 nM resulted in 93% of the tEnd.1cells as Alexa-fluor positive. AMOs carried by LDH resulted in a significant depletion of miR-196b-5p transcripts at 48 h after the transfection as measured by RT- qPCR ($p=0.0079$). We developed stable Mg-Al-LDH nanoparticles and nanosystem suitable for functional interference of microRNAs studies. As a perspective, we will investigate the role of miR-196b-5p in temporal lobe epilepsy models using treatment with the Mg-Al-LDH nanosystem.

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EVALUATION OF CYTOTOXIC ACTIVITY OF *Dalbergia ecastaphyllum* HYDROALCOHOLIC EXTRACT

Bianca Silva Alves[1]; Lucas Henrique Domingos da Silva[1]; Iara Silva Squarisi[1]; Heloiza Diniz Nicolella[1]; Jairo Kenupp Bastos[2]; Denise Crispim Tavares[1]

[1]University of Franca, Franca, São Paulo; [2]Faculty of Pharmaceutical Sciences of Ribeirão Preto, University of São Paulo, Ribeirão Preto, São Paulo

bsalvesb@gmail.com

Keywords: *Dalbergia ecastaphyllum*; cytotoxicity; XTT colorimetric assay.

Over time, humans have relied on nature for their basic needs, such as for the production of food, shelter, clothing, means of transport, fertilizers, aromas and fragrances and medicines. Natural products and their derivatives have historically been invaluable as a source of therapeutic agents. *Dalbergia* is a botanical genus that has about 500 species, of which 39 are found in Brazil and are distributed in practically all the vegetation, such as the Cerrado, Caatinga, Mata Atlântica and Campos Rupestres. This genus presents several chemical constituents, being characteristic of the presence of isoflavonoids. Among the species of this genus, it can highlight *Dalbergia ecastaphyllum*, popularly known as "rabo-de-bugio" or "marmelo". This plant species has as phytogeographic domains the North and Northeast regions of Brazil. In folk medicine, its roots and barks are used in the fight against uterine inflammation and anemia. *D. ecastaphyllum* is considered the main botanical source of red propolis because it presents similar chemical composition. The objective of this study was to evaluate the cytotoxicity of the *D. ecastaphyllum* hydroalcoholic extract (DEHE). For this purpose, the cultures of *Rattus novergicus* intestinal epithelial cells (IEC-6) were treated with extract concentrations ranging from 19.53 to 2.500 µg/mL. Cytotoxicity was assessed by XTT colorimetric assay. The results showed that there was a significant reduction of the cellular viability at extract concentrations greater or equal to 312.5 µg/mL. The concentration that inhibited 50% of cell viability was 373.2 µg/mL. Further studies are needed to understand the action of the extract on cell viability.

Financial support: São Paulo Research Foundation (FAPESP), Coordination of Improvement of Higher Level Personnel (CAPES) and National Council for Scientific and Technological Development (CNPq).

THE GENOME SEQUENCE OF SPORISORIUM PANICI- LEUCOPHAEI, THE CAUSAL AGENT OF SOURGRASS SMUT DISEASE

Gustavo Schiavone Crestana[1]; Lucas Mitsuo Taniguti[2]; Claudia Barros Monteiro-Vitorello[1]

[1]Universidadede São Paulo,Escola Superior de Agricultura"Luiz de Queiroz"(ESALQ), Departamento de Genética, Piracicaba, Brasil. [2]Mendelics Análise Genômica S.A, São Paulo, Brasil.

gustavo.crestana@usp.br

Keywords: comparative genomics, plant-microbe interaction, smut disease.

Fungi from the Ustilaginaceae family are the main causal agents of smut diseases in Poaceae plant species, which compromises crop quality and productivity. Smut species present a biotrophic lifestyle, dependent on an intimate interaction with the host to complete its life cycle and produce spores (2n). The fungus *Sporisorium panici-* leucophaei infects and colonizes seedlings of *Digitaria insularis*, a perennial weed resistant to glyphosate, responsible for the commonly known sourgrass smut disease. Symptomatically, the disease resembles another one caused by *S. scitamineum* in sugarcane, producing a whip-like structure from the apex of the plants where sporogenesis take place. Despite similarities (life cycle and symptom induction) with sugarcane smut little is known about this pathosystem and whip induction. The objective of our work was to sequence the entire genome of *S. panici-leucophaei* and compare to that of *S. scitamineum* searching for species-specific genes and those shared between them that may help to explain similarities in the symptom development. The genome of the haploid isolated SPL10A was sequenced using paired-end strategy in the Illumina HiSeq 2000 platform. A total of 6.454.014 million reads were assembled using SPAdes software into an 18,0 Mbp sequence distributed in 567 contigs. We used Augustus to predict 6,423 ORFs, of which 40% had the closest orthologs in *S. scitamineum* genome among all smuts sequenced to date. So far, we have analyzed the secreted and candidate effector proteins using SignalP/TMHMM and Effector P, respectively, defining the set of shared and species-specific proteins. The amount of secreted proteins and effector candidates encoded by the *S. panici-leucophaei* genome (7% and 18%) were similar to those encoded by *S. scitaminuem* (6% and 23%). However, out of 77 potential effectors of *S. panici-leucophaei*, only 21 had orthologs in *S. scitamineum*. We will further characterize these set of proteins in order to provide candidates for future investigations and *S. panici-leucophaei* genome will further be sequenced using the Oxford MinION platform to obtain the complete sequence of all chromosomes.

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Identification of antimicrobial resistance genes in metagenomic databases

Felipe Marcelo P. dos Santos[1], Rafael Silva-Rocha[1], Maria Eugenia Guazzaroni[2]

[1]Universidade de São Paulo - Faculdade de Medicina de Ribeirão Preto. [1]Departamento de Biologia Celular e Molecular e Bioagentes Patogênicos, Faculdade de Medicina de Ribeirão Preto - Universidade de São Paulo.[2]Departamento de Biologia,Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto - Universidade de São Paulo.

felipemarcelo@usp.br

Key-words: resistance genes, metagenomics, bioinformatics.

Antimicrobial resistance mechanisms are a menace to the survival of humanity due to the prolific resistance gene dissemination between critically health related pathogen microorganisms. Perhaps the core of the problem is the large scale production linked to promoting a high selective pressure on microorganisms, and this pressure has been promoting the amplification, diversification and dissemination of genes that promote resistance to diverse antibiotics. With the aid of bioinformatic tools and metagenomic databases available on the web, candidate sequences were identified as new resistance genes, in addition to the identification of resistance genes cataloged. Our aim is to understand the distribution of these genes across the Brazilian territory, the relation of the environment to the quantity of genes, and to classify them as generators of resistance genes. Genome sequencing of the entire community of a sample, the metagenomes, is stored in online repositories such as MG-RAST, which was used to obtain the sequences for analysis. For the characterization of genes, we used the DeepArg, because it demonstrates a high predictive potential, in addition to a low false negative rate, compared to other tools. From a sample of Av. Chagas Filho from the university city of the Federal University of Rio de Janeiro, we analyzed a metagenome in which 15 classes of antibiotics were identified. It can be inferred from this sample also that this environment is not a potential generator of resistance genes because most of the genes identified in it are genes with high identity with the databases used by the software. However, the sequences that were related to the antibiotics of the tetracycline class had low identity with the databases, that is, the environment has the potential to generate genes related to a specific class. We obtained about 15 samples of assembled and unmounted metagenomes, and we will increase this quantity in order to have more expressive conclusions. It is also intended to seek a relationship of the environment with the antibiotic class of the resistance genes present in it.

Funding Agency: FAPESP.

Presence of C-262T polymorphism in catalase gene in patients with Down Syndrome at APAE of Araras-SP

Marcos Vinicius da Cruz[1], Leticia Roberta Leme Sapatini[1]; Flavia Lima Costa Faldoni[1], Fernando Russo Costa do Bomfim[1*]

[1]Molecular Biology Laboratory,Centro Universitário da Fundação Herminio Ometto-FHO UNIARARAS.

*fernandobomfim@fho.edu.br

Palavras-chave: Down Syndrome; Catalase; Polymorphism

Background Down's Syndrome (DS) is the most prevalent chromosomal disease in the world, extra copy of chromosome 21, which turns cells less stable and more susceptible to oxidative stress. Oxidative balance is fundamental for homeostasis and is mediated by enzymatic complexes as catalase (CAT) enzyme that is encoded by catalase gene found on chromosome 11 (11p13), Its main activity is to neutralize free radicals and reduce effects on aging, although the polymorphism C-262T (SNP-262 C>T) is recurrent and reaches the promoter region of the CAT gene that can alter the enzymatic function. Objective The aim of this study was to evaluate the prevalence of the C-262T polymorphism of catalase gene in patients with DS treated at APAE of Araras, SP, Brazil. Methods After approval by the Ethics Committee, number 2.590.998, and signed of the terms of free and informed consent by those responsible, thirteen patients were volunteers for the study, nine males and four females, all were diagnosed clinically and by karyotype with DS. Blood samples were collected by puncture of the cephalic vein and collected in tubes containing EDTA. DNA extraction was performed following cell lysis and proteinase K (10mg/mL) incubation at 65ºC. The samples were submitted to polymerase chain reaction (PCR) for the CAT gene with the following reagents, in final concentration, buffer containing MgCl2 1X, 0.2mM dNTP, Forward Primer 0.2pmol (5'- CTGATAACCGGGAGCCCCGCCCTGGGTTCGGATAT-3', Reverse Primer 0.2pmol (5'- CTAGGCAGGCCAAGATTGGGAGCCCAATGG-3'), 0.05U Taq DNA polymerase and 5ng of DNA. PCR was performed under the following conditions, initial denaturation at 95°C for 5 minutes, thirty-five denaturation cycles at 95°C for one minute, annealing at 70°C for one minute and extension at 72°C for one minute and final extension at 72°C for ten minutes. PCR samples were submitted to enzymatic digestion with the following reagents in final concentration: buffer 1X, EcoRV enzyme 10U, DNA-PCR 0.3µg and incubated at 37°C for 16 hours. The presence of the C-262T polymorphism was evaluated by 3% agarose gel electrophoresis for CC (190bp, wild homozygote), normal gene function, TT (157bp and 33bb, homozygous variant), and CT (190bp, 157bp and 33bp, heterozygote), both for polymorphism presence. Results Eleven samples (84.61%) presented variant homozygous (TT), whereas two samples (15.39%) presented heterozygosis (CT) and none sample showed wild homozygous (CC). Of the eleven TT samples, eight were male (72.72%) and three female (27.27%), while one CT sample was male (50%) and one female (50%). Conclusion The presence of C-262T polymorphism in CAT gene occurs in one or both alleles in patients with DS that can lead to altered gene function and may be associated with decrease of catalase activity and increase of oxidative stress. It is necessary to follow these patients for the prevention of aging and diseases related to oxidative stress.

CHEMOPREVENTIVE EFFECT OF MANOOL IN RODENTS

Tábata Rodrigues Esperandim; Heloiza Diniz Nicolella; Gabriela Fernandes; Saulo Duarte Ozelin; Arthur Barcelos Ribeiro; Francisco Rinaldi Neto; Rodrigo Cassio Sola Veneziani; Denise Crispim Tavares

University of Franca, Franca, São Paulo, Brazil.

tabataesperandim@hotmail.com

Keywords: Manool; *Salvia officinalis*; chemoprevention.

Due to the various difficulties in the treatment of cancer, natural products are important sources in the development of new drugs. Phytochemical studies of *Salvia officinallis* allowed the identification of terpenoids and phenolic compounds, with diterpenes being their most characteristic micrometabolites. Among the diterpenes, it is noteworthy the manool (Mo) that demonstrated cariogenic potential against several bacteria associated with periodontitis and a selective cytotoxic effect between normal cell line and tumor cell lines. In addition, Mo presented protective effect against genotoxicity induced by methyl methanesulfonate in human hepatocarcinoma cell line. In this sense, the present study aimed to evaluate the effect of Mo on pre-neoplastic lesions in rodent colon. For this, the aberrant crypt foci (ACF) assay in Wistar rats was used. The animals were treated five times a week for two weeks with the different doses of Mo (0.312, 1.25 and 5.0 mg/kg b.w.) via gavage. The 1,2-dimethylhydrazine carcinogen (DMH, 160 mg/kg b.w., total dose) was administered subcutaneously twice weekly in the first two experimental weeks. Body mass and water consumption were assessed throughout the experimental period. In addition, the systemic toxicity of the treatments was evaluated by analyzing biochemical markers such as alanine aminotransferase, aspartate aminotransferase, creatinine and urea. The results revealed that the animals treated with Mo plus DMH showed significantly lower ACF frequencies than those treated only with the carcinogen. Furthermore, no significant differences were observed in the biochemical parameters analyzed in the animals of the different groups, revealing absence of hepatotoxicity and nephrotoxicity of the treatments. Therefore. Mo showed showed a promising protective effect on the colon carcinogenesis. The data obtained contribute to a better understanding of the biological activities of Mo, aimed at its use in human therapy.

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THE RECENT EVOLUTION OF GUT IgA PRODUCTION NETWORK IN COMPARISON TO THE OTHER ADAPTIVE IMMUNE SYSTEM PROCESSES

Maikel Varal[1]; Alana Castro Panzenhagen[1]; Alexandre Kleber Silveira[1]; Álvaro de Oliveira Franco[1]; José Cláudio Fonseca Moreira[1]

[1]Centro de Estudos em Estresse Oxidativo, Programa de Pós-graduação em Ciências Biológicas: Bioquímica, Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil.

*maikelvaral@yahoo.com.br.

Key-words: systems biology; evolutionary genetics; adaptive immunity.

Thousands of microorganisms that inhabit the human digestive tract are crucial to several physiological processes of the host. The adaptive immune response, not only allows recognition with specificity and diversity, but also suppresses and/or stimulates local immune responses. Immunoglobulin A (IgA) is the central antibody secreted by mucosal tissues and its synthesis is regulated by a variety of mechanisms. Although we are aware that IgA is present in reptiles, birds, and mammals, it is important to elucidate this process scenario and evolutionary roots, as well as its different types of synthesis mechanisms. Therefore, the systems biology approach fits perfectly in this context, providing means to a holistic analysis of the processes. The aim of this study was to analyze how the intestinal immune network for IgA production (IgA network) evolved and what is its relationship with other processes of the adaptive immune system. The genes that compose the IgA network were collected through the *Kyoto encyclopedia of Genes and Genomes* (KEGG) database. Among the other compared processes were: T cell receptor signaling pathway, B cell receptor signaling pathway, Th1 and Th2 cell differentiation and Th17 cell differentiation (KEGG – genome.jp/kegg), and all the genes listed as part of the adaptive immune system (Reactome – reactome.org). The orthologous groups of each respective gene was searched in the StringDB database (string-db.org). The emergence of each group of genes was inferred by the orthologous distribution and their most parsimonious scenario using the package *geneplast* in the R environment. The data were analyzed through the Kolmogorov-Smirnov test through GraphPad Prism 7 software. The results revealed that the IgA network is the most recent among the selected processes. This holds statistically true when comparing IgA with the processes of B cell receptor signaling pathway ($p<0.0002$), T cell receptor signaling pathway ($p<0.0002$), Th1 and Th2 cell differentiation ($p<0.0005$), and Th17 cell differentiation ($p<0.001$), as well as the adaptive immune system as a whole ($p<0.0005$). None of the latter presented significant differences between each other. While the IgA network was composed of less than 20% of its orthologs at the bottom of Vertebrata, B cell receptor signaling pathway already presented more than 80%. This indicates that the IgA pathway had a later evolution in the adaptive immune system group. IgA recent development is evidenced by an acute increase around the roots of Vertebrata and Eutheria, a point in time where all its orthologs were finally present. The sudden evolution of this process suggests a strong selective pressure, likely related to the parasite-host dynamics and the repression of immune responses against commensal microorganisms. More studies are needed in order to further comprehend IgA synthesis and how the manifestation of this process interaction occurs in a molecular level.

IMIDAZOLINONE TOLERANCE IN RICE PLANTS CAN BE ENHANCED BY GENETIC ENGENEERING OF NOVEL/SPECIFIC AMINO ACIDS IN THE ACETOLACTATE SYNTHASE ENZYME

Giseli Buffon[1], Mara Cristina Barbosa Lopes[3], Raul Antonio Sperotto[1,2], Luis Fernando Saraiva Macedo Timmers[1,2]

[1]Graduate Program in Biotechnology, [2]Biological Sciences and Health Center, University of Taquari Valley - Univates, Lajeado, RS, Brazil; [3]Rice Research Institute, Cachoeirinha, RS, Brazil.

rasperotto@univates.br

Key words: alanine-scanning, herbicide, point mutation

Advancements in genetically modified herbicide resistance technology opened a new way to manage weed populations in crop fields. Since then, many important genetically modified crops that are tolerant to various herbicides have been developed and commercialized. Herbicides primarily act by disrupting key enzymes involved in essential metabolic or physiological processes associated with growth and development of plants. Most of the herbicide tolerant plants have been developed by introducing point mutations (non-GM approach) in the target site of herbicide action, due to the advantage of easier registration/release for commercial cultivation as well as wider public acceptance. Of the various herbicides, imidazolinones are probably the most widely targeted ones for developing herbicide tolerant crops through non-GM approach. In rice, several mutant lines carrying specific amino acids changes in ALS are able to tolerate different imidazolinones, including a Glycine to Glutamic acid substitution in position 628, a Serine to Asparagine in position 627, and a double substitution Tryptophan to Leucine in position 548 / Serine to Isoleucine in position 627. The combination of these mutant lines with the specific herbicides they tolerate provides a reliable method to eliminate weeds in the fields. However, the continuous overuse of a single herbicide multiple times in a growing season increases the potential risk of evolution of resistant weeds, which has become a major concern in agriculture worldwide. For this reason, the discovery of novel mutations in ALS enzyme that can generate rice plants more tolerant to imidazolinones than the available mutant rice lines is still a hot topic in plant-herbicide interaction field. With that in mind, we performed molecular docking analysis of imidazolinone herbicides imazapic, imazapyr, imazaquin, and imazethapyr on the binding cavity of ALS enzyme (Os02g30630) from rice, being able to identify the specific amino acids residues that interact with the four herbicides. After introducing point mutations in these specific amino acid residues (one at a time) using Alanine scanning mutagenesis methodology and recalculating the herbicide-ALS interaction affinity, we were able to detect novel amino acid residues (especially Lysine in position 230, and Arginine in position 351) on the structure of ALS that can inhibit more substantially the binding of imidazolinones to ALS than the already known amino acid mutations. This rational approach allows the researcher/farmer to choose the number of point mutations to be inserted in a rice cultivar, which will be dependent on the type of imidazolinone used. In order to obtain a rice cultivar able to tolerate the four imidazolinone tested concomitantly, we suggest mutation of the six amino acid residues that most affect their interaction with OsALS (Val170, Phe180, Lys230, Arg351, Trp548, and Ser627).

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OPTIMIZATION OF AN AUTOINDUCTION SYSTEM FOR GENE EXPRESSION DEVELOPED FOR *Bacillus subtilis*

Graciely Gomes Corrêa[1]; Milca Rachel da Costa Ribeiro Lins[1]; Bruna Fernandes Silva[1]; Gabriela Barbosa de Paiva[1]; Nathan Vinícius Ribeiro; Danielle Biscaro Pedrolli[1*]

[1]Universidade Estadual Paulista – UNESP, Faculdade de Ciências Farmacêuticas, Departamento de Bioprocessos e Biotecnologia (Rodovia Araraquara-Jau km1, 14800- 903 Araraquara, Brasil).

*danielle.pedrolli@unesp.br

Key-words: *luxRI* system; *Bacillus subtilis*; regulation of gene expression.

Demands for industrial strains that efficiently produce of biocompounds is increasing. Development of autoinduction systems based on bacterial *quorum-sensing* aims at preserving the natural metabolic balance of the bacteria during the *lag* and early exponential growth phases, while triggering high production levels at the mid-exponential phase. In addition, it eliminates the costs for industrial production related to the addition of inducing substances such as isopropyl β-D-1-thiogalactopyranoside (IPTG) and subsequent removal of them from in the downstream process. The need for monitoring culture density prior to induction is eliminated too, as bacteria will take on this task. The objective of the present work was to construct and optimize synthetic autoinduction devices showing different fold change and promoter strengths to generate a toolbox with options for approaching genetic and metabolic engineering. The *luxRI* genes, carrying or not histidine tags, were amplified by PCR from *Aliivibrio fischeri* genomic DNA template. Plux promoter optimizations were rationally designed and purchased as complementary oligonucleotides for extension PCR. Plasmid pBs3Clux, containing a bioluminescence reporter operon and BioBricks cloning standards were used. Growth and bioluminescence were assayed in a microplate reader. Data were processed in the program Excel 2010 and Spyder (Python 3.7). The srfA promoter was used as a positive control. *A. fischeri luxRI* genes integrated into *Bacillus subtilis* genome were proven functional. Although efficient in its task of inducing gene expression during the exponential growth phase, the autoinduction system initially constructed showed low levels of gene expression. Optimization of the autoinduction system initiated by the separation of the induction device (*luxRI* transcription unit) from the response device (responsive promoter and reporter operon). Therefore, the Plux promoter located between the *luxR* and *luxI* genes was duplicated, and the copy placed upstream of the reporter. Synthetic Plux promoters were constructed based on promoters of different forces in *B. subtilis*. As a result, six promoter variations and ten new gene circuits were designed and built. The optimization process was successful and variations of the system were obtained with different points of induction and variations regarding the activation fold change and promoter strengths. We have created an autoinduction toolbox with great applicability to metabolic engineering. The toolbox user will be able to apply the same autoinduction process to different genes in the cell, choosing the most appropriate promoter strength and fold of activation depending on the desired gene product dose.

Funding Agencies: CAPES and FAPESP

INVESTIGATION OF THE ASSOCIATION BETWEEN -31G/C POLYMORPHISM OF *BIRC5* GENE AND THE RISK OF BREAST CANCER IN AN AMAZONIAN POPULATION

Caio Dantas Alves[1,2], Ana Carolina Pinheiro[1,2]; Anna Carolina Lima Rodrigues[1,], Brenda Suelen Jardim Oliveira[1], Deyse Dayane Chaves Borges[1,2], Mariana Diniz Araújo[1], Danilo do Rosário Pinheiro[1,2], Rommel Rodríguez Burbano[3], Bárbara do Nascimento Borges[1]

[1]Laboratory of Molecular Biology, Institute of Biological Sciences, Federal University of Pará, Belém, Pará. [2]University of the Amazon, Belém, Pará, [3]Laboratory of Molecular Biology of Hospital Ophir Loyola, Belém, Pará. *Correspondence: Laboratory of Molecular Biology, Institute of Biological Sciences, Federal University of Pará, Belém, Pará. R. Augusto Corrêa, 01. Guamá, Belém, Pará.

*caiodantasalves@icloud.com

Key-words: Breast cancer, SNPs, susceptibility.

Breast cancer (BC) is the most common type of tumor among women, and the second leading cause of death worldwide. Its etiology is multifactorial, and the main factors involved are environmental, such as diet and age, and genetics. *BIRC5* is responsible for the synthesis of the SURVIVIN protein, involved in several biological pathways such as cell proliferation and apoptosis, playing a critical role in cancer development and thus considered as a target for chemotherapy. Therefore, the present study aimed to evaluate the association between -31G/C polymorphism of *BIRC5* gene, in patients with breast cancer of ductal carcinoma type in the population of Belém, Pará, a city located in the brazilian amazon region. For this, tumoral samples were collected from women subjected to mastectomy at Ophir Loyola Hospital. Control blood samples were obtained at Laboratório de Análises Clínicas at UFPA from women with no history of cancer. DNA extraction was perfomed using commercial kit, followed by Polymerase Chain Reaction (PCR) of the target fragment, and automatic sequencing. Statistical analyzes were carried out in BioEstat and GraphPad Prism softwares. The allele frequencies for case and control samples were the same, and no allele was associated with an increased risk of develop breast cancer in the population. When considering only the tumoral samples, none of the analyzed alleles were associated with any of the clinical features of the patients (molecular subtype nor Ki67 value). We can conclude that the -31 G/C is not associated with breast cancer risk in our population.

Funding Agency: CNPq, CAPES, UFPA, HOL

IMPROVING EDNA METABARCODING IN THE NEOTROPICS: A NEW PRIMER SET AND IMPROVED REFERENCE DATABASE TO UNCOVER THE HIGH FISH BIODIVERSITY IN BRAZIL

Izabela Santos Mendes[1, 2], David Travassos Milan[1], Naiara Guimarães Sales[3], Daniel Fonseca Teixeira[1], Júnio Damasceno de Souza[1,4], Daniel Cardoso Carvalho[1,2]

[1]Conservation Genetics Lab, Post-graduation Program of Biology of Vertebrates, Pontifical Catholic University of Minas Gerais, Belo Horizonte, Brazil. [2]Post-graduation Program in Genetics, Federal University of Minas Gerais, Belo Horizonte, Brazil. [3]Ecosystems & Environment Research Centre, School of Environment & Life Sciences, University of Salford, UK. [4]State University of Minas Gerais.

*izabelasantosmendes@hotmail.com

Keywords: environmental DNA, noninvasive tool, biodiversity assessment

The mega diversity found in the Neotropics represents a challenge for species detection and monitoring using non-invasive methods such as environmental DNA (i.e. DNA extracted from environmental samples). Challenges range from the lack of a comprehensive reference DNA databases for taxonomic assignment to standardized methods for eDNA detection and molecular markers able to detect the entire biodiversity. Here, we built a reference database using the 12S mitochondrial region and developed and tested primers (<200pb) for eDNA analyses using 67 fish species (70 Operational Molecular Taxonomic Units - MOTUs), representing 54 genera, 25 families and six orders from the São Francisco River Basin (South-eastern/Brazil). We obtained 132 DNA sequences from the 12S region (565pb) and used it as a reference for developing new primers sets targeting a 193 bp fragment. To test amplification efficiency of the primers, we applied *in silico* and *in vitro* approaches. *In vitro* tests using tissue samples demonstrated an efficient amplification for all species analyzed and also, for eDNA retrieved from water samples from an aquarium containing *Geophagus brasiliensis*. To evaluate the efficacy of the 12S mini-barcode region in delimiting species, we conducted four delimitation species analyses based on Bayesian approach (GMYC/bPTP) and genetic distance (ABGD/Tamura-Nei). The Bayesian analyses, GMYC and bPTP, identified 70 and 76 MOTUs, respectively. Distance-based analyses, ABGD and Tamura- Nei, recovered 62 and 72 MOTUs, respectively. Thus, GMYC was accurate in pointing out the 70 MOTUs previously identified within the 67 morpho-species. *In silico* PCR did not detect non-targeted organisms' amplification such as arthropod, bacteria, mollusks or mammalian (including *Homo sapiens*). *In silico* analyses demonstrated that the fragment analyzed contain enough resolution to differentiate all 67 species and may be useful for an ecoregion scale eDNA metabarcoding biodiversity evaluation, helping with the complex task of monitoring and conserving the Neotropical ichthyofauna.

QUANTITATIVE ASPECTS OF NFκB ACTIVITY IN *Drosophila melanogaster* INNATE IMMUNITY

Maira Arruda Cardoso[1,2]; Paulo Mascarello Bisch[1]; Helena Araujo[2]*

[1]Laboratório de Física Biológica, IBCCF, UFRJ, RJ, Brazil. [2]Laboratório de Biologia Molecular e do Desenvolvimento, ICB, UFRJ, RJ, Brazil.

*haraujo@histo.ufrj.br

Palavras-chave: *Drosophila melanogaster*; NFκB; Innate Immunity

The elements that control innate immunity are evolutionarily conserved, thus studies in animals with low complexity may serve as basis to understand human immunity. Assays using the model *Drosophila melanogaster* have revealed fundamental aspects of the humoral immune response, regarding the control of NFκB/c-Rel activity. Drosophila presents two c-Rel proteins, both inhibited by Cactus (Cact), the sole IκB homologue in Drosophila. The Dorsal transcription factor (Dl), a member of the NFκB/c- Rel family, regulates several genes responsible for dorsoventral axis patterning of the Drosophila embryo, as well as genes encoding antimicrobial (AMP) and antifungal peptides during the innate immune response. Two mechanisms regulate the levels of Cact and thus control Dl activity: activation of the Toll pathway promotes the phosphorylation and ubiquitination of Cact molecules complexed to Dl (Cact/Dl) and consequent degradation of Cact by the proteasome; the second mechanism that regulates Cact levels is mediated by Calpain A (CalpA), a Ca^{+2}-dependent cysteine protease. Our group has shown that CalpA cleaves free Cact molecules present as homodimers in the cytosol, releasing a fragment that is unresponsive to the Toll Pathway (CactE10) and a Cact monomer that may form new signal-responsive complexes with Dl molecules. These effects were observed during embryogenesis, and recently we have identified a similar role in the humoral response in larvae and adult flies. During embryogenesis, both regulatory mechanisms may be required to promote the correct balance of Cact molecules in the cytoplasm in order to refine the Dl nuclear gradient. To explore a similar role in immunity, we have quantitatively analyzed NFκB activity in fat body tissue from Drosophila larvae after immune challenge, using different genetic backgrounds. As a result, we have verified that CalpA impacts the survival and the production of AMPs both in the basal context and during the activation of the Toll pathway after infection by *Beauveria bassiana*. CalpA activity is also important for the redistribution of pre-signaling complexes in different contexts. In addition, our data demonstrate that not only CalpA, but also members of the Bone Morphogenetic Protein (BMP) pathway, aid in the immune response during the activation of the Toll pathway, favoring the survival of adult flies.

BIOPROSPECTING FOR POTENTIAL BIFUNCTIONAL α-L-ARABINOFURANOSIDASE/XYLOBIOHYDROLASE OF *Trichoderma harzianum*

Maria Lorenza Leal Motta[1], *; Clelton Aparecido dos Santos[1]; Jaire Alves Ferreira Filho[1]; Ricardo Rodrigues de Melo[2]; Letícia Maria Zanphorlin[2], Anete Pereira de Souza[1]

[1]Centro de Biologia Molecular e Engenharia Genética, Universidade Estadual de Campinas, Campinas, São Paulo, Brasil; [2]Laboratório Nacional de Biorrenováveis, Centro Nacional de Pesquisa em Energia e Materiais, Campinas, São Paulo, Brasil.

* mah.lorenza.leal@gmail.com

Keywords: Bioethanol; α-L-arabinofuranosidases; *T. harzianum.*

The bio-conversion of plant biomass into value-added bio-chemical products, including biofuels is currently being studied as a renewable alternative to fossil fuels. However, the efficient conversion of lignocellulosic substrates into fermentable sugars requires steps in which hydrolytic enzymes play a major role. The filamentous fungi, especially those belonging to the genus *Trichoderma* stand out as producers of carbohydrate- active enzymes, as they are being explored biotechnologically with regard to the production of enzymatic cocktails for the degradation of biomass. Thus, enzymes having different catalytic activities may be a solution for obtaining improved enzymatic cocktails. Therefore, bifunctional enzymes such as α-L- arabinofuranosidase/xylobiohydrolases (ABFs) are an interesting target for such studies. In the present study, we aim at bioprospecting a potential ABF of *Trichoderma harzianum* IOC-3844 and evaluating their potential to improve the plant biomass degradation and its consequent use for the production of bioethanol. Initially, *in silico* analyses were performed, using bioinformatics tools to select a potential bifunctional ABF through phylogenetic analysis, RNA-Seq and a search in co-regulatory networks. Subsequently, the target gene was amplified from the cDNA of *T. harzianum* and cloned into a protein expression vector pET-28a(+). The recombinant ABF was expressed into the inclusion bodies of *Escherichia coli* that motivated us to optimize a protein refolding method for the solubilization and purification of the target protein. The recombinant ABF was successfully solubilized and purified in high yield using nickel affinity and size-exclusion chromatography. The presence of α-helix and β-sheets secondary folding of the recombinant purified protein, which was obtained by refolding, was evaluated by circular dichroism, whereas the tertiary folding was confirmed by analytical size-exclusion chromatography. Initial biochemical characterization using the substrate 4-nitrophenyl-α-L-arabinofuranoside confirmed that the purified protein is active and shows optimum catalysis activity at 45°C, pH 6.5. Preliminary assays indicate that the recombinant ABF appears to have catalytic activity for non-canonical substrates, although further analyses are still required. Our work will shed new light on the mechanisms behind the bifunctionality of ABFs and their use to improve enzymatic cocktails designed for the conversion of plant biomass to bioethanol.

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MITOCHONDRIAL UNCOUPLING PROTEINS: PHENOTYPICAL AND PHYSIOLOGICAL CHARACTERIZATION OF *ARABIDOPSIS* DOUBLE-INSERTION MUTANTS REVEAL AN IMPORTANT DISTURBANCE DURING THE VEGETATIVE PHASE

Rômulo Pedro Macêdo Lima[1]*; Alessandra Vasconcellos Nunes Laitz[2]; Ivan de Godoy Maia[1]

1Departamento de Genética, Instituto de Biociências de Botucatu, UNESP, São Paulo, Brasil. 2Instituto Federal de Educação, Ciência e Tecnologia de Rondônia, Colorado do Oeste, Rondônia, Brasil.

*romulo.lima@unesp.br

Keywords: mitochondria; uncoupling protein; double mutant.

Mitochondrial uncoupling proteins (UCPs) are specialized proteins capable of dissipating the proton electrochemical gradient generated in respiration, thus reducing the efficiency of oxidative phosphorylation. In *Arabidopsis thaliana*, three genes encoding UCPs (*AtUCP1-3*) have been described. Previous studies using single T-DNA insertion mutants revealed a decrease in photosynthetic efficiency as well as in water content (T_{H^2O}), seedling root length (SRL), seed germination rate (G) and average germination speed index (aGSI), mainly in the *atucp2* mutant. Here, aiming to exacerbate the aforementioned phenotypes and evaluate possible functional redundancy between the *AtUCP* genes, double-insertion mutants for different gene combinations (*atucp1/atucp2*, *atucp1/atucp3* and *atucp2/atucp3*) were obtained. Phenotypic analyzes were performed to investigate the growth and development of these double-mutants under normal and stressed conditions [salt (NaCl) and osmotic (Mannitol)]. As a result, the *atucp2/atucp3* seeds showed decreased G and GSI values under stress. Moreover, based on SRL evaluation, an inhibition on root elongation was observed in the *atucp1/atucp2* and *atucp1/atucp3* seedlings under stress. Overall, these results indicate attenuated tolerance to the imposed stresses during early development. The T_{H^2O} analysis revealed drastic changes in internal water levels both in roots and aerial parts, especially in *atucp1/atucp3*, which also showed a clear decrease in leaf growth. In parallel, gas exchange analyzes performed under normal conditions using a portable IRGA photosynthesis system (LICOR Environmental) revealed drastic changes in photosynthetic efficiency compared with the wild-type Col- 0, which was particularly evident in the *atucp2/atucp3* double-mutant. Overall, these data point to an important role of the three isoforms in vegetative growth, abiotic stress tolerance and in photosynthetic efficiency.

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STUDY OF THE DYNAMICS OF EMBRYONIC TRANSCRIPTION FACTOR GRADIENTS IN THE BEETLE *TRIBOLIUM CASTANEUM*

Joao Vieira[1,2];José Nepomuceno[1]; Helena Araujo[2];Lupis Ribeiro[1]; Mateus Berni[2]; Rodrigo Nunes-da-Fonseca[1]

[1]Laboratório Integrado de Ciências Morfofuncionais, Universidade Federal do Rio de Janeiro, Instituto de Biodiversidade e Sustentabilidade, Macaé, Rio de Janeiro, Brazil. Laboratório de Biologia Molecular do Desenvolvimento, Universidade Federal do Rio de Janeiro, Instituto de Ciências Biomédicas, Rio de Janeiro, Rio de Janeiro, Brazil.

jpaulovieira@hotmail.com

Key-words: Evo-devo; HDR; Tribolium

The evolutionary developmental biology (Evo-Devo) provides a comparative study of the evolution of regulatory networks and signaling pathways that act in the control of gene expression throughout the development of living beings. The present project seeks to analyze the *in vivo* spatial distribution of Dorsal, Zelda and MAD (Mothers Against Decapentaplegic) transcription factors (TFs) along the embryonic development of the beetle *Tribolium castaneum* characterizing their spatio-temporal interactions during the developmental processes. Plasmid constructs of TF and fluorescent fusion proteins will be developed using the Homologous Directed Repair (HDR) coupled with the Cas9/CRISPR system already established in the *T. castaneum*. We also intend to analyze the functions of these molecules in situations where their concentrations are limited, as for example in embryos whose expression has been reduced experimentally by RNA interference (RNAi). These results will contribute to a better understanding of the importance of these molecules and their interactions for the embryonic development of *T. castaneum.* Evolutionarily, while the dorsal function in dorsoventral axis formation appears to be restricted to insects, Decapentaplegic has vertebrate orthologs with similar functions in embryogenesis, such as the Bone Morphogenetic Protein (BMP), which acts on the formation of this axis in vertebrates. In addition to the control of bone formation in vertebrates, the family of BMP-related proteins plays a key role in establishing the central nervous system in vertebrate and invertebrate embryos. It is possible that the studied Dorsal, Zelda and Dpp genes present different functions in beetles than those studied in vinegar flies. Some evidence suggests that these divergences are: (i) the Dorsal nuclear gradient in *T. castaneum* rapidly forms and disappears when compared to *D. melonogaster*, where it is more stable; (ii) the receptor of the Toll pathway in *T. castaneum* appears to be zygotically activated by Dorsal, whereas in *D. melanogaster* the mRNA deposited by the mother is essential for early embryonic development; (iii) in *T. castaneum* Dorsal does not repress the expression of Dpp or Zerknüllt, two genes responsible for the formation of the dorsal region in *D. melanogaster* and that in the beetle have strong control of the anteroposterior system; (iv) there is no nuclear dorsal in the posterior region of the embryo, where new segments are added, suggesting different mechanisms of patterning of this region; (v) a self-regulatory feedback mechanism between Dorsal and its Cactus inhibitor, an I-κB that holds Dorsal in the cytoplasm, outside the nucleus, has been been described. In *T. castaneum* Dorsal activates zygotically the expression of its inhibitor *cactus*, which together with another TF called *twist* terminates the Dorsal gradient. However, definitive evidence of how this activation-inhibition process occurs in the embryo of *T. castaneum* has not yet been obtained and is one of the goals.

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PROTEIN ADP-RIBOSYLATION, GENOMIC STABILITY AND HUMAN DISEASE

Nicolas C. Hoch[1,2], Hana Hanzlikova[1], Stuart L. Rulten[1], Emilia Komulainen[1], Limei Ju[1], Grace Yoon[3] & Keith W. Caldecott[1]

[1]Genome Damage and Stability Centre, University of Sussex, Brighton, UK [2]Department of Biochemistry, University of São Paulo, São Paulo, Brazil
[3]The Hospital for Sick Children, University of Toronto, Toronto, Canada.

*E-mail: nicolas@iq.usp.br

Keywords: Human Genetics, Neurodegeneration, DNA damage signalling

Mutations in several DNA repair genes are associated with rare hereditary diseases characterized by neurodevelopmental and/or neurodegenerative phenotypes. However, the underlying molecular mechanisms are largely unknown, precluding the identification of potential therapeutic strategies. Here we describe a novel disorder caused by biallelic mutations in the human XRCC1 gene and implicate elevated levels of protein ADP- ribosylation in the associated neuropathology. DNA single-strand breaks (SSBs) are arguably the most abundant form of DNA damage in cells and their repair is initiated by poly-ADP-ribose polymerases (PARPs), which modify proteins surrounding the break site with poly-ADP-ribose (PAR) chains. This leads to the recruitment of XRCC1, which binds, stabilizes and stimulates the DNA end- processing enzymes that process and subsequently ligate damaged DNA termini. Single-strand break repair plays critical roles in the brain, particularly the cerebellum, as mutations in many of these factors result in rare hereditary diseases characterized by cerebellar atrophy, ataxia and oculomotor apraxia. We describe the first XRCC1-deficient patient with these clinical features and show that patient-derived cells exhibit not only reduced rates of single-strand break repair but also increased levels of DNA damage- induced protein ADP-ribosylation. Interestingly, aberrant ADP-ribose levels were also observed in cells from a patient with a related syndrome caused by mutations in the XRCC1 partner protein PNKP and in the cerebellum of untreated Xrcc1-deficient mice. Remarkably, genetic deletion of PARP1 rescued normal ADP-ribose levels both in human cells and mouse cerebellum, suggesting that delayed repair of SSBs leads to overt PARP1 signalling of these lesions. Strikingly, PARP1 loss also reduced the loss of cerebellar neurons and ataxia in these animals, identifying a molecular mechanism by which endogenous single-strand breaks trigger neuropathology. Collectively, these data establish the importance of XRCC1 protein complexes for normal neurological function and identify PARP1 as a therapeutic target in DNA strand break repair-defective disease.

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Toxicogenetic study of asiatic acid and its influence on genomic instability

Saulo Duarte Ozelin; Arthur Barcelos Ribeiro; Lucas Henrique Domingos da Silva; Francisco Rinaldi-Neto; Karoline Soares de Freitas; Larissa Daniela Ribeiro de Souza; Ricardo Andrade Furtado; Wilson Roberto Cunha; Denise Crispim Tavares.

Universidade de Franca, Franca, São Paulo, Brazil.

sauloozelin@hotmail.com

Key-words: Asiatic acid; cytotoxicity; antigenotoxicity

Asiatic acid (AA) is a pentacyclic triterpene that occurs in various fruits and herbs. It is particularly abundant in *Centella asiatica* and has been reported its healing, anti-diabetic, antioxidant and cytotoxic activities. Bioassays allow the determination of chemoprevention parameters, such as antigenotoxicity, antioxidant activity and control of cell death. These effects contribute to the prevention and treatment of biochemical disorders caused by DNA mutations, such as cancer. In the present study, *in vitro* assays were conducted aiming at understanding the mechanisms involved in cytotoxic, genotoxic, antigenotoxic and antioxidant activities of AA. The antiproliferative activity was evaluated by XTT colorimetric assay using human tumor cell lines (M059J, HeLa and MCF-7) and non-tumor cells (GM07492A), with concentrations of AA ranging from 3.9 to 500 µg/mL, during 24, 48 and 72 hours. Genotoxic and antigenotoxic activities were assessed by micronucleus test in V79 cells, using different concentrations (10-40 µg/mL). The ability to interfere in the cell cycle, oxidative stress and induction of apoptosis were evaluated by flow cytometry. According to results, AA showed cytotoxic effect after 24, 48 and 72 hours of treatment in GM07492A (IC_{50}- 48.6 to 0.4 µg/mL), M059J (IC_{50} - 49.1 to 6.8 µg/mL), HeLa (IC_{50} - 54.6 to 9.0 µg/mL) and MCF-7 (IC_{50} - 54.3 to 13.2 µg/mL). The selectivity index (SI) was obtained by the ratio between the IC_{50} observed in the non-tumor and those observed in tumor cell lines. In this way, selective cytotoxicity was detected for the M059J cell line, with SI equal to 2.2 in the 48 hours of treatment. Cultures treated with AA showed no significant increase in micronucleus frequencies at the concentrations tested. On the other hand, AA led to significant reductions in the micronucleus frequencies induced by the mutagens doxorubicin (DXR, 0.5 µg/mL), methyl methanesulfonate (44 µg/mL) and hydrogen peroxide (4.25 µg/mL). AA (12 µg/mL) induced apoptosis, decreased the percentage of cells in the G0/G1 and increased the percentage of cells in G2/M cell cycle phase in M059J cells after 48h of treatment. Reduction of DXR-induced intracellular free radicals was also observed in M059J cells treated with AA (5 µg/mL). In conclusion, AA showed cytotoxic effect in tumor and non-tumor cell lines, particularly in glioblastoma cells (M059J). The reduction of cell viability should be due to the apoptotic activity of AA. In the experimental conditions used, AA did not show genotoxic activity, but revealed an antigenotoxic effect against chromosomal damage induced by mutagens with different mechanisms of action. The preventive activity of AA may be due, at least in part, to its antioxidant capacity. The obtained data corroborate to better understand the mechanisms of action involved in AA biological activities.

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INTRA AND INTER-POPULATION DIVERSITY OF *Heliconius erato phyllis* (LEPIDOPTERA: NYMPHALIDAE: HELICONIINI) IN THE ATLANTIC FOREST

Patrícia Avelino Machado[1*]; Karina Lucas Silva-Brandão[2]; Anete Pereira de Souza[1]; André Victor Lucci Freitas[1,3]

[1] Instituto de Biologia, Universidade Estadual de Campinas, Campinas, SP, Brasil. [2] Centro de Ciências Naturais e Humanas (CCNH), Universidade Federal do ABC, Santo André, SP, Brasil. [3] Museu de Zoologia, Instituto de Biologia, Universidade Estadual de Campinas, Campinas, SP, Brasil

*patyavelino95@gmail.com

Key-words: Phylogeography, mtDNA, Structuring

The Atlantic Forest (AF) is one of the most threatened biomes in the world, characterized by high biodiversity and high rates of endemism. Almost five centuries of anthropic degradation resulted in habitat loss and forest fragmentation, reducing the AF to less than 12% of its original cover. Phylogeographic studies in this biome can contribute for answering important questions about species distribution and their relation to its geological history, and studies with butterflies have longly contributed for our understanding of evolutionary and ecological patterns. The butterfly *Heliconius erato* comprises 29 subspecies distributed across the Neotropics, with *Heliconius erato phyllis* occuring throughout the AF. Considering the high climatic and geographic heterogeneity along *H. erato phyllis* distribution, understanding the genetic structure of this subspecies in different climatic domains and the importance of geographical barriers in the processes associated to this structure is highly relevant. In this study, we present data obtained from mtDNA COI sequences of 364 individuals from 64 AF localities. The F_{ST} indicates moderate to high structuring among populations (F_{ST} = 0.27), but genetic differentiation is not correlated to geographic distance (Mantel test, F = 0.10, p = 0.42). The haplotype network recovered three major haplogroups of individuals that share more haplotypes with each other than with individuals from other groups. Each haplogroup is associated with three different regions of the AF, Northeast, Southeast and South regions. These results suggest that both geographical and climatic barriers have contributed to the genetic structure currently found in *H. erato phyllis*.

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Landscape of HOX driven regulation in cancer

Jessica Rodrigues Plaça[1,2,3]; Josane de Freitas Sousa[4]; Israel Tojal da Silva[5]; Wilson Araújo da Silva Jr.[1,2,3,6*]

[1]National Institute of Science and Technology in Stem Cell and Cell Therapy and Center for Cell-Based Therapy, Ribeirão Preto, São Paulo, Brazil. [2]Clinical Oncology, Stem Cell and Cell Therapy Program, Ribeirão Preto Medical School, Ribeirão Preto, São Paulo, Brazil. [3]Center for Medical Genomics at General Hospital of the Ribeirão Preto Medical School, University of São Paulo, Ribeirão Preto, SP, Brazil; [4]Institute of Biological Science, Federal University of Pará (UFPA), Belém, Pará, Brazil. [5]Internacional Center of Research and Education, AC Camargo Cancer Center, São Paulo, São Paulo, Brazil. [6]Department of Genetics at Ribeirão Preto Medical School, and Center for Integrative System Biology (CISBi-NAP/USP), University of São Paulo, Ribeirão Preto, São Paulo, Brazil.

*wilsonjr@usp.br

Palavras-chave: HOX; cancer; gene regulation

The family of HOX genes comprises a set of 39 transcription factors highly evolutionarily conserved divided into four clusters: HOXA, HOXB, HOXC and HOXD. These genes act on embryonic development by regulating biological processes, such as proliferation, differentiation, migration, angiogenesis, and apoptosis, which are reactivated during carcinogenesis. Recent studies have shown some HOX genes acting in the progression of various tumors and include the HOX family in a restricted group of genes that regulate tumorigenesis. However, is not known which members of them are predominantly active and how they regulate the cancer environment. So, the aim of the study was to apply a *in silico* approach with public data to investigate the expression regulation of all members of the HOX genes in 19 carcinomas of TCGA (*The Cancer Genome Atlas*). To accomplish this goal the differential profile of HOX genes was identified between normal and tumor samples. HOX gene targets were identified and, when differentially expressed, were associated with HOX genes expression. Finally, the final associations among the HOX genes and their targets were enriched with the Molecular Signatures Database (MSigDB) of hallmark gene set collection of cancer. A HOX gene expression alteration signature was identified among the tumors. This signature was associated with the anteroposterior axis of the human body, as well as the embryonic leaflets originating from the tumor tissues, compatible with the expression pattern in the embryonic development. A total of 12615 HOX gene targets were detected, which the most of them are common among the HOX genes and are activated in at least 4 different tumors. Interestingly, a large number of genes of HOXA family work as inhibitors of proliferative hallmarks (HOXA2, HOXA4, HOXA5, HOXA7, HOXA9), including in breast cancer. Interestingly, a considerable number of HOX genes preferentially act via enhancers in the regulation of their associated targets. As an example, the HOXB7 and HOXC11 genes, which function as pro-tumor modulators. Additionally, the epithelial mesenchymal transition signature was activated in 6 different tumors. Finally, the study shows that in view of the growing number of public genomic data, more can be discovered about HOX genes and their implication in cancer.

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INFLUENCE OF BETULINIC ACID ON THE CHROMOSOMAL DAMAGE INDUCED BY DOXORUBICIN IN MICE

Lucas Teixeira Souza de Oliveira, Matheus Reis Santos de Melo, Nayanne Larissa Cunha, Karoline Soares de Freitas, Samuel do Nascimento, Fernanda Santos Fernandes, Wilson Roberto Cunha, Natália Helen Ferreira, Denise Crispim Tavares.

Universidade de Franca, Franca, São Paulo.

lucasteixeirasouza@gmail.com

Keyword: Betulinic acid; antigenotoxicity; micronucleus assay.

The betulinic acid (BA) is a triterpene usually isolated from *Betula alba* trees. This triterpene has various biological activities as anti-inflammatory, hepatoprotective, analgesic, cardiotonic, sedative, bactericidal, fungicidal, antiviral and antitumor. In view of its pharmacological potential, the present study evaluated the effect of BA on genotoxicity induced by doxorubicin (DXR). For this, the micronucleus assay in Swiss mice peripheral blood was used. The doses used of BA were 0.25, 0.50 and 1.0 mg/kg body weight (b.w.) and 10 mg/kg b.w. of DXR. The frequency of micronuclei was obtained from the analysis of 25,000 polychromatic erythrocytes (PCE) per treatment group. The cytotoxicity was evaluated by the ratio between PCE and total erythrocytes, being analyzed 10,000 cells per treatment. The results showed that the treatments with BA led to significant reductions in the frequencies of DXR-induced micronuclei. Furthermore, no significant differences were observed in the PCE/total erythrocytes ratio between the different treatment groups, revealing absence of cytotoxicity. In conclusion, the BA showed antigenotoxic effect, under the experimental conditions used. The protective effect observed may be due to the action of triterpene as a free radical scavenger causing the reduction of oxidative stress induced by DXR. Further studies are required for a better understanding of the mechanisms of action involved in the chemopreventive action of BA.

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Evaluation of the antiproliferative activity of the red propolis hydroalcoholic extract and its fractions obtained by partition

Karoline Soares de Freitas[1]; Iara Silva Squarisi[1]; Danieli Cristina Lemes[1]; Rodrigo Cassio Sola Veneziani[1]; Jairo Kenupp Bastos[2] and Denise Crispim Tavares[1].

[1]Universidade de Franca, Franca, SP. [2]Faculdade de Ciências Farmacêuticas de Ribeirão Preto, Universidade de São Paulo, Ribeirão Preto, SP.

*karolinesoaresfreitas@gmail.com

Palavras-chave: red propolis; cytotoxicity; XTT colorimetric assay.

The Brazilian red propolis is a product synthesized by *Apis melífera* bees, from exudates collected from *Dalbergia ecastaphyllum* (L) Taub plant, found mainly in Alagoas state. This propolis is rich in flavonoids, tannins, xanthones and terpenoids. The red propolis presents antibacterial, antifungal, antiparasitic, anti-inflammatory, antioxidant and antitumor activities. In this sense, the present study aimed to evaluate the cytotoxicity of the red propolis hydroalcoholic extract (RPHE) and its fractions, dichloromethane (DF), hexanic (HF), *n*-butanol (BF) and ethyl acetate (AF), on tumor and non-tumor cell lines. For this purpose, the XTT colorimetric assay was performed with human lung fibroblasts (GM07492A, non-tumor cells), breast adenocarcinoma (MCF-7), glioblastoma (U-343) and cervix adenocarcinoma (HeLa) cells. The cultures were treated with concentrations ranging from 9.77 to 1,250 µg/mL of each sample. Negative (no treatment), solvent (dimethylsulfoxide, DMSO, 0.4%) and positive (DMSO, 25%) controls were included. The cell viability was determined as a percentage of viable cells in relation to the negative control group, which was designated as 100% of viability. The cytotoxicity was also evaluated using IC_{50}, the concentration that inhibits 50% of cell viability. The results showed that, for each cell line - GM07492A, MCF-7, U-343 and HeLa - RPHE revealed IC_{50} of 144.5, 266.3, 144.0 and 141.5 µg/mL, the DF presented IC_{50} of 78.9, 222.4, 71.6 and 83.9 µg/mL, and the HF demonstrated IC_{50} of 33.8, 34.5, 133.3 and 33.8 µg/mL. BF and AF revealed IC_{50} higher than 1250 µg/mL in all cell lines. Therefore, RPHE, DF and HF showed cytotoxic potential to all tumor cell lines, but also to normal cell line, indicating absence of selectivity. HF presented the highest cytotoxic potential with the lowest IC_{50}, which might be attributed to its chemical composition, consisting predominantly of the benzophenones guttiferone E, xanthochymol and oblongifolin B, described by the literature as pro apoptotic compounds. These results allowed to better understand the cytotoxic potential of the red propolis hydroalcoholic extract.

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FUNCTIONAL ANNOTATION OF SNPs ASSOCIATED WITH GDF15 LEVELS IN A PUTATIVE ENHANCER REGION ACTIVATED BY METFORMIN

Daniela A. Pereira[1]; Natália D. Linhares[1]; Izabela M. C. A. Conceição[2]; Glória R. Franco[3]; Walter L. Eckalbar[4,5]; Nadav Ahituv[4,5]; Marcelo R. Luizon[1,2,*]

[1]Programa de Pós-Graduação em Genética, Instituto de Ciências Biológicas (ICB), Universidade Federal de Minas Gerais (UFMG), Belo Horizonte, MG, Brazil. [2]Departamento de Genética, Ecologia e Evolução, ICB/UFMG, Belo Horizonte, MG, Brazil. [3] Departamento de Bioquímica e Imunologia, ICB/UFMG, Belo Horizonte, Minas Gerais, Brazil. [4,5]Institute for Human Genetics and Department of Bioengineering and Therapeutic Sciences, University of California San Francisco, San Francisco (UCSF), California, United States of America.

* mrluizon@ufmg.br

Keywords: Enhancers, Growth differentiation factor 15 (GDF15), Metformin

Introduction: Metformin is the first-line oral therapy for type 2 diabetes, and it is also used as a treatment for other diseases, such as polycystic ovary syndrome, gestational diabetes, obesity and cancer. However, no strong clinical biomarker for the use of metformin had been identified apart from glycemic measures. Recently, growth differentiation factor 15 (*GDF15*) levels were recently identified as a novel biomarker for metformin use. Moreover, single nucleotide polymorphisms (SNPs) were found to be associated with plasma GDF15 concentrations. Nevertheless, many of these SNPs are located in noncoding regions. **Objectives:** We thus searched for metformin-responsive regulatory elements in the GDF15 locus and linked SNPs within them that may be associated with GDF15 levels. **Methods:** We examined publicly available RNA-seq and ChIP-seq data for the acetylation of histone H3 on lysine 27 (H3K27ac) in human hepatocytes treated with metformin and several functional genomics data to search for genes coding for the potential biomarkers of metformin use and for metformin-responsive regulatory elements in the *GDF15* locus. GeneHancer database was used to identify active regulatory elements that inferred target GDF15. The 4DGenome database was also used to consult integrated method for predicting enhancer targets (IM-PET) algorithm data, which integrates four types of genomic features to predict enhancer- promoter interactions. We generated a BED file with these database results. Additionally, we used Genotype Tissue Expression (GTEx) Project data for SNPs linked to *GDF15* expression levels in whole blood tissue and SNPs previously found to be associated with GDF15 levels by candidate gene studies and GWAS to perform the comprehensive functional annotation for the noncoding SNPs located in the *GDF15* locus and their overlap with metformin responsive-regulatory elements. **Results:** The main novel findings reported here were that (1) *GDF15* and other genes coding for potential biomarkers for metformin use (galectin-3, *LGALS3* and alpha-2-macroglobulin, *A2M*) were found to be upregulated by metformin using RNA-seq; (2) SNPs rs62122429 and rs62122430 associated with GDF15 levels are located nearby the *GDF15*locus within a metformin-responsive region enriched for the active histone mark H3K27ac upon metformin treatment according to previous ChIP-seq data; and (3) this metformin H3K27ac-enriched region shows high linkage disequilibrium (LD) with the GWAS lead SNP rs888663 and eQTLs for *GDF15* expression levels in Europeans and East Asians. **Discussion and Conclusions:** We identified a putative metformin-responsive enhancer region near *GDF15*. In Europeans and East Asians, this region shows high LD with GWAS lead SNPs and eQTLs for *GDF15* expression levels. Our findings may guide functional assays to test whether the SNPs within this putative metformin H3K27ac- enriched region may upregulate *GDF15* expression levels. The perspectives are that these SNPs could help to predict GDF15 levels in patients with type 2 diabetes or other diseases who are treated with Metformin.

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CHARACTERIZATION OF SsaA PROTEIN IN *Staphylococcus saprophyticus*

Guilherme Algusto Alves Silva[1]; Andrea Santana de Oliveira[1]; Bianca Silva Vieira de Souza[1]; Clayton Luiz Borges[1], Célia Maria de Almeida Soares[1]; Marcia Giambiagi de Marval[2], Juliana Alves Parente Rocha[1]

[1]Laboratório de Biologia Molecular, Instituto de Ciências Biológicas, Universidade Federal de Goiás, Av. Esperança, ICB2, 74690-900, Goiânia - Goiás, Brazil. [2]Laboratório de Microbiologia Molecular, Instituto de Microbiologia Prof. Paulo de Góes, Universidade Federal do Rio de Janeiro, Cidade Universitária, 21941-970, Rio de Janeiro – Rio de Janeiro, Brasil.

algustoguilherme1@gmail.com

Key-words: *Staphylococcus saprophyticus*; SsaA protein; immunogenic proteins.

Staphylococcus saprophyticus is a Gram-positive bacterium and stands out as the second pathogen responsible for diagnosed cases of urinary tract infection (UTI), affecting mainly young women. Some factors may explain the ability of *Staphylococcus saprophyticus* to colonize periurethral, urinary and genital regions, such as its ability to attach to the epithelial tissue of the genitourinary tract and the high activity of the urease enzyme. However, few mechanisms that this bacterium uses to efficiently infect and colonize the host are fully elucidated. In species of the genus *Staphylococcus* most of the virulence factors are considered as proteins secreted during the process of infection. Our research group identified proteins secreted from *Staphylococcus saprophyticus* isolates that demonstrated a strong ability to stimulate the immune response in mice, one of the major immunogenic proteins identified was the Staphylococcal A Secretory Antigen (SsaA). In other species of the genus *Staphylococcus* the SsaA protein seems to be related to virulence factors being regulated by the same systems, however its specific role during the infection has not yet been fully elucidated. In this sense, we propose the characterization of the SsaA protein in *Staphylococcus saprophyticus*. SsaA is apparently conserved in the genus *Staphylococcus* and in our study has been identified in different strains of *Staphylococcus saprophyticus*, such as food sample (cheese mines), environmental sample (Leblon beach sand) and clinical sample (microbiota of pregnant women) through the technique Western Blotting. Bioinformatics analyzes using the BioCyc database revealed that the SsaA protein has a CHAP domain with a predicted amidase function. The 3D structure of the SsaA protein was predicted through protein modeling by the I-TASSER server following the validation protocols. Cloning, heterologous expression and purification of the SsaA protein are under progress and will contribute to confirm the SsaA function. Our work will contribute to elucidate biological function of SsaA protein, an immunogenic protein that can be useful as diagnostic and/or vaccine target.

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INSIGHTS INTO A RARE EMBRYONAL LIVER CANCER OPEN NOVEL AVENUES OF STUDY: NOT ALL HEPATOBLASTOMAS ARE EQUAL

Juliana Sobral de Barros[1], Talita Aguiar[1,2], Silvia Souza da Costa[1], Anne Caroline Barbosa[1], Maria Prates Rivas[1], Estela Novak[6], Vicente Odone-Filho[6], Silvia Regina Caminada de Toledo[5], Monica Cypriano[5], Dirce Maria Carraro[2], Isabela Werneck da Cunha[4], Cecília Maria Lima da Costa[3], Carla Rosenberg[1], Ana C.V. Krepischi[1]

[1]Human Genome and Stem-Cell Research Center, Department of Genetics and Evolutionary Biology, University of São Paulo – Institute of Biosciences, São Paulo, Brazil, [2]International Research Center, AC Camargo Cancer Center, São Paulo, Brazil. [3]Department of Pediatric Oncology, AC Camargo Cancer Center, São Paulo, Brazil. [4]Department of Pathology, AC Camargo Cancer Center, São Paulo, Brazil. [5]Pediatric Oncology Institute (GRAACC), Department of Pediatrics, Federal University of São Paulo, São Paulo, Brazil. [6]Institute of Childhood Cancer Treatment (ITACI), Pediatric Department, Medical School of University of São Paulo, Hematology–Oncology Division, São Paulo, Brazil

juliana.sobral@ib.usp.br

Key words: Hepatoblastoma, Copy number alteration, Cytogenomics

Despite being the most common liver cancer in children, accounting for approximately 80% of the total hepatic cancer, hepatoblastoma (HB) is a rare embryonal tumor, with an annual incidence rate of 1.5 cases per million children. Due to its rarity, underlying molecular mechanisms of origin are poorly understood, and currently there are few validated biomarkers for HB patients. Cytogenetic alterations are hallmarks of cancer cells and can provide clues about relevant biological pathways. The aim of this study was to perform a cytogenomic investigation in 25 HB tumors provided by three Brazilian reference centers in childhood cancer. Chromosomal microarray analysis (CMA; 180K platform) and NGS data (mendeliome panel and whole-exome) were used for calling copy number alterations (CNA). Our results revealed a quite stable genome in most HBs, with seven of them presenting no detectable chromosomal imbalances, and two with focal gains ranging in size from 300-500 Kb. Eleven HBs exhibited mainly whole- chromosome and arm aneuploidies, mostly in a mosaic state, with prevalence of gains affecting the chromosome arms 1q and 2q as well as chromosome 20, in addition to 1pter and 4q losses. Altogether, this CNA profile had been already described in the literature for HB tumors, but we were able to delimitated six minimal common regions, highlighting a group of 16 candidate genes possibly related to the HB genesis. Additionally, remarkable complex genomes were observed in five tumors (20% of the cohort), presenting a very unusual CNA profile in comparison to the group, with a higher load of chromosomal rearrangements. Two of these male patients developed HBs carrying several non-contiguous deletions clustered in a single chromosome arm, in a complex pattern resembling chromothripsis; these patients shared clinical signs such as prematurity, metastases at diagnosis and global developmental delay. Therefore, our data showed that whole-chromosome and arm aneuploidies play a major role driving HB tumorigenesis, but specific regions of the genome could be pinpointed as potentially relevant. More outstandingly, our results highlighted that not all hepatoblastomas are equal, revealing intertumoral heterogeneity at least in a structural level, as well as disclosed examples of high chromosomal complexity such as those possibly derived from chromothripsis. These insights can provide a future framework to further stratify hepatoblastomas based on their cytogenomic profile.

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INTEGRATIVE TRANSCRIPTOMIC AND EPIGENOMIC ANALYSIS REVEALS LONG NONCODING RNAs WITH BOTH ALTERED GENE EXPRESSION AND METHYLATION PATTERN DURING MELANOMAGENESIS

Ádamo Davi Diógenes Siena[1,2], Jéssica Rodrigues Plaça[2], Isabela Ichihara Barros[1,2], Carlos Alberto Oliveira de Biagi Júnior[1,2], Wilson Araújo Silva Jr[1,2,3]

[1]Department of Genetics at Ribeirão Preto Medical School, University of São Paulo, Ribeirão Preto, Brazil. [2]Center for Cell-Based Therapy (CEPID/FAPESP); National institute of Science and Technology in Stem Cell and Cell Therapy (INCTC/CNPq), Regional Blood Center of Ribeirão Preto, Riberão Preto, Brazil.[3]Center for Medical Genomics, HCFMRP/USP. Riberão Preto, Brazil.

*adamo@usp.br

Keywords: long noncoding RNA; Melanoma; Integrative analysis.

Melanoma is by far the most lethal skin cancer. Its emergence and progression have been widely associated with genetic alterations, as the very known BRAFV600E point mutation. However, melanoma progression could not be fully explained only by DNA mutations. Recently, many changes in regulatory gene expression have been revealed with key roles in melanoma progression. In this sense, long noncoding RNAs (lncRNAs) became important molecules acting in gene expression regulation and development of cancer. In this work, our objective is to analyze RNA-Seq and methylation data to search for differentially expressed lncRNAs, which also harbored promoter regions differentially methylated, and that could impact in melanoma progression. We performed RNA-Seq in melanoma cell lines, and we also downloaded public RNA-Seq and genome-wide methylation data to accomplish an integrated bioinformatics analysis. The RNA-Seq dataset consists of melanocytes (n=2), primary melanoma cell lines (n=6) and metastatic melanoma cell lines (n=9). Genome-wide methylation data are derived from a cohort of patients with advanced melanoma (n=50, stage III/IV tumors) and which also was included data from melanoma cell lines (n=9) and two technical replicates of light, medium and darkly pigmented melanocyte. Our results of differential gene expression from RNA-Seq comparing melanocytes versus metastatic melanoma cell lines demonstrated 47 differentially expressed lncRNA, whereas melanocytes versus primary melanoma cell lines revealed 29 differentially expressed lncRNAs, and primary versus metastatic melanoma cell lines only showed five differentially expressed lncRNA. Besides, we analyzed differentially methylated regions and/or CpG islands using two different R packages (COHCAP and DMRcate) that revealed a total of 196 differentially methylated CpG islands. After reannotation of probes from differentially methylated regions, we found 83 unique lncRNAs with Transcription Start Site (TSS) differentially methylated in melanoma versus melanocyte comparison. Then, we crossed the results from lncRNAs differentially expressed and lncRNAs with differentially methylated TSS. Our analysis showed ten lncRNAs with both alterations in gene expression and methylation at the promoter site. Interestingly, three of these lncRNAs (AGAP2-AS1, LINC00462, and SATB2-AS1) are altered in comparisons of melanocyte versus primary melanoma and melanocyte versus metastatic melanoma. Each of these lncRNAs was found upregulated or downregulated, which could reflect their features as oncogenic or as tumor suppressor genes. Together, these results revealed many lncRNAs with promoter regions influenced by epigenetic repression or activation, which could lead to deregulated gene expression in melanoma progression towards more aggressive phases. Furthermore, our results demonstrated a new integrative approach using different data, platforms, and analysis to get insights into the regulation of lncRNAs gene expression and their consequence in cancer development.

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SEQUESTOSOME 1 GENE VARIANTS IN OBESITY

Marina Andrade Tomaz[1,2], Johanna Giuranna[1]; Carla Ivane Ganz Vogel[2]; Anke Hinney[1]

[1]Department of Child and Adolescent Psychiatry, Psychosomatics and Psychotherapy, University Hospital Essen, University of Duisburg-Essen, Essen, NW, Germany. [2]Department of Food and Animal Production, Faculty of Veterinary Medicine, State University of Santa Catarina, Lages, SC, Brazil.

marinatomaz@hotmail.com

Keywords: SQSTM1; Obesity.

Monogenic forms of obesity represent approximately 3% of the cases of this worldwide high prevalent disease. Genes known to be associated with the condition are mostly part in the melanocortinergic pathway, although genes involved in other biological processes than food suppression have been shown to be good candidates. The *SQSTM1* gene encodes p62, a protein that participates in several process including apoptosis, autophagy, cell differentiation and immunity pathways, and is related to body weight regulation in the signaling cascades that control adipogenesis. Adipocyte-specific p62- deficient mice present higher body weight, fat mass and serum triglyceride levels than p62-wild type mice. Consequently mutations in *SQSTM1* gene are considered to be a plausible cause of obesity in humans. With the aim to search for potential obese causing mutations in *SQSTM1*, the complete gene coding sequence was screened using Sanger sequencing on 191 obese children and adolescents, 191 underweight individuals and 96 patients diagnosed with anorexia. A total of 10 variants were detected, in which four were polymorphisms, four were rare missense mutations and two were rare synonymous variants. Difference between frequency of rare variants in obese and other phenotypes were not statistically significant ($p>0.05$). No rare missense mutation found in theobese group was predicted to be pathogenic in *in silico* analysis. At present, our data suggests that *SQSTM1* mutations are not associated with obesity, yet larger study groups are required to determined this correlation.

HISTONE DEACETYLASES AND THEIR ROLE IN HONEY BEE, *APIS MELLIFERA* L., CASTE DEVELOPMENT

Giovana Cravero[1]; Carlos Antônio Mendes Cardoso Júnior[2]; Klaus Hartfelder[2]

[1]Departament of Genetics – Ribeirão Preto School of Medicine (FMRP) – University of São Paulo (USP), Ribeirão Preto, São Paulo, Brazil. [2] Departament of Cell, Molecular Biology and Pathogenic Bioagents – Ribeirão Preto School of Medicine (FMRP) – University of São Paulo (USP), Ribeirão Preto, São Paulo, Brazil

Keywords: sodium butyrate; caste development; histone deacetylases.

Honey bees are characterized by a very advanced social organization, where the queen and worker castes perform specific functions in the hive. The queen is responsible for reproduction, while the workers are responsible for performing all the tasks of maintaining the colony. Despite their morphological and functional differences, queens and workers do not differ genetically, but represent a polyphenism triggered by the differential feeding of queen and worker larvae. Royal jelly that nurse bees feed in copious amounts to queen larvae contains compounds that are able to regulate the epigenetic state of the larvae, altering DNA methylation levels and modifying histones. One such molecule is 10-hydroxy-2-decenoic acid (10HDA) acid, which is present in high amounts in royal jelly. 10HDA has been identified as an inhibitor of histone deacetylase (HDAC) activity in mammalian cells. Histone deacetylases remove acetyl groups from lysine residues in histones, mainly in histone 3, thus leading to greater chromatin compaction and, consequently, hindering the access of the transcriptional machinery. To address the question of whether HDACs play a role in honey bee caste development we first quantified the expression levels of the four HDACs encoding genes of *Apis mellifera* (HDAC-1,3, 4 and 6) by means of RT-qPCR, comparing worker and queen larvae during their development. We found that in the fourth instar queen larvae the transcript levels of HDAC-1, 3, and 6 were significantly higher than in workers, indicating that this stage is possibly susceptible to alterations in the chromatin epigenetic state related to caste development. To test this we designed a functional assay feeding the generic HDACs inhibitor sodium butyrate to honey bee larvae. In this assay, larvae were reared from the earliest larval stage (L1) on an artificial diet containing 1 mM or 10 mM sodium butyrate, to test its effects on total development time and weight at adult eclosion. The results of this pilot study revealed differences in developmental time until the adult stage between the control and treatment groups, but there was no significant difference in weight. The next step now is to analyze the effect of sodium butyrate on HDACs transcript levels and to compare the effects of sodium butyrate with those of 10HDA.

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COMPARISON OF PROTEOMIC PROFILES BETWEEN *Xanthomonas citri* subsp. *citri* AND treA DELETION MUTANT AND *IN VITRO* PROTEIN INTERACTION OF THE RECOMBINANT TREHALASE

Solange Cristina Antão[1]; André Vessoni Alexandrino[1]; Celia Regina Câmara[1]; Maria Teresa Marques Novo Mansur[1]

[1]Laboratório de Bioquímica e Biologia Molecular Aplicada – Departamento de Genética e Evolução - Universidade Federal de São Carlos.

solange.cristina@ufscar.br

Keywords: Citrus canker; 2D-DIGE; *Pull Down.*

Citrus canker provides a drop in citrus fruit yield and quality because of the lack of efficient control and eradication measures. It is caused by the bacterium *Xanthomonas citri* subsp *citri* (Xcc), of fast dispersion and high degree of virulence. In a work previously performed by our research group, an Xcc mutant was produced by deleting the treA gene, present in a single copy in the genome of Xcc strain 306 and encoding the enzyme trehalase. This enzyme catalyzes the hydrolysis of the disaccharide trehalose in two glucose monomers. There are studies relating trehalose with mechanisms of protection of the bacterium, and in this sense, the deletion of the treA gene led to an apparent greater virulence of Xcc. The objective of this work was to compare the proteomes of Xcc wild-type (strain 306) and mutant strain (XccΔtreA) for a better understanding of the role of trehalase in the infectious process of Xcc, by identification of proteins affected by gene deletion. Growth curves were performed in XAM-M pathogenicity- inducing medium and were similar for both strains. Cells were collected at the logarithmic phase of bacterial growth (DO_{595nm} 0.6) for differential proteomic analysis. Total proteins were extracted and quantified, and 50 µg were fluorescent-labeled for 2D-DIGE analysis. After analysis performed in DeCyder v. 7.2, 11 spots with statistically relevant differential abundance between wild and mutant strains (p-value <0.05 in the Student's T-Test) were isolated for further protein identification by mass spectrometry. A *Pull Down* assay was also performed to investigate possible *in vitro* interactions of the immobilized Xcc recombinant trehalase, produced in our research group, with other Xcc proteins from the cell lysate of both wild and mutant strains. It was possible to identify by SDS-PAGE two bands of wild-type lysate which co-eluted only with the recombinant protein, indicating possible metabolic relation with trehalase. These bands were isolated, trypsin-digested and will be subjected to mass spectrometry for identification of the proteins by searching Xcc genome. In conclusion, deletion of the gene encoding the periplasmic trehalase generates changes in the total proteome of Xcc, and at least two proteins from lysate demonstrated to have *in vitro* interaction with trehalase. Future identification of the proteins detected in both approaches may lead to a better understanding of the role of trehalase in the metabolism and pathogenicity of Xcc.

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SCREENING OF GERMLINE MUTATIONS IN GENES RELATED TO DNA REPAIR AMONG LYNCH-LIKE PATIENTS DIAGNOSED WITH ENDOMETRIAL CANCER

Reginaldo C A Rosa[1]; Fernando Chahud[2]; Alfredo R Silva[2]; Mariângela O Brunaldi[2]; Wilson A Silva-Júnior[1,3]; Victor E F Ferraz[1*]

[1] Department of Genetics – Ribeirão Preto Medical School at The University of São Paulo. [2] Department of Pathology and Legal Medicine – Ribeirão Preto Medical School at The University of São Paulo. [3] Center for Genomics Medicine – Ribeirão Preto Clinics Hospital.

*vferraz@usp.br

keywords: Endometrial cancer; Targeted Next Generation Sequencing; Lynch-like syndrome.

Introduction: Lynch syndrome (LS) is a hereditary disease that increases the risk for several tumors, notably colorectal and endometrial. This syndrome is caused by germline mutations in *MLH1, MSH2, MSH6* or *PMS2* genes which encode for the main proteins of the DNA-mismatch repair pathway (MMR). Although MMR deficiency (dMMR) is considered a hallmark of tumors associated to LS, up to sixty percent of all patients diagnosed with dMMR cancer have no detected germline mutation in the LS associated genes. These cases are called Lynch-Like syndrome (LLS) and the etiology of them remain unclear. **Objective:** to investigate the occurrence of germline mutations in genes associated with DNA repair in LLS patients diagnosed with dMMR endometrial cancer. **Methodology:** 38 patients diagnosed with endometrial endometrioid adenocarcinoma at the Ribeirão Preto Clinics Hospital previously investigated for LS, with no germline mutations on LS-associated genes, were included in this study. The occurrence of germline mutation in 24 genes implicated in DNA repair was evaluated using customized probes and reagents of SureSelectQXT Kit (Agilent Technologies) and posterior sequencing on the NexSeq (Illumina) platform in a paired end assay. Variants were called using SureCall v.4.1 software (Agilent Techologies). The American College of Medical Genetics Guidelines were used to classify mutations for pathogenicity. Variants classified as Pathogenic; Probably Pathogenic and Variants of Unclear Significance (VUS) were validated by Sanger sequencing. **Results and Discussion:** it was found a total of 23 mutations distributed among 16/24 genes which were present in a total of 18/38 patients. 2/23 mutations are Pathogenic (*FAN1*:p.P52Pfs and *ATR*:p. R2089*) and the remaining 21/23 mutations are VUS. The protein encoded by *FAN1* interacts with MMR proteins and its nuclease function is required for fully functional MMR activity. *ATR* somatic mutations have already been observed in MMR endometrial tumors, but these variants were found to occur as a consequence of MMR deficiency since *ATR* gene harbors microsatellites in its coding sequence. All VUS found in this study have a rare frequency (minor allele frequency < 1%) in the Brazilian Genomics Database (ABraOM) and are predicted as pathogenic in *in silico* predictors of pathogenicity (Mutation Taster, Sift, Polyphen, Provean and CADD). It was found two mutations in *PMS1* (p. E59K and p.R202K); one in *MSH3* (p.P63A) and one in *EXO1* (p.R93G). The occurrence of germline mutations in other genes of DNA-mismatch repair have already been observed among individuals of LS families, however, the clinical significance of mutations in these genes in this disease remains unclear. **Conclusion:** germline mutations in genes associated to genome integrity can play a role on the etiology of some LLS cases. However, further studies are required to clarify the real impact of this genes in the MMR pathway.

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PRESENCE OF MRN COMPLEX SUBUNITS AND THEIR RELATIONSHIP WITH DOUBLE STRAND BREAK REPAIR IN HUMAN MITOCHONDRIA

Laís Y. M. Muta[1*]; Mateus P. Mori[1]; Felippe T. Machado[1]; Nadja C. Souza- Pinto[1.]

[1] Department of Biochemistry, Institute of Chemistry, São Paulo University - USP, São Paulo, SP.

*lais.muta@usp.br

Keywords:DNA repair, Mitochondria, MRN complex.

In the nucleus, DNA double strand breaks (DSBs) are predominantly repaired by the Non-Homologous End Joining and the Homologous Recombination repair pathways. In mammals, the MRN complex, formed by the Mre11, Rad50 and Nbs1 subunits, signals for subsequent DNA damage response while binds to and stabilizes DSBs for further processing. While Mre11 has been previously localized in mitochondria, mitochondrial localization of Rad50 and Nbs1 subunits has not been demonstrated and the mechanistic understanding of DSB Repair in mitochondria remains poor. Here we demonstrate, by qPCR analysis, that Hek293T cells can repair mtDNA after treatment with bleomycin, a radiomimetic which induces mostly DSBs. Analysis *in silico* using the IPSORT and Mitofates software indicate that Mre11, Nbs1 and Rad50 do not show canonical mitochondrial localization presequences. However, putative Mitochondrial Processing Peptidases (MPP) cleavage sites and Tom20 - recognizing motifs, a sequence that is recognized by Tom20 subunit of the Translocase of Outer Membrane complex (TOM), were identified in all 3 proteins. *Western Blot* analysis of mitochondria from untreated cells indicated that Nbs1 is found associated with mitochondria, but not in the mitochondrial matrix, raising the possibility that MRN complex subunits stay at intermembrane space and are translocated to the matrix upon DNA damage. Furthermore, a possible mitochondria-specific Nbs1 isoform was identified by *Western Blot* analysis in HeLa mitochondrial extracts, but the nature of this isoform still needs to be confirmed. In conclusion, (i) DSB are efficiently repaired in human mitochondria, as indicated by qPCR analysis; (ii) peptide sequences of MRN subunits indicate possible ways of importing these proteins into mitochondria and (iii) the mitochondrial localization of MRN subunits, in absence or presence of mtDNA damage, needs to be confirmed by *western blot* analysis of highly purified mitochondrial extracts from Hek293T and HeLa cells. Thanks to financial support: FAPESP grants 2017/04372-0 and 2018/04471-1.

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TSPY GENE COPY NUMBER IN URBAN, INDIGENOUS, AND QUILOMBOLA POPULATIONS

Juliana O. Cruz[1,2¶], Jorge A. P. Marchesi[1], Sandra M.B. Sousa[2¶], Aguinaldo L. Simões[1¶], Ester S. Ramos[1¶*]

[1]Department of Genetics, Ribeirão Preto Medical School, University of São Paulo, Ribeirão Preto, São Paulo, Brazil. [2]Department of Genetics, Institute of Biological Sciences, Federal University of Minas Gerais, Belo Horizonte, Brazil. [3]Department of Natural Sciences, University Estadual do Sudoeste da Bahia, Vitória da Conquista, Bahia, Brazil.

juftoc@gmail.com

Key words: *Testis-specific protein Y-encoded*; Traditional populations; CNVs.

The *Testis-specific protein Y-encoded* (*TSPY*) is a multiple copy gene, ranging from 11 to 74 copies in humans. It is assumed that its protein participates in the regulation of male meiosis (mediating meiotic division) and in early stages of tumorigenesis. The Y Alu polymorphic (YAP) may influence reproductive gains or losses, due to its insertion site in a region rich in replicates, and also as determinant in spermatogenesis. This polymorphism is used in population studies as ancestral informational marker. The aim of our study was to verify inter- and intra-individual copy number (CN) variation of the *TSPY* gene, and Alu YAP insert in males from different populations. We analyzed DNA (blood) from 125 subjects from the urban population of Ribeirão Preto (São Paulo), 69 from the quilombola population São Gonçalo (Bahia), and 91 indigenous Pataxó (Bahia), totalizing 285 unrelated males, by the Quantitative Polymerase Chain Reaction (qPCR) using primers for sequences of the *TSPY*, *SRY*, and *GAPDH* genes. We identify the Alu YAP insert by conventional PCR with subsequent visualization in agarose gel. Additionally, we compared the results of 17 pairs (fathers and sons), and, for 16 individuals, the results in three body fluids (blood, saliva, and sperm). The CN ranged from 15 to 123, with a mean of 39. The CN of the *TSPY* differed among the three groups studied (p <0.05). There was a difference in the CN of the *TSPY* gene for the absence and presence of the Alu YAP marker (p<0.05), having a higher number of copies in the presence of Alu YAP insert. There was no influence of age increase with *TSPY* CN, and there was no statistical difference between the results of fathers and sons (p>0.05) in relation to the populations, but in 13 of 17 (76%) there was no concordance between the results of parents and their respective children, even though there was agreement of the results of Alu YAP. There was a difference among the three fluids of the same individual (p <0.05), indicating an intra-individual variation. This is the first study that describes CNVs of the *TSPY* gene in healthy individuals of the Brazilian populations. The data allow us to conclude that the Brazilian population is heterogeneous with a high variation of the number of copies at the population level, among males and even in the same subject. The *TSPY* copy number is not a good marker for forensic studies, or for paternity tests. In another hand our results highlight a singular comportment of a gene in different tissues and generations of the same family, that probably have a functional and physiological role.

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The effect of microRNA 100 and anti microRNA 100 and their target genes in metastatic prostate cancer.

Vanessa R Guimarães[1]; Alexandre Iscaife[1]; Sabrina T dos Reis[1]; Nayara I Viana[1]; Ruan C A Pimenta[1]; Denis R Morais[1]; Juliana A Camargo [1]; William C Nahas[2]; Miguel Srougi[1]; Katia R M Leite[1].

[1] Faculdade de Medicina da Universidade de São Paulo, Disciplina de Urologia, Laboratório de Investigação Medica (LIM55) – (São Paulo - SP) [2] Instituto do Câncer do Estado de São Paulo. (ICESP) – (São Paulo- SP)

v.guimaraes@usp.br

Key-words: Metastasis Prostate Cancer; microRNA-100; Target therapy.

Introduction: Metastatic Prostate Cancer (PC) is an incurable disease which traditional and more efficient treatment is androgen deprivation (AD). However, the efficiency is limited, and, after an average period of five years, tumor acquires a status of castration resistance (CR), when therapeutic options are limited. microRNAs (miRNAs) are small (~19 nucleotides), non-coding RNA molecules fundamental for the control of gene expression. Changing of their expression during the PC carcinogenesis process has been studied in recent years with the objective of identifying new diagnostic and prognostic biomarkers, as well as therapeutic agents. **Objective:** To analyze the expression of miRNA100 and anti miRNA 100 and their target genes SMARCA5 and mTOR in metastatic PC in an in vivo model. **Method:** For induction of metastatic PC, 19 Balb/c nude mice were inoculated with PC- 3M-luc-C6 bioluminescent cell line, derived from a metastatic, castration-resistant PC. After the establishment of metastatic disease, at day 21st five animals were treated with three injections into the caudal vein of miRNA100 and five animals with anti-microRNA 100. Atelocollagen was used to stabilize miRNA molecules. Nine animals were used as controls treated with "scramble miRNA" injections. Images were obtained using IVIS Spectrum. Expression analysis of miRNAs and their target genes was performed by the qRT-PCR in metastatic tumor tissue. **Results:** Treatment with anti-miRNA100 promoted a reduction in the tumor growth rate compared to the control and miR100 groups. The mean bioluminescent signal was 8,910 for anti-miRNA100, 30,84 for miRNA100 and 91,98 for controls on the last day of treatment. SMARCA5 and mTOR genes were overexpressed in animals treated with miRNA 100 when compared with the animals treated with anti-miRNA100. SMARCA5 2.2 and 1.2 and mTOR 1.4 and 0.6 respectively. **Conclusion:** Treatment with anti-miRNA100 in the animal model of metastatic PC showed a decrease in tumor growth rate. We observed an increase in the expression of SMARCA5 and mTOR oncogenes in animals treated with miRNA 100, confirming they as miR100 targets and the role of miR100 as an oncomiR in an animal model of metastatic PC.

ACETIC ACID APPLICATION TO ENHANCE DROUGHT TOLERANCE IN SOYBEAN

Elizandra Carneiro Andreata[1];Isabel Duarte Coutinho[2]; Silvana Regina Rockenbach Marin[4]; Caroline Fonceca Belinato[3]; Mayla Daiane Corrêa Molinari[1];Daniel de Amorin Barbosa[1];Alexandre Lima Nepomuceno[4], Luiz Alberto Colnago[2]; Liliane Marcia Mertz-Henning[4]*

[1]State University of Londrina - UEL; PPGGBM - Postgraduate Program in Genetics and Molecular Biology, Londrina - PR. [2]Embrapa Instrumentation Nuclear Magnetic Resonance Laboratory, São Carlos - SP. [3] State University of Maringá - UEM; PBA - Postgraduate Program in Environmental Biotechnology.[4]Brazilian Agricultural Research Corporation - Embrapa Soja, Londrina - PR.

* elizandraandreatta12@gmail.com

key words: Glycine max; chemical-priming; drought stress.

Soybean is one of the commodities of major economic importance in nowadays. However, the presence of abiotic factors, such as water deficit (WD), is one of the impasses to be overcome by crops in the agricultural sector. Studies conducted up to now suggest that the exogenous application of acetic acid may be an interesting alternative to mitigate the effects of drought on the plants how maize, colza, and Arabidopsis thaliana. Thus, tolerance to water deficit (WD), is mediated by a chemical priming due to the application of acetic acid causing a mechanism of "Epigenetic memory", due to pre-exposure to stress, allowing plants to prepare for coping with an adverse condition. The objective of the present investigation was to evaluate the effect and changes of the metabolites in plants of soybean subjected to a previous treatment of exogenous acetic acid followed by water deficit. The experiment was carried out in a greenhouse in a 2 x 4 x 2 factorial scheme, with two genotypes (BR 16 and Embrapa 48), four doses of acetic acid (0, 10, 20 and 40 mmol) and two water conditions (WD and control), in randomized blocks with nine replicates. When they reach the stage V3, the pretreatment of the acetic acid solution was applied by capillarity, in the concentrations of 0, 10, 20 and 40 mmol, for nine days, while the control group remained under ideal irrigation. After 7 days under stress, foliar samples were collected, lyophilized, for further analyses of metabolites by nuclear magnetic resonance (1H NMR) spectroscopy, the data obtained were subjected to analysis of main components. The present study revealed that the cultivar BR16 did not present a cluster between the different treatments. On the other hand, for the samples of the cultivar Embrapa 48, wherever differences were identified between the WD and control treatments, mainly in the concentrations of 20 and 40 mmol, showing higher levels in the spectrum reading in plants subjected to acetic acid application and grown under ideal irrigation (control). In general, significant changes signals were observed levels in the of control samples, were corresponding to citric acid, while the WD samples showed a higher correlation with fumaric acid, lactic acid, glucose, and amino acids.

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THE *CRNN* TUMOR SUPPRESSOR AND THE *DCD* ONCOGENE ARE NOVEL CANDIDATE IMPRINTED LOCI

Alan Tardin da Silva[1]; Cristina dos Santos Ferreira[1], Douglas Terra Machado[1], Enrique Medina-Acosta[1]

[1]Núcleo de Diagnóstico e Investigação Molecular, Laboratório de Biotecnologia, Universidade Estadual do Norte Fluminense, Campos dos Goytacazes, RJ, Brazil.

*alantardins@gmail.com

Keywords: Genomic imprinting; Tumor suppressor; Oncogenes

Genomic imprinting is an epigenetic mechanism that leads to the parent-of-origin- dependent monoallelic expression of a limited set of genes. Imprinted genes are implicated in a wide array of biological processes, required in embryo development to adulthood. Deregulation of the imprinted genes can cause several genetic diseases. The epigenetic marks associated with the monoallelic expression of imprinted genes can be altered by cancer. Only 2% (38/1890) of all oncogenes and tumor suppressor genes are known to be imprinted. The objectives of this study were to identify oncogenes and tumor suppressor genes that are subjected to monoallelic expression and to appraise the effect of cancer on the epigenetic profiles of candidate imprinted genes. We assessed, through computational meta-analysis, the allele-specific expression across single nucleotide polymorphism (SNPs) in 1890 known oncogenes and tumor suppressor genes using primary RNA-Seq data from 2015 experiments from 24 tissues from healthy individuals and 2424 experiments from 23 tumor tissues, and secondary data from the Genotype- Tissue Expression (GTEx) project, all from public repositories. We required ≥3 informative SNPs, with a coverage of ≥ 12 reads per SNP, with a strictly monoallelic profile in at least one tissue to call a hit. For the candidate imprinted genes, we integrated public data for the methylation statuses at CpG sites from healthy (n=6) and tumor tissues (n=10) profiled with the Infinium HumanMethylation450 bead array. We also examined the candidate imprinted genes for the enrichment of active and repressive histone modification marks by integrating with data from the RoadMap Epigenomics project. The mutation load in the candidate imprinted genes and the disease burden (tested as survival rates) were estimated from processed data available from the cBioPortal for Cancer Genomics. We identified two candidate hits with strictly monoallelic expression profile: the *CRNN* tumor suppressor in thyroid and the *DCD* oncogene in the esophagus. In the *DCD* oncogene, we detected a single differentially methylated CpG (cg01687040) site, being hypermethylated in the healthy esophagus, but hypomethylated in the esophageal tumor. *CRNN* and *DCD* contain each a reported candidate maternally methylated CpG (cg19370451 and cg25372195, respectively). There was the enrichment of the H3K36me3, H3K4me1, and H3k27ac activation marks only in the *CRNN* gene body in healthy esophagus samples. There is no reported accumulation of mutations in the *DCD* gene in esophageal tumors. In contrast, for the *CRNN* tumor suppressor gene, there is a high accumulation of mutations (gain, diploidization, and shallow deletions) reported in thyroid cancer. Patients with thyroid or esophagus tumor, who presented alterations in the *CRNN* tumor suppressor gene, had higher survival rates. Therefore, mutations in the *CRNN* may be protective effect.

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EFFECT OF COVERAGE DEPTH ON ALLELE FREQUENCY OF THE TAMBAQUI (*Colossoma macropomum*) GENOTYPED WITH GENOTYPING-BY-SEQUENCING (GBS)

Alex Alberto Vela Facundo[1]; José Dirley Mendes Alborado[1]; Vanessa Tourinho da Costa[1]; Gilvan da Costa Ramos[2]; José de Ribamar da Silva Nunes[1,2] *

[1]Instituto de Natureza e Cultura – Universidade Federal do Amazonas. [2]Programa de Pós-graduação em Ciência Animal – Universidade Federal do Amazonas.

*ribamarnunes@ufam.edu.br

Key-words: Segregation; Genotyping-by-sequencing; SNP coverage.

The tambaqui (Colossoma macropomum) is the largest native Characiforme species from the Amazon and Orinoco river basins, and is the most important native aquaculture species in Brazil. The next-generation sequencing (NGS) allowed a great advance in genomic and transcriptomic approaches, especially related to non-model species. The genotyping-by-sequencing (GBS) method is efficient for the discovery of tens of thousands of SNPs in the genome without a reference sequence. However, if the GBS experiment not be correctly carried out, the coverage will decrease. Low quality reads result in more reduced read counts consequently SNP coverage will decrease. Here, we identified a large number of SNPs from a full-sib family of 124 individuals and their parents using GBS. A total of 68,584 SNPs were initially identified using minimum MAF of 5%. To evaluate the GBS approach capability, as it is related to accurate determination of allelic frequencies, we compared the allelic frequency obtained in our genotyping with the expected for a diploid cross. We also analyzed allele frequency calls into population using different SNP coverage thresholds. The SNPs were placed into six groups depending on the coverage (5-10X, 10-20X, 20-40X, 40-80X, 80-160X, ≥160X). With the coverage ≥ 160X we observed three peaks of the allele frequency distribution for the SNPs. The first and third peak represents the crosses of AAxAa, with allele frequencies of 0.25 and 0.75, respectively, with 1:1 segregation of AA and Aa genotypes. The second peak represents the crosses of AaxAa, with allele frequency of 0.5 and 1:2:1 segregation of AA, Aa and aa genotypes. Lower coverage resulted in allelic frequency deviations from the expected. The results showed that higher SNP coverage results in an allele frequency distribution that is consistent with a diploid expected crossing. For instance, the coverage of ≥160X provided smaller levels of missing data and a higher percentage of called genotypes.

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GENOME-WIDE TRANSCRIPTOME SCAN FOR MONOALLELICALLY EXPRESSED lncRNAs ASSOCIATED WITH DIFFERENTIALLY METHYLATED REGIONS IN HUMANS

Amanda Pereira Vasconcelos[1*]; Cristina dos Santos Ferreira[1]; Juan Carlo Santos e Silva[1]; Enrique Medina-Acosta[1]

[1]Núcleo de Diagnóstico e Investigação Molecular, Laboratório de Biotecnologia, Universidade Estadual do Norte Fluminense Darcy Ribeiro – UENF

* amapereira19@gmail.com

Keywords: epigenomic; genomic imprinting; lncRNA

Genomic imprinting entails the parent-of-origin-dependent silencing of allele expression. The monoallelic expression of imprinted genes is regulated by epigenetic marks, which include differential DNA methylation of CpG islands, allele-specific enrichment of histone activation and repression marks, and long non-coding RNAs (lncRNAs). We previously identified 125 candidate differentially methylated regions (DMRs) in the human genome, which may be associated with monoallelic expression of genes. This study aimed to identify monoallelically expressed lncRNAs associated with the 125 DMRs. We carried a genome-wide transcriptome scan for allele-specific expression (ASE) for 7030 lncRNAs annotated at least 4 Mb around the candidate DMRs. We used in-house R scripts to integrate RNA-Seq secondary data for ASE across heterozygous single nucleotide polymorphisms (SNPs) from the Genotype-Tissue Expression (GTEx) project. We excluded from the analysis lncRNAs exhibiting ASE sites (expressed SNPs) that overlap segmental duplications, with allele frequency < 0.01 or with flanking sequences (14 nucleotides at each side) that were not unique in the genome. We found twenty-eight lncRNAs monoallelically expressed (≥ 3 informative eSNPs per lncRNA) in at least one tissue from at least two donors in the GTEx database. By cross-referencing with public data about biological processes, we selected the lncRNAs *LINC01138* (long intergenic non-coding RNA 01138 located on 1q21.2) and *LINC02470* (located on 12p13.31) for further analysis. *LINC01138* maps near the candidate DMR at the *PDIA3P* gene and it was monoallelically expressed in whole blood, esophagus and brain cortex. Its expression is reported to be linked to the oncogenic features and poor outcomes of hepatocellular carcinoma patients via interaction with arginine methyltransferase 5 (*PRMT5*). *LINC01138* is also known to accelerate tumor growth and invasion in gastric cancer by regulating miR-1273 and promotes the proliferation and inhibited apoptosis of prostate cancer. *LINC02470* maps near the candidate DMR of the *retro-PTMA,* and it was monoallelically expressed in whole blood, brain cortex, spinal cord, and putamen. Deregulation of its expression is reported to be linked to ovarian and lung cancers. However, its biological function is still unknown. The monoallelic expression of these two oncogenic lncRNAs suggests that they are subject to genomic imprinting.

Funding Agencies: CNPq, FAPERJ, UENF

DISTRIBUTION OF MERLIN TRANSPOSON ACROSS EUKARYOTIC GROUPS.

Ana Luisa Kalb Lopes[1]; Adriana Ludwig[2]; Marco Aurélio Krieger[1,2].

[1]Universidade Federal do Paraná, [2]Instituto Carlos Chagas, Fiocruz-PR, Curitiba.

Keywords: Merlin**;** DNA transposon; Phylogenetic analysis

Transposable elements (TEs) are defined as repeated DNA sequences that can move in the host genome. TEs exhibit a broad diversity in their structure and transposition mechanism and were classified into two classes according to these characteristics. Class II elements, or DNA transposons, move by "cut and paste" mechanism and are divided into seven main superfamilies: *Tc1/mariner, hAT, PiggyBac, Mutador, Merlin, Transib, P*. The Merlin superfamily was first described in 2004 and were detected in a wide range of animal genomes. However, in the last years, a massive number of new genomes have been sequenced, allowing the identification of transposable elements. Here, we described a global analysis of Merlin elements in Eukaryote. We performed a search for the Merlin elements deposited in the Repbase and selected the sequence MERLIN1SM of the planarian *Schmidtea mediterranea* to be used as a query in NCBI serverBLASTp searches against eukaryotes. A hit was considered significant when the e-value was lower than 10^{-4}. Moreover, these sequences were evaluated for the presence of conserved domains in CD-search from NCBI server. These sequences were used for multiple sequence alignment using ClustalW on the platform Galaxy. Phylogeny of the elements was inferred by the Neighbor-Joining method using the MEGA7 software and *Maximum likelihood* as implemented on CIPRES. We identified 31 Merlin sequences with DDE motif in Repbase. In Blast search, we find an additional of 273 sequences after filtering. Our results show a wide distribution of Merlin in eukaryotes, including animals, fungi, plants and some protozoa. In some species, Merlin was more representative like *Anncaliia algerae* (fungi) with 338 sequences, *Octopus bimaculoides* (octopus) with 199, *Trichuris suis* (nematode) with 104 and *Centruroides sculpturatus* (scorpion) with 54. Merlin elements were more widely distributed in animals with 91 species identified including 44 arthropods, 14 nematodes, 6 fish, 6 cnidarians, 4 molluscs, 3 annelids, 3 flat worms, 1 amphibian, 1 ascidian and 1 echinoderm. A single *Merlin* element was identified in plants, *Selaginella moellendorffii*. Also, we identified 7 species of fungus and 4 species of oomycetes. In the previous work, no Merlin element was identified in fungi and plants. The phylogenetic trees showed no resolution for the more basal relationships. Although we observed a more extensive distribution of Merlin than it was observed before, it is rather discontinuous. This may indicate the Merlin is an ancient element in eukaryotes with the possibility of past occurrence of horizontal transfer of the element between species of distinct groups and/or loss of the element in some lineages.

Funding Agency: CAPES, Fiocruz, UFPR

Aptamer-based serological detection of dengue virus

Amanda G. da Silva[1]; Raíssa M. Chini[1]; Luiz R. Goulart[2]; Adriana F. Neves[1*]

[1]Universidade Federal de Goiás, Laboratório de Biologia Molecular, Catalão - GO. [2]Universidade Federal de Uberlândia, Laboratório de Nanobiotecnologia, Uberlândia- MG.

*neves.af@gmail.com

Keyword: apta-RT-PCR, magnetic beads, oligonucleotide, serum samples, RNA.

The high incidence and mortality rates due to dengue fever in tropical and subtropical regions of the world require urgent interventions in health public programs with a more accurate laboratory surveillance. Current serological analyses may result in cross- reactions among other virus of the Flaviviridae family, especially Dengue and Zika, which leads not only to the failure of epidemiological surveillance, but also in appropriate measures to treat and prevent clinical outcomes. There is no definitive assay to detect dengue virus to date; however, nucleic acids aptamers have arisen as theranostic molecules with very high selectivity and specificity. We present in this investigation a novel aptamer-based RT-PCR detection assay for serological detection of dengue virus. The apta-RT-PCR consists of incubating serum samples with the aptamer APTA11 and its genetic variants APTA11-M2 and APTA11-M3. For the proof- of-concept, three positive serum samples with DENV-2 were evaluated, and paramagnetic microparticle alone were used as negative control. Biotinylated aptamers were immobilized onto paramagnetic particles complexed with streptavidin in a magnetic platform. The captured RNA from serum samples was eluted in ultrapure water treated with DEPC and subjected to RT using M-MLV enzyme at 37°C for one hour. The qPCR assays were performed in duplicates and were performed by using the cDNA of each sample and the 5X HOT FIREPol® EvaGreen® Mix plus (ROX) buffer. Our results demonstrated that all aptamers were able to capture the viral RNA from serum samples. The APTA11 showed the lowest Ct due to primer dimers as indicated by melting amplification curves. Agarose gel electrophoresis of apta-RT-qPCR amplicons evidenced by the superior performance of the APTA11-M3 variant. Briefly, we propose a novel Apta-RT-qPCR serological detection of dengue virus based on the APTA11-M3 probe, which can be used as a hybrid capturing system to improve viral RNA detection through RT-PCR.

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Melatonin action in modulating cell proliferation in cervical cancer

Barbara Maria Frigieri[1-2], Myllena Mayla Santos de Oliveira [2], Mayra Carolina da Silva Ferreira[2], Luana Pereira Cardoso[2], Flávia Cristina Rodrigues-Lisoni[2]

[1] São Paulo State University (UNESP), Institute of Biosciences, Humanities and Exact Science (IBILCE), Campus São José do Rio Preto, SP, Department of Biology.[2]São Paulo State University (UNESP), School of Engineering (FEIS), Campus Ilha Solteira, Department of Biology and Animal Science.

bafrigieri@live.com

Keywords: cervix cancer, cell culture, pineal gland, circadian rhythm.

Cervical carcinoma is the second most common form of neoplasm worldwide in women. Treatment for cervical cancer includes surgery, radiation therapy and chemotherapy, but researchers have been looking for new antitumor drugs to help with the effectiveness of chemotherapy treatment. Melatonin would be one of those drugs because it has antitumor activity through various mechanisms, including its antiproliferative and pro-apoptotic effects, as well as its potent pro-oxidant action in tumor cells. Melatonin is a natural hormone produced by the pineal gland, regulates the circadian rhythm, possessing immunomodulatory properties, anti-inflammatory, antioxidants and it is still important in actions of pathological processes, among them, cancer. The present work was proposed to evaluate its potential effect on morphology, proliferation and cytotoxicity, observing how it acts and how these alterations can participate in the tumorigenic process. For this, we treated the uterine cervix squamous carcinoma (SiHa) and adenocarcinoma (HeLa) cell lines. The melatonin was applied at the concentrations of 1mM and 100nM and the effects were observed at times of 4, 24, 48 and 72 hours. The melatonin did not alter the cellular morphology, diminished the cellular proliferation and was not considered cytotoxicity in both cell lines studied. Thus, melatonin acted in the inhibition of proliferation from mechanisms that may involve cell death, being a compound with antiproliferative potential and open new possibilities for cervical cancer therapy.

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MCR-1 GENE AND RESISTANCE TO COLISTINE IN ISOLATES OF *E. coli* OBTAINED OF PIG FROM PERNAMBUCO

Beatriz Nascimento de Araujo[1]; Ingrid Giovanna Vieira Santos[2]; João José de Simoni Gouveia[3]; Mateus Matiuzzi da Costa[3]; André de Souza Santos[4]; Rinaldo Aparecido Mota; Gisele Veneroni Gouveia[3*]

[1]Programa de Pós-graduação em Ciência Animal, Universidade Federal do Vale do São Francisco, Petrolina-PE, Brasil. [2] Ciências Biológicas, Universidade Federal do Vale do São Francisco, Petrolina-PE, Brasil. [3]Colegiado Acadêmico de Zootecnia, Universidade Federal do Vale do São Francisco, Petrolina-PE, Brasil. [4] Centro Universitário Brasileiro, Recife-PE, Brasil. [5]Departamento de Medicina Veterinária, Universidade Federal Rural de Pernambuco, Recife-PE, Brasil.

gisele.veneroni@univasf.edu.br

Key-words: mcr-1 gene; antimicrobial resistance; *E. coli*.

Among the diseases that cause losses to swine breeding are those caused by *Escherichia coli*. For a long time, the colistin antibiotic was used not only in the treatment of these diseases, but also as zootechnical additive with the aim of preventing infections and promoting animal growth. Cases of resistance to colistin have been reported in *E. coli* isolated from animals and humans, which causes a public health problem. Thus, the objective of the present study was to characterize phenotypically and genotypically the resistance to colistin of *E. coli* obtained of swine feces from Pernambuco, Brazil. The colistin resistance of 85 *E. coli* isolates was analyzed by the Mueller Hinton broth (MH) dilution method using the colistin breakpoint. Subsequently, PCRs were performed to verify the presence of the mcr-1 gene (marker of resistance to colistin presented in the literature) in the isolates. The Fisher's test was performed to verify the relationship of resistance to colistin with the presence of themcr-1 gene. The phenotypic tests showed that 36.05% of the *E. coli* isolates tested showed resistance to colistin. Thus, it was possible to observe the presence of resistant isolates even though the use of colistin in Brazil is prohibited. In the present study it was also found that 36.05% (n = 31) of the *E. coli* strains had the mcr-1 gene. However, among the isolates that had the mcr-1 gene, 24.42% were sensitive to the action of colistin and only 11.63% were resistant to the action of the antibiotic evaluated. The results of Fisher's exact test did not demonstrate a relationship of resistance to colistin with the presence of the mcr-1 gene. These results suggest that other genes may be involved in this resistance phenomenon or that polymorphisms in the mcr-1 gene may lead to altered of the encoded protein.

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GENOTÓXIC ANALYSIS OF FISH SAMPLES EXPOSED TO CHEMICAL REJECTING LEAKS OF HYDRO ALUNORTE MINING MACHINE IN THE MUNICIPALITY OF BARCARENA – PA

Benilson Silva Rodigues[1]; Paula Sabrina Bronze Campos[2]; Kayury Serrão da Silva[3]

[1,2,3] Instituto Federal de Educação, Ciência e Tecnologia do Pará – IFPA – *Campus* Abaetetuba, Pará, Brazil.

benilson.rodrigues@ifpa.edu.br

Key - words: Micronucleus test; mutagenesis; genotoxic agents.

Increasing urbanization and industrialization have intensified water contamination and become a concern worldwide. In February 2018, in the municipality of Barcarena - PA, a leak of the bauxite tailings dam from Hydro Alunorte occurred, and the analysis of the water of the rivers affected revealed high levels of lead, nitrate, sodium and aluminum. Therefore, the objective of this work was to verify the existence of micronuclei in fish samples as a bioindicator of the presence of genotoxic agents in rivers affected by the tailings of the mining company Hydro Alunorte that could jeopardize the health of the local population. Thus, fish belonging to the species *Hoplerythrinus unitaeniatus* and *Astyanax bimaculatus* were collected in the Murucupi river, which was reached by the chemical rejects, in the city of Barcarena - PA, and samples of the species *Pimelodus maculatus* were also used in the Jarumã river, which was not apparently affected by chemical rejects , in the neighboring municipality of Abaetetuba - PA to allow the comparison of the results of the micronucleus test between different areas. For the micronucleus test (MN) fish blood samples were used and after this procedure the fish were released in the wild. The preparation and analysis of the blood followed the methodology proposed by Ranzani-Paiva et al. (2013), and MN analyzes were performed according to Fenech (2000) and Salvadori (2003), with 3,000 erythrocytes analyzed per animal. Statistical analysis of the results was done using the non- parametric Mann-Whitney test ($p < 0.05$) using the BioEstat 5.0 program. The results showed that the species *A. bimaculatus*, *H. unitaeniatus* and *P. maculatus* presented 0.16%; 0.1% and 0.16% of the total cells analyzed with MN. When comparing the *P. maculatus* species of Abaetetuba with the species of the city of Barcarena, it is observed that the number of micronuclei of *P. maculatus* is equal to that of *A. bimaculatus* and greater than that of *H. unitaeniatus*, indicating that the species of fish that were analyzed in this research showed similar levels of damage to their genetic material, possibly by some genotoxic agent, even though they were captured in rivers of different cities. Statistical analysis revealed a $P > 0.05$ (0.12) also indicating that there is no significant statistical difference between the micronuclei results of fish samples from both cities. These results also suggest that probably some genotoxic agent may have reached the Jarumã river and caused damage to aquatic ecosystems, similar to that observed in the Murucupi river in the city of Barcarena, and it is also necessary to investigate water quality in the Jarumã river. In addition, people living near these rivers should avoid the consumption of fish removed from these waters to avoid diseases caused by these polluting agents.

Funding Agency: IFPA.

ENGINEERING *Pantoea agglomerans* 33.1 TO KNOCK-DOW RNASE III ACTIVITY BY GENE EDITING

Bruna Garbatti Factor[1]; Paul Dyson[2]; Maria Carolina Quecine Verdi[1], Antonio Figueira[3]

[1]Departamento de Genética e Melhoramento de Plantas, Universidade de São Paulo - Escola Superior de Agricultura "Luiz de Queiroz", Piracicaba, Brazil. [2]Institute of Life Science - Swansea University, Swansea, Wales. [3]Laboratório de Melhoramento de Plantas, Universidade de São Paulo - Centro de Energia Nuclear na Agricultura, Piracicaba, Brazil.

bruna.factor@usp.br

Key words: gene editing; *Pantoea agglomerans* 33.1; RNase III.

RNase III, encoded by the *rnc* gene, plays a crucial role in processing rRNA and other types of RNA, and it is characterized by the ability to bind to double stranded RNAs (dsRNAs). Strains of *Escherichia coli* with *rnc* deleted have been reported, but attempts to disrupt the *Bacillus subtilis rnc* gene, for example, were not successful. Thus, RNase III is considered to be non-essential for some bacteria, but essential for others, such as *B. subtilis*. However, it is not possible to predict the essentiality of RNAse III to other bacteria species. *Pantoea agglomerans* is a Gram-negative bacterium from the Enterobacteriaceae group, and it is frequently found associated with a wide range of host plant species as an endophyte, colonizing leaves, stems and roots. This colonization brings benefits to their plant hosts, since they contribute to the promotion of plant growth either through the production of indole-acetic acid growth hormone (IAA), phosphate solubilization or nitrogen fixation. Here, we identified and characterized the RNase III *rnc* gene of the *P. agglomerans* strain 33.1 originally isolated from *Eucalyptus grandis* but also founded in sugarcane (*Saccharum* sp). Subsequently, we edited the *rnc* gene by λ- red homologous recombination to insert the kanamycin resistance gene (*kanR*) into de *P. agglomerans* 33.1 *rnc*. Transformants of *P. agglomerans* 33.1 cells were successfully obtained with the edited *rnc*. We showed that this knock-down bacterium is viable and able to colonize plant tissues as an endophyte.

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New report of the PcP190 SATELLITE DNA in *Cycloramphus bolitoglossus* (ANURA, CYCLORAMPHIDAE) BROAD THE OCURRENCE OF THIS REPETITIVE SEQUENCE IN ANURA GENOME

Gislayne de Paula Bueno[1*]; Kaleb Pretto Gatto[2]; Daniel Pacheco Bruschi[3];

[1] Universidade Federal do Paraná, Curitiba, Brazil. [2]Departamento de Zoologia,Instituto de Biociências, Universidade Estadual Paulista, Rio Claro, Brazil. [3]Departamento de Genética, Universidade Federal do Paraná, Curitiba, Brazil.

gislayne.bueno@gmail.com

Key-words: Satellite DNA ; molecular evolution; amphibia.

Satellite DNA (satDNA) are abundant repetitive DNA component in the eukaryote genome and a number of studies have evidenced the active role in the karyotype diversification. The PcP190 sequence is a satDNA derived from 5S ribosomal DNA and have been found in Anura genomes of the representatives of Hylidae, Hylodidae and Leptodactylidae families. PcP190 is also associated with sex chromosomes evolutionin the genus *Pseudis*. Here, we investigate the occurrence of the PcP190 satDNA in genome of the *Cycloramphus bolitoglossus,* an endemic specie from Atlantic Forest in Brazil. The satDNA PcP190 was isolated by PCR from *C. bolitoglossus* genomic DNA, and amplicons showed the size of 150 to 700 bp. The nine clone sequences included partial PCP190 monomer (198 pb) and fragments includes trimers monomers (607 pb). Ten complete monomers showed high similarity each other (95.68%), recovered two distinct regions (conserved and hypervariable regions) within each monomers, defined by sequence variations. The PcP190 sequences from *C. bolitoglossus* showed highest similarity with the firsts 120bp from other anurans PcP190 sequences, corroborated with conserved domain already related for this satDNA family. Additionally, a hypervariable region have been recognize within the PCP190 monomer, with species-specific pattern among species. For this, we suggest that the PcP190 from *C. bolitoglossus* constitutes a new sequence group of this satDNA family. This first report of this satDNA in Cycloramphidae family broad the occurrence of this satDNA family in Anuran and open the new perspectives about their evolutionary maintenance in genomes and, for consequence the role in this satDNA in the Anura genomes.

A PHYSICAL BARRIER (PCH's) AND ITS INTERFERENCE IN A POPULATION DIVERSITY OF Astyanax altiparanae

Caio Felipe da Silva[1]; Fernanda Dotti do Prado[1,2]; Ricardo Utsunomia[1]; Diogo Teruo Hashimoto[3]; Sérgio Ricardo Bautloni[3]; Fausto Foresti[1]; Fábio Porto-Foresti[1,2*]

[1]Universidade Estadual Paulista (Bauru), Faculdade de Ciências de Bauru. [2]Universidade Estadual Paulista (Botucatu), Instituto de Biociências de Botucatu. [3]Centro de Aquicultura da Unesp (CAUNESP, Jaboticabal, São Paulo).

Key-words: neotropical fish; microsatellite markers; dams

Astyanax altiparanae, popularly known as "lambari-do-rabo-amarelo", has ecological and economical importance and is also used as bait for sport fishing. This species is widely distributed through Sapucaí-Mirim river, tributary of the Upper Paraná River basin, which has numerous dams for energy production. Considering that the consequences of dam constructions on the levels of species diversity remains unclear, microsatellites markers can be a valuable tool to analyze genetic variability with applications to conservation and population genetics. Therefore, this study aimed to analyze the genetic diversity of *A. altiparanae* using microsatellite. For this purpose, 270 individuals of *A. altiparanae* were collected in the reservoir of a small hydropower plant of Palmeiras, located northwest of the São Paulo, Brazil, over four years (2015, 2016, 2018, 2019). Genomic DNA was extracted and their quality was checked in 1% agarose gel. Seven microsatellite loci were genotyped using the ABI3730 system. The Cervus 3.0 software was used to analyze the total number of alleles (*A*), expected heterozygosity (H_E) and observed heterozygosity (H_O). Statistical analysis was performed using the Hardy- Weinberg Equilibrium (HWE) and the fixation index (F_{IS}) was tested in Genepop 4.0. Null alleles were detected using the software Microchecker 2.2. The mean allele per locus ranged from 16 to 25.27, the observed heterozygosity from 0.686 to 0.771 and the expected heterozygosity from 0.826 to 0.892, showing a high genetic diversity through the four years, corroborating with results found in the literature in populations of the same species in rivers of the Upper Paraná basin. The average values of fixation index (F_{IS}) were positive and similar during the four years. Besides, approximate values of expected heterozygosity, observed heterozygosity and fixation index were also very similar to those found in the literature. The high genetic diversity of *A. altiparanae* in the Palmeiras reservoir over the four years was expected due to its recent construction and supportive breeding practices that has been carried out since 2013, besides the *A. altiparanae* ability to adapt to different environments. In general, we conclude that this study will serve as a basis for a continuous monitoring of the diversity of this species, since the monitoring studies in reservoirs are scarce.

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GENETIC STUDY OF POSSIBLE HYBRIDS OF *LEONTOPITHECUS ROSALIA* AND *LEONTOPITHECUS CHRYSOMELAS*

Camila Bueno Rodrigues[1]; Paola A. Ayala-Burbano[1]; Patrícia Domingues de Freitas[1]; Cecília Kiefulff[2].

[1]Departamento de Genética e Evolução, Universidade Federal de São Carlos, São Carlos SP, Brazil; [2]Instituto Pri-Matas para a Conservação da Biodiversidade, Belo Horizonte MG, Brazil.

*camilabuenorodrigues@gmail.com

Key-words: *Leontopithecus chrysomelas; Leontopithecus rosalia;* hybridization.

The hybridization process involves the crossbreeding between individuals of genetically distinct populations or species, which may or may not harm the parental species. In this sense, hybridization may increase genetic diversity, leading to the emergence of new lineages or so resulting in loss of one or both parental species. In nature, this process occurs in zones that allow the encounter and crossing between these individuals. However, anthropic actions may result in the formation of artificial hybrid zones, as occurred in the city of Niterói (RJ), where two couples of *Leontopithecus chrysomelas* and one of *Leontopithecus rosalia* were released in 1994. The genus *Leontopithecus* includes four allopathic species that are distributed in different areas of the Brazilian Atlantic Forest, and natural contact between them is not common. Due to favorable conditions at the release site, where these couples were introduced, the population expanded to about 1000 individuals. However, the phenotype of the animals born during the population expansion period did not allow an accurate diagnosis of the species involved to generate the successive offspring. Thus, it is possible the existence of hybrids originated from these unknown crosses. Once some individuals from this Niteroi area have been translocated to Bahia, occurrence area of *L. chrysomelas*, it is urgent the investigation about genetic contribution to form such population. Thus, the present work aims to perform a genetic analysis, using sex-specific genetic markers, in order to verify the presence of hybrids in this population. To date, we performed preliminary analyzes with mtDNA cytochrome oxidase I (COI) gene in DNA samples from Niterói individuals, from *L. chrysomelas* and from *L. rosalia*, being the last ones representatives of possible parental species. The multiple alignment and the editing of the sequences were implemented in the Geneoius, using the ClustalW tool, and in the BioEdit software, respectively. The distance analysis was performed in the Mega 6 program, based on the Kimura 2-parameter model (K2P). A Neighbor-Joining (NJ) topology was constructed under this same model, graphing the divergences between pure species and potential hybrids. As results, we obtained sequences of 573 bp with 14 variable sites. The cladogram allowed to conclude that the only maternal contribution was from *L. chrysomelas*. Now we are analyzing the paternal contribution through SRY gene of the Y chromosome, which will allow us to identify if there are individuals with different parental species, confirming the existence of hybrids.

Molecular Study of a Family with Usher Syndrome and Otosclerosis

Camila Cristina Avila Martins[1]; Uirá Souto Melo[1]; Guilherme Lopes Yamamoto[1]; Silvia Souza Costa[1]; Regina Célia Mingroni-Netto[1]

[1]Departamento de Genética e Biologia Evolutiva, Instituto de Biociências / Universidade de São Paulo, Brazil.

camila.cristina.martins@usp.br

Keywords: Usher Syndrome; Otosclerosis; Genetics.

We have ascertained in Laboratório de Genética Humana, IB-USP, a family with three individuals presenting sensorineural hearing loss associated to retinitis pigmentosa, thus characterizing Usher syndrome (US, OMIM #276900), and one affected individual with hearing loss but presenting clinical features of otosclerosis (OTSC, OMIM #166800). Otosclerosis is characterized by abnormal bone remodeling in the otic capsule of middle ear, which leads to conductive hearing loss. When it has a genetic cause, it presents autosomal dominant inheritance. Using massively parallel DNA sequencing of a standardized panel of 98 deafness genes, it was detected, in the sample from the proband with US, one mutation in the *USH2A* gene (USH2A:NM_007123:exon7:c.1214delA:p.N405fs). The mutation, found in only one of the alleles, does not explain US, an autosomal recessive condition. The purpose of this study was the molecular investigation of the family in order to find the causative mutation(s) that explains the Usher syndrome and the otosclerosis phenotypes. Array- CGH, made through the "*CytosureTM, ISCA v2 array 4X180K*" (OGT©, Oxfordshire, UK) platform, containing approximately 180,000 oligonucleotides, allowed to eliminate the hypothesis of duplications or deletions in Usher syndrome related genes in the proband. Massively parallel sequencing of the exome in the HiSeq2500 equipment (Illumina, San Diego, California, USA) with the reagents present in the kit "*HiSeq v2 Reagent Kit*" (Illumina, San Diego, California, USA) was performed with samples from three individuals. The exome capture was performed through kit "*Sure Select QXT Target Enrichment*" (Agilent Technologies, Santa Clara, California). We performed variant filtering from the Annotated VCF computational file, using the computational pathogenicity prediction packages Polyphen, SIFT and Mutation Taster, and the frequency data of the variants in four main genomic databases: Exome Aggregation Consortium (ExAC), NHLBI Exome Sequencing Project (ESP), 1000 Genomes Project and Brazilian Online Archive of Brazilian Mutations (ABraOM). After filtering variants detected through exome sequence, we investigated the segregation of different candidate variants in *USH2A*, *DIAPH1* and *MYH9* genes by Sanger sequencing in samples from the remaining individuals, but none of the selected variants explained the occurrence of US in the family. The search for candidate variants to explain the OTSC in the affected individual was performed on the *MEPE*, *TGFB1* and *SERPINF1* genes, also with Sanger sequencing. To date, no relevant candidate variant has been found to explain OTSC. Although we have not been able to find the molecular causes of the phenotypes observed in the family, our results strongly indicate that there are unknown genes related to the Usher syndrome yet to be described.

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FOLIC ACID ON OOCYTE MATURATION ALTERS PRODUCTION RATE AND GENE EXPRESSION OF *IN VITRO* PRODUCED BOVINE EMBRYOS

Carolina G. Verruma[1]; Cristiana L. Miranda-Furtado[2]; Marcia D. Hayaxibara[3]; Reginaldo A. Vila[1]; Ester S. Ramos[1]; Raysildo B. Lôbo[1]

[1]Departamento de Genética, Faculdade de Medicina de Ribeirão Preto, Universidade de São Paulo, Ribeirão Preto, SP, Brasil. [2]Núcleo de Pesquisa e Desenvolvimento de Medicamento, Universidade Federal do Ceará, Fortaleza, CE, Brasil. [3]Serviço de Genética Médica do Hospital das Clínicas de Ribeirão Preto, Universidade de São Paulo, Ribeirão Preto, SP, Brasil.

verruma.cg@usp.br

Key-words: Bovine embryo; Epigenetics; Gene expression.

Assisted reproductive technologies (ARTs) are often associated to epigenetics errors during embryo development and this may lead to abnormal gene expression causing, in humans, imprinted syndromes like Prader-Willy and Beckwith-Wiedemann syndromes, and the Large offspring syndrome, in bovines. Besides the components present in ARTs stages, the morphology and quality of oocytes are important to embryo success rate. Folic acid (FA) is a cofactor that acts as methyl donor, and its supplementation during oocyte maturation may influence epigenetic mechanisms, influencing bovine oocyte maturation, embryo production and gene expression. Our study aimed to add FA on oocyte *in vitro* maturation and to analyze its influence on embryo *in vitro* production and expression of the *Long intronic transcript 1* (*LIT1*) and *Insulin-like growth factor 2* (*IGF2*) genes. Different FA concentrations [0µM (FA-0), 10µM (FA-10), 30µM (FA-30) and 100µM (FA-100)] were added on maturation medium of 2159 bovine oocytes (classified as GI and GIII). On average, 720 oocytes (biological triplicates) were used in each FA treatment for embryo *in vitro* production. The embryo production and gene expression, from each treatment, was evaluated on R software using the Student *t*-test. Moderate doses of FA (FA-30) improved embryo production from GIII oocytes (p=0.012), but did not influence embryo production from GI oocytes, while high FA doses (FA-100) decreased embryo production from GI oocytes (p=0.010) but did not influence embryos from GIII oocytes. In embryos derived from GIII oocytes, 10µM of FA (FA-10) reduced *LIT1* gene expression (p=0.004). The expression of the *IGF2* gene was detected only on embryos derived from GI (FA-0, FA-10 and FA-30) and GIII (FA-0) oocytes and there was no statistical difference. In conclusion, FA had different effects on bovine embryo production and gene expression, depending on the oocyte quality and FA concentration. These results may contribute to understanding on how FA acts and may influence epigenetic mechanisms, reinforcing the hypothesis that components stored by the oocyte during the maturation period are important to the preimplantation embryo development.

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SEX-BIASED TRANSCRIPTOME PROFILE IN THE FISH *Megaleporinus macrocephalus* (CHARACIFORMES: ANOSTOMIDAE)

Carolina Heloisa de Souza Borges[1]; Lieschen Valeria Guerra Lira[1]; Arno Juliano Butzge[2]; Vito Antonio Mastrochirico Filho[1]; Raquel Belini Ariede[1]; Milena Vieira de Freitas[1]; Ricardo Sohei Hattori[3]; Ricardo Utsunomia[4]; Diogo Teruo Hashimoto[1]

[1]Centro de Aquicultura, Universidade Estadual Paulista, Jaboticabal, Brazil. [2]Instituto de Biociências, Universidade Estadual Paulista, Botucatu, Brazil. [3]Department of Marine Biosciences, Tokyo University of Marine Science and Technology, Tokyo, Japan.[4]Departamento de Ciências Biológicas, Universidade Estadual Paulista, Bauru, Brazil.

* chs.borges@unesp.br

Key-words: sexual determination; transcriptome; *Dmrt1*

Unlike other vertebrates, teleost fish show a variety of sex determination mechanisms, even in closely related species (*e.g.* medaka species). To date, seven sex-determining (SD) master genes have been identified in fish: *Dmy*, *Dmrt1*, *GsdfY*, *Sox3*, *Amhy*, *Gdf6Y*, and *SdY*. In the present work, we investigated the transcriptomic profile of males (ZZ), females (ZW), and sex-reversed individuals (phenotypic males ZW) of *Megaleporinus macrocephalus*, a teleost fish with heteromorphic sex-linked chromosomes ZZ/ZW. Briefly, treated fish were fed with 17ĸ- methyltestosterone up to 150 days after hatching (dah) (previously determined period of sex differentiation in this species). All fish were euthanized and the two gonads (one used for histology and the other for RNA-seq) and kidney were dissected immediately. The genotypic and phenotypic sex was accessed through cytogenetic and gonadal histology, respectively. After sex identification, samples were clustered in three replicates of four pools of gonads (10 gonads/pool) for RNA extraction: ZZ control males, ZW control females, ZZ treated males, ZW treated males (neomales). RNA-seq was performed in Illumina HiSeq 2500 platform. After trimming adapters and poor-quality reads, the transcriptome was assembled *de novo* with Trinity package (parameters: no normalize reads and min. contig length=200). The assembly presented 211.536 transcripts, largest length=17.917 bp, mean length=819 bp, and N50=1.611 bp. Functional annotation was conducted through BLAST similarity search against UniProtKB/SwissProt/PFAM database (*e*-value threshold 10^{-3}). Differential expression levels in each pool and downstream analysis was performed with DESeq2. Principal component analysis (PCA) plot obtained for the global gene expression pattern showed the formation of two separate clusters: the phenotypic males, regardless of genotypic sex (ZZ control males, ZZ treated males, ZW treated males), and females (ZW control females), highlighting different transcriptomic profiles of phenotypic males and females. Neomales presented similar males ZZ transcriptome profile. The heatmap plot analysis of SD related genes displayed *Foxl2*, *Sox3*, *Gtsf1* and *Zar1* upregulated in ZW control females. Also, a consistent *Dmrt1* up- regulation was found in ZZ control males, but not in other phenotypic males. This may be due to the more advanced stage of development observed in treated fish gonads, probably lacking *Dmrt1* overexpression. *Dmrt1* has been pointed out as a master male SD gene in different species of birds, frog, and fish (half-smooth tongue sole), species that also exhibits ZZ/ZW SD system. Our findings suggest that *Dmrt1, Foxl2*, *Sox3*, *Gtsf1*, and *Zar1* could play a critical role in *M. macrocephalus* sex determination and differentiation.

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MODEL BASED SPECIES DELIMITATION AS A TOOL TO IDENTIFY UNDESCRIBED ANURANS IN SOUTHERN ATLANTIC FOREST

Caroline Batistim Oswald[1*]; Rafael Félix de Magalhães[1]; Fabrício Rodrigues dos Santos[1]; Paulo Christiano de Anchietta Garcia[1]; Selvino Neckel-Oliveira[2]

[1]Universidade Federal de Minas Gerais. [2]Universidade Federal de Santa Catarina.

* carolbatistim@gmail.com

Palavras-chave: Evolutionarily significant units (ESU); *Ischnocnema*; threatened anuran

The delimitation of taxonomic units is an important theme for biodiversity conservation, influencing efforts on preserving species. Cryptic species are morphologically indistinguishable and have the potential to affect human interpretations,. Anurans seem to show high cryptic diversity, and phylogeographical methods could help to identify new taxa. Indeed, model-based species delimitation can provide important results for systematics and conservation, increasing precision and replicability in taxonomical decisions. The threatened leaf-litter frog *Ischnocnema manezinho* is a species known only from Santa Catarina Island, southern Brazil, its type-locality. However, some studies have recorded new continental populations that are morphologically similar to it, which would likely chance its conservation status in red list if it were confirmed. We want to define the genealogical and geographical limits of *Ischnocnema manezinho,* The specific goals of our study were to: (i) delimit the lineages of *I. manezinho* above the species level, (ii) determine the genealogical relationships between the delimitated lineages, and (iii) evaluate the impact of the results in the categorization of *I. manezinho* in red lists, suggesting conservation and taxonomic research priorities. The delimitation analyses were done in two steps, first the discovery and then the validation methods. The final scheme was obtained through the strict consensus between all methods. Two independent methods were applied in the first step, one for mitochondrial and other for four nuclear genes, independently. The mitochondrial discovery was made using a Generalized Mixed Yule Coalescent model and the nuclear discovery was done through the haplowebs approach, aiming search for groups of individuals that form reciprocally exclusive allelic pools. The validation step was implemented using all markers through the Bayesian Phylogenetics and Phylogeography method. Lastly, to estimate most recent common ancestors of the candidate species obtained through the consensus, a dated species tree was generated in StarBEAST2. Our results reveal five entities, including *I. manezinho*, which is restricted to Santa Catarina Island and four unconfirmed candidate species, that ramain as undescribed cryptic species. The area of occupancy and the extent of occurrence of *I. manezinho* are both smaller than 500km^2. Aggravatingly, the species is distributed in isolated fragments of forest surrounded by urban areas. The status of *Ischnocnema manezinho* should be re-evaluated, with our results indicating that it is Endangered. Similar results of cryptic diversification were observed for several species of anurans, including several from the same genus of our object of study. This indicates that many widely distributed species may be a mosaic of undescribed taxa. Therefore, model-based taxonomy could help to discover and preserve them, as it is a powerful tool to improve the discovery and conservation of cryptic species. This approach should be applied for any other cryptic species complexes with the aim of emphasizing the reproductive isolation.

DUPLICATION OF 11P15.1P11.2 IN A MALE WITH SEVERE INTELECTUAL DISABILITY AND DYSMORPHIC FEATURES

Mariana Luiza Junta Ferro [1*]; Marina Candido Visontai Cormedi [1]; Julia Teixeira Luitti[1], Thaliane Buranello[1], Luissa Hikari Hayashi Araujo[1], Marcelo Szeremeta Ayres Correia[1], Lisandra Mesquita Batista[1], João Monteiro de Pina Neto[1]

[1]Serviço de Genética Médica, Hospital das Clínicas da Faculdade de Medicina de Ribeirão Preto - Universidade de São Paulo, Ribeirão Preto/SP, Brasil

*marina_ferro92@hotmail.com

Key-words: Chromosomal Duplication; Mental Retardation; Developmental Disability

Chromossomal abnormalities are a relevant cause of severe intelectual disability. Nevertheless, duplication of chromosome 11p is very rarely reported in the literature. Our aim was to describe the phenotype of a chromosome 11p duplication not previously reported. Karyotype study was performed for the proband and her mother from blood samples. Array comparative genomic hybridization (arrayCGH) for the proband. We report a case of a 19-years-old male with a history of developmental delay that evolved into a serious mental disability. He presented with developmental delay, cryptorchidism, umbilical hernia and bilateral inguinal hernia and important dysmorphism features such as dolichocephaly, down-slanting palpebral fissures, synophrys, hypertelorism, telecanthus, broad nasal bridge, prominent ears, clinodactyly of fifth fingers, sacral sulcus. He also presented 2 seizures at ages 3 and 4 years. He was the first child of healthy unrelated parents and had no relevant family history. Karyotype study resulted 46, XY, add (11) (p15) and arrayCGH revealed a chromosome gain of 11p15.1p11.2. The mother's karyotype study resulted normal and the father was not available to be tested and therefore it was not possible to determine whether this was a de novo or a familial abnormality. To the best of our knowledge this is the first report duplication of chromosome11p15.1p11.2. Excluding reports of duplications of 11p15.5 which are associated with well described syndromes, literature shows that 11p duplications phenotype is very variable. Descriptions included epilepsy, mental retardation, eye disorders and various dysmorphic features. We present the clinical features of a male with duplication of 11p15.1p11 which had never been reported. We believe this case may contribute to better understand the phenotype associated with this chromosomal abnormality.

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FAMILIAL CHROMOSOME 1P32.2P22.2 DUPLICATION IN A FEMALE WITH GROWTH RETARDATION, DEVELOPMENTAL DELAY AND DYSMORPHIC FEATURES

Marina Candido Visontai Cormedi[1*]; Mariana Luiza Junta Ferro[1]; Julia Teixeira Luitti[1], Thaliane Buranello[1], Luissa Hikari Hayashi Araujo[1], Marcelo Szeremeta Ayres Correia[1], Victor Evangelista de Faria Ferraz[1], Jair Huber[1]

[1]Serviço de Genética Médica, Hospital das Clínicas da Faculdade de Medicina de Ribeirão Preto - Universidade de São Paulo, Ribeirão Preto/SP, Brasil

* marinacormedi@gmail.com

Key words: Chromosomal Duplication; Growth Disorder; Developmental Disability

Duplication of chromosome 1p is rarely reported in literature and its phenotype is largely diverse. We aim to describe clinical features of a chromosome 1p duplication not previously reported. Karyotype study was performed for the proband and her parents from blood samples. High Density SNP Array was performed for the proband using CytoSNP - 850K BeadChip (Illumina Technologies). We report a two-years-old female who was the first child of unrelated parents presenting with growth retardation, developmental delay, microcephaly, hearing loss and dysmorphic features (hypertelorism, epicanthal folds, bilateral fifth finger clinodactyly). She was delivered by cesarean section due to acute fetal distress. She was born with low birth weight and was small for gestational age. She had an altered hearing test at birth. The mother had personal history of three abortions in the first trimester of pregnancy and no other relevant familial history was informed. At age 2 years a genetic consultation was performed. Karyotype study resulted 46,XX,ins(7)(q11.2) and High Density SNP Array revealed a duplicated chromosomal segment on 1p32.2p22.2. Karyotype study of the mother resulted 46,XX,ins(7;1)(q21.2;p32.2p22.2) demonstrating the abnormal chromosome of the proband was inherited from her. Father's karyotype study was normal. To the best of our knowledge this is the first report duplication of chromosome 1p32.2p22.2. Other reports of 1p duplications include descriptions of the following characteristics: growth retardation, microcephaly, males with sex differentiation disorders, developmental delay, cardiac defects, and various dysmorphic features. We described clinical characteristics of a two-years-old girl with a maternally inherited duplication of chromosome 1p32.2p22.2 which had never been reported. We believe this case may contribute to better understand the phenotype associated with this chromosomal abnormality.

TEMPORAL STUDY OF THE MICROBIAL COMMUNITY FLOW AMONG INTERNAL AND EXTERNAL HOSPITAL ENVIRONMENTS

Catarina dos Santos Gomes[1], Marlon Fortes Rocha[1], Mirian Felix de Carvalho[1], Leticia Andrade Costa[1], Giovanna Orlovski Nogueira[1], Julia Roberta Degrande Machado[1], Naira Lopes Bibo[1], Juliana Reis Souza[1], Ana Clara Lagazzi Cressoni[1], Carolinne Tomarchio Fogagnolo[1], Gabriel Servilha Menezes[1], Yurie Sato[1], Arthur Alves Coelho[1], Brenda Cristina Vitti, Nubia Sabrina Martins[1], Debora Akemi Endo Colodete[1], Larissa Pinto de Andrade[1], Carolina Buzzulini[1], Gabriel Martins da Costa Manso[1], Giseli Furlan Corrêa[1], Daniel Rodrigues[1], Andressa Mayara dos Santos[1], Bruna Felippe Ferreira[1], Gilberto Gambero Gaspar[3], Maria Fernanda Zaneli Campanari[6], Rafael Correa da Silva[6], Daniel Guariz Pinheiro[6], Emmanuel Dias-Neto[7], Valdes Bollela Roberto[3], Victoria Simionatto Zucherato[1], João Mesquita Luiz, Vanessa da Silva Silveira[2], Aparecida Maria Fontes[2], Nilce Maria Martinez-Rossi[2], Wilson Araújo Silva Jr[2,4,5]

1 Biomedical Sciences - RCB0300 Biotechnology III, Ribeirão Preto Medical School, University of São Paulo, Brazil; 2 Department of Genetics at Ribeirão Preto Medical School, University of São Paulo, Brazil; 3 Department of Internal Medicine, Ribeirão Preto Medical School, University of São Paulo, Brazil; 4 Center for Medical Genomics at Clinics Hospital of Ribeirão Preto Medical School, University of São Paulo, Brazil; 5 Regional Blood Center at Clinics Hospital of Ribeirão Preto Medical School, University of São Paulo, Brazil; 6 Department of Technology, School of Agricultural and Veterinarian Sciences, São Paulo State University (UNESP), Sao Paulo, Brazil; 7 International Research Center, A.C.Camargo Cancer Center, Lab. of Medical Genomics, São Paulo, São Paulo, Brazil.

Keywords: Metagenomics, Hospital Microbiome, hospital-acquired infection

The microbiome refers to the microbiota and the collective genetic information those organisms carry. The investigation of the microbiome composition and the screening of different areas inside and outside the hospital can result in important implication for the management of practices to control hospital-associated infections. Moreover, the comparative study of the phylogenetic diversity of microbial communities in 2018 and 2019 will provide information for exploring whether or not gradually changes in abundances occurs over this period. In this study, we collected samples from 24 different places each year (11 external and 13 internal surface areas of the University Hospital (HC). After extraction of all DNA from every place we assessed bacterial diversity by sequencing 16S rRNA genes. The vast majority of the annotated sequences, 87%, comprise three phyla: Actinobacteria, Firmicutes, and Proteobacteria, which had the same distribution in the two years of study. However, distinct frequencies of some phyla were found when we compared the inside and outside areas in both years. In 2018, we observed a predominance of the phyla Actinobacteria in outside HC area (36%) while in 2019 we found the predominance of Proteobacteria (36%). This last one, the results obtained for the internal area show that after one year, the abundance of proteobacteria increased from 17% to 28%. Alpha and beta diversity analysis related to genus and species are being investigated to discriminate these bacterial communities better. In summary, the results of this study may aid in the development of hospital infection reduction programs, based on information on the dynamics of pathogen abundance over the years.

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GLOBAL EXPRESSION PROFILE of microRNAs in DIABETES MELLITUS TYPE 1

Lucas Bezerra[1,2]; Cintia Silva[1,2]; João Soares Felício[3,4]; Natércia Marques[4], Tatiana Vinasco[1]; Arthur Santos[1]; Adenilson Pereira[1]; Amanda Vidal[1,2];Ândrea Ribeiro-dos-Santos[1,2,3]

1Laboratório de Genética Humana e Médica, Instituto de Ciências Biológicas, Universidade Federal do Pará, Belém-PA, Brasil. 2Programa de Pós-Graduação em Genética e Biologia Molecular, Instituto de Ciências Biológicas, Universidade Federal do Pará, Belém-PA, Brasil. 3Programa de Pós-Graduação em Oncologia e Ciências Médicas, Núcleo de Pesquisas em Oncologia; Universidade Federal do Pará, Belém-PA, Brasil. 4Unidade de Endocrinologia e Metabologia/Diabetes, Hospital Universitário João de Barros Barreto, Universidade Federal do Pará, Belém-PA, Belém, Brasil.

cauebezerrasantos@gmail.com

Keywords: miRNoma, type 1 diabetes mellitus, differential expression, biomarkers.

Brazil occupies the third position in the ranking of countries with the highest absolute number of children affected by type 1 diabetes mellitus (DM1). This disease is characterized by deficiency of insulin production due to the destruction of pancreatic beta (β) cells through autoimmune mechanisms. There are no efficient biomarkers to diagnosis of DM1 early stages, which could improve prognosis and treatment of patients. Some microRNAs (miRNAs) are directly associated with DM1 and may be useful for diagnosis; however, the expression profile of these markers is still unknown. The aim this study was to characterize the global miRNA expression profile and its functional role in DM1. For this, 16 blood samples were used: 12 samples from patients with DM1 and four from individuals without the disease, all from the north of Brazil. The sixteen miRNome were generated using MiSeq Platform (Illumina). Differential expression analysis was developed using DESeq2 statistical package within the R environment, and Log2 | (fold change) |> 2 and P-adjust <0.05 were used as parameters to indicate differentially expressed miRNAs. For the identification of target genes and functional analysis/gene enrichment in KEGG pathways, miRTargetLink and STRING online tools were used considering only data of interactions validated experimentally with strong evidence. We identified 60 differentially expressed miRNAs in DM1. The search for their target genes has shown that 194 genes can be regulated by at least two different miRNAs. Functional analysis of these target genes demonstrated that they regulate important biological pathways for the development and progression of DM1, highlighting the innate immune system, MAPK, apoptosis, insulin and neurotrophin signaling pathways. Therefore, our study demonstrates that miRNAs are directly associated with DM1 and can assist in disease progression and pathogenesis.

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n-BUTANOL FRACTION FROM *SANIONIA UNCINATA* PROTECTS HUMAN KERATINOCYTES AGAINST UVA-INDUCED APOPTOSIS AND SCAVENGES REACTIVE OXYGEN SPECIES

Andréia da Silva Fernandes[1*], Adilson Fonseca Teixeira[1,2], Heitor Evangelista[1], Elisa Raquel Anastácio Ferraz[3], José Luiz Mazzei[4], André Luiz Mencalha[1], Israel Felzenszwalb[1].

[1]Department of Biophysics and Biometrics, Roberto Alcantara Gomes Biology Institute, Rio de Janeiro State University, Rio de Janeiro, Brazil. [2]Department of Surgery, University of Melbourne, Australia. [3]Department of Pharmacy and Pharmaceutical Administration, Pharmacy College, Fluminense Federal University, Niteroi, Brazil.[4]Department of Natural Products, Institute of Drug Technology, Oswaldo Cruz Foundation, Rio de Janeiro, Brazil.

*andreiadasilvaf@gmail.com

Key-words: *Sanionia uncinata,* UV-induced apoptosis inhibition, ROS scavenging

Sanionia uncinata (Hedw.) Loeske is a moss native to the Antarctic Peninsula. Our group has shown by *in vitro* models that dermal formulations containing the *n*-butanol fraction (Bf) from hydroalcoholic extract of *S. uncinata* protects the DNA bases against the induction to cyclobutane pyrimidine dimers (CPDs) and oxidation. The association of Bf with UV-filter benzophenone-3 (BZ-3) was ~ 5 times more effective than BZ-3 alone in the reduction of CPDs. We also showed that human HaCaT keratinocytes were more resistant to cell death induced by Bf, using 3D cell culture compared to monolayers. The Bf did not induce mutagenicity, genotoxicity, and phototoxicity even in association with UV-filters. Furthermore, Bf protects the HaCaT cells against UV-induced damage via cell membrane disruption. In the present study, we aim to evaluate the potential of Bf in scavenging intracellular Reactive Oxygen Species (ROS) and on the interference of the cell cycle (CC) phases. HaCaT cells were treated with either Bf (0.4-40 µg/mL) or UVA (10 J/cm^2/sec [CC] and 30 J/cm^2/sec [ROS]) or both. For CC analysis, the cells were fixed with ice-cold 70% ethanol, incubated with 7-AAD Viability Staining Solution and RNase solution. For measurements of intracellular ROS, HaCaT cells were incubated with 10 ĸM of 2,7-dichlorofluorescein diacetate for 30 min at 37°C. The cells were analyzed in a flow cytometer (BD FACSCalibur, USA). No significant change in the CC phases (G_0/G_1, S, G_2/M) was observed after exposure of Bf comparatively to untreated cells. It was detected that UVA-irradiation induces cellular Sub-G1 arrest (24.48±0.19%) and decreases G_2/M phase, whilst dose-dependent (≥ 1 µg/mL) treatment with Bf significantly (p<0.01) induces the decrease in arrest in Sub-G1 phase reaching 0.81±0.15% (at 40 µg/mL), and keeps G_2/M phase without changes, comparatively to the untreated cells. Furthermore, the Bf alone (without UVA-irradiation) had no effect on the generation of ROS. The ROS levels were significantly (p<0.01) reduced in a dose- dependent manner (≥ 4 µg/mL) on Bf-pretreated HaCaT cells, reaching 4.3 times lower (at 40 µg/mL) compared to that exposed by UVA. These results demonstrate that Bf can scavenge UVA-induced ROS, corroborating with results using *Salmonella* strains in which Bf reduced the ROS damage formation, acting as a strong reducing agent. Therefore, Bf can effectively revert the UVA-induced apoptosis of HaCaT cells, protecting them to cell death. In addition, the Bf decreases UVA-induced oxidative damage, confirming the hypothesis that Bf can act as a chemopreventive agent by downregulation of the redox status, reducing the genomic instability.

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Single-cell gene expression reveals a landscape of HOX genes in placental cell subtypes

Danielle Barbosa Brotto[1,2]; Carlos Alberto Oliveira de Biagi Junior[1,2]; Wilson Araújo da Silva Junior[1,2,3,4]

[1]Department of Genetics, Ribeirão Preto Medical School-FMRP, University of São Paulo-USP, Ribeirão Preto, SP, Brazil. [2]National Institute of Science and Technology in Stem Cell and Cell Therapy (INCT/CNPq); Center for Cell-based Therapy-CEPID/FAPESP, Ribeirão Preto, Brazil. [3]Center for Integrative System Biology - CISBi-NAP/USP, University of São Paulo, Ribeirão Preto, São Paulo, Brazil. [4]Center for Medical genomics at Clinical Hospital of Ribeirão Preto Medical School, University of São Paulo, Ribeirão Preto, São Paulo, Brazil.

*daniellebrotto@gmail.com

Keywords: HOX genes, placenta, single-cell.

HOX genes encode highly conserved proteins, which act as transcriptional factors through binding to downstream targets, to activate or repress their function. In humans, there are 39 HOX genes clustered on four different chromosomes and 13 paralogues groups. HOX genes play role as morphogenesis and cell fate regulators, driving embryonic development in animals. In addition, several members of the HOX gene family were also found expressed in some normal adult organs, as well as during placentation. The placenta is a dynamic tissue with an invasive structure whose growth is directed towards the formation of a transport system that will provide the fetus with metabolic requirements, gas transport and an excretory and endocrine system necessary for embryo development. To get novel insights into how HOX genes act in trophoblast differentiation and placental development, we aimed to explore the transcriptome of placenta at single-cell resolution, during the early stages of development. Through in silico analysis from single-cell RNA sequencing (scRNA-Seq) data available at Gene Expression Omnibus (GEO: GSE89497), we were able to identify and evaluate the expression of 39 HOX genes in 764 cells of different placental cell subtypes during the first and second trimester of pregnancy (8 and 24 weeks). The data provided came from isolation by MACS – magnetic-activated cell sorting, of the following four cell types: cytotrophoblasts (CTB), syncytiotrophoblast (STB), extravillous trophoblasts (EVT) and stromal cells (STRs). For the single-cell analysis, we used FastQC and MultiQC, as reads quality control tool and TrimGalore! for trimming the reads. Mapping and alignment against the reference genome (GRCh38/hg38) were performed using the software STAR. For differential gene expression analysis (DGEA) between groups of samples, we used Seurat package (v3.0) in R. Furthermore, DGEA showed a variation on specific HOX genes between biological groups. Among the 39 HOX genes, HOXC12, HOXC13 and HOXA3 showed differential expression between cell subtypes and developmental stages. Interestingly, the genes HOXC12 and HOXC13 stands out because they are highly expressed in CTB when compared to other cells subtypes. These results may indicate a role in the proliferative function of cytotrophoblasts in early placental development. Taken together, our findings support that a few HOX genes have differential expression between placental cell subtypes. Although further analysis should be performed in order to validate this information, our analysis might be an indicator of HOX function in regulating targets related to placenta development, once it is a transient organ with development accentuated during initial stages, where most of the mechanisms and pathways must be requested.

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B CHROMOSOME EFFECTS ON THE GENERATION OF tRNA-DERIVED FRAGMENTS IN THE CICHLID *Astatotilapia latifasciata*

Adauto Lima Cardoso[1*]; Luiz Augusto Bovolenta[1]; Rafael Takahiro Nakajima[1]; Jordana Inácio Nascimento de Oliveira[1]; Cesar Martins[1]

[1]Integrative Genomics Laboratory, Departament of Morphology, Institute of Biosciences, Sao Paulo State University.

Palavras-chave: non coding RNA, transfer RNA, supernumerary chromosome.

B chromosomes are extra and dispensable elements found in eukaryotes genomes. In the past, these chromosomes were thought as non-functional units, but this view was changed with growing information about their biology including genetic content, origin, evolution, transmission and effects in hosts. In individuals of *Astatotilapia latifasciata* 0, 1 or 2 B chromosomes can be observed. In this species, the B chromosome is composed by several classes of repetitive DNA and protein coding genes, and impacts cell physiology in different ways. Transfer RNAs (tRNA) are a class of non-coding RNAs involved in translation and recent discoveries indicate that enzymatic cleavage of them promote the formation of fragments. These tRNA-derived fragments (tRFs) consitute a class of small RNAs and are associated with several bilogical proccess. Here we conducted a genomic and transcriptomic approach to assess the impact of B chromosomes in tRFs generation in *A. latifasciata*. First, we did a search for tRNA genes in a genome assembly without B chromosome (B-) and in a genome with B (B+) using homology and prediction strategies. After, we identified the types of tRFs and the differentially expressed (DE) tRFs in sequenced libraries of small RNAs of gill, encephalon, muscle and gonads of individual B- and B+, including males and females. Finally, we calculated the entropy of tRNA fragmentation. We found 1007 and 1120 copies of tRNAs in B- and B+ assemblies, respectivelly. Moreover, we identified increased copy number of several tRNA types in B+ assemblies. These data suggest the presence of tRNA genes in the B chromosome. Our DE analyses revealed that B chromosome promotes heterogeneous effects on the expresion of several types of tRFs in gills of both sexes, in muscle of males and in encephalon of females. Furthermore, we found that B chromosome increases the variability of the entropy of tRNA cleavage. These data indicate that the B chromosome affects the formation of tRFs by expression and cleavage of its tRNA copies or by trans-acting regulation of the A chromosome copies. Together our data expand the knowledge about the content and the effect levels of B chromosome.

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INTERSPECIFIC HYBRIDIZATION BETWEEN SEA TURTLES IS ASSOCIATED TO THE LOW HATCHLING SUCCESS IN ABROLHOS ARCHIPELAGO

Larissa Souza Arantes[1*]; Lucas Cabral Lage Ferreira[2]; Camila Junqueira Mazzoni[3,4]; Fabrício Rodrigues dos Santos[1]

[1]Departamento de Genética, Ecologia e Evolução, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais (UFMG), Belo Horizonte, Brasil. [2]Parque Nacional Marinho de Abrolhos. [3]Berlin Center for Genomics in Biodiversity Research (BeGenDiv), Berlin, Germany. [4]Evolutionary Genetics Department, Leibniz Institute for Zoo and Wildlife Research (IZW), Berlin, Germany.

*larissarantes1@hotmail.com

Key-words: reproductive output of hybrids, 3RAD method, conservation genetics

Hybridization between sea turtle species is an important conservation concern, which occurs particularly in high frequency in two hybrid zones in Brazil. We investigated the hypothesis of the existence of a new hybrid spot in the Abrolhos Archipelago, which is surrounded by the largest and richest coral reefs in the South Atlantic, located in the southern coast of Bahia state, and is a well-known nesting area for loggerhead turtles (*Caretta caretta*). We performed a multidisciplinary investigation of the sea turtles and their reproductive output in the Abrolhos beaches. First, we sequenced mtDNA and six nuclear markers of six nesting females and 19 hatchlings, each representing 19 distinct nests. SNP data analysed through Bayesian hybrid-index showed that five out of the six females are loggerhead turtles and one female is a first-generation hybrid between a male hawksbill turtle (*Eretmochelys imbricata*) and a female loggerhead turtle (HxL). From the 19 hatchlings analysed, 10 are loggerhead turtles, but four hybrids are backcrosses of HxL with loggerhead turtles and other five are backcrosses of HxL with hawksbill turtles, showing that the introgression process is occurring with either parental species. Second, we used a 3RADseq method to generate genomic data to characterise the introgression level of 14 hatchlings from six different nests. After filtering, 3RAD sequences were analysed using the *Chelonia mydas* genome as reference, to call SNPs using the program STACKS. Haplotypic data was analysed in the software STRUCTURE and confirmed that the hybrid hatchlings analysed with 3RAD haplotypes are backcrosses with loggerhead. Finally, we compared the reproductive output of 29 nesting events for loggerhead turtles and 16 for hybrid females, whose species assignment was confirmed by either female morphology and/or genetic analysis. The clutch size was significantly greater for hybrids (137±20.06) than loggerhead (114.9±25.2). Incubation period was also greater for hybrids (55±3.06) compared to loggerheads (52.5±3.4). Temporal distribution of nests analysed in this work revealed that the reproductive season for loggerhead females finish earlier than hybrids. The hatchling success (HS) was significantly greater for loggerheads (59.8±29.9) compared to hybrids (27±26.97), which suggests a reproductive disadvantage of hybrids (outbreeding depression) in Abrolhos. The HS for loggerhead turtles in Abrolhos is also lower than other Brazilian populations, which may be associated to the Abrolhos' beach features. The integration of genetics and reproductive biology data analyses showed for the first time the association between hybridization process and low reproductive success, that can represent a threat to the sea turtles' conservation.

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GENOTOXIC EVALUATION OF RURAL WORKERS EXPOSED TO AGROCHEMICALS FROM MISSAL-PR CITY

Christopher William Lee[1]; Maria Claudia Gross[1]; Maria Leandra Terencio[1]; Carlos Henrique Schneider[2]

[1]UNILA - Universidade Federal da Integração Latino-Americana. [2]UDC – Centro Universitário Dinâmica das Cataratas.

biologolee@gmail.com

Key-words: Mutagenesis; Pesticides; Micronucleus

The exposure to agrochemicals can result in genotoxic injuries and increase micronucleus (MN) formation in individuals through aneugenic or clastogenic events. The presence of these biomarkers in individuals can be used as a chromosomic instability indicator. Thus, the analysis of micronucleus can be used as a tool to predict cancer predisposition. The objective of this study was to assess the presence of micronucleus in inhabitants of Missal city, Paraná state, exposed actively or passively to agrochemicals. Ethics approval for the study was given by the Research Ethics Committee Research Ethics Committees/CEPs of the National Research Ethics Commission/ CONEP (CAAE: 00698918.8.0000.8527, parecer opinion: 3.141.519). Slides of lymphocytes culture treated with cytochalasin B were examined from 57 individuals (16 females and 39 males); 27 were actively exposed to agrochemicals and 28 passively (control group). The individuals were separated in the intervals classes of age: 25-29, 30-34, 35-39, 40-44, 45-49, 50-54, 55-59 and over than 60 years. From each individual 50 binucleated lymphocytes was analyzed. For the statistical analyses test-t were used to check if there is any statistically significant difference between two samples means, non-parametric tests were used to compare two or more samples categories and the simple linear regression was used to analyze the connection of age and micronucleus. There is no statistically significance difference regarding gender and no statistical differences were observed regarding urban or rural living. There are statistically significant differences between: active and passive exposure (p=0.0018), active and passive exposure (last 3 months) (p=0.015) and the type of agrochemical application (p=0.000017). The application with costal sprayer and open tractor shower is twice more harmful than the application with cabined tractor. The preparation of the agrochemical for the future application also implies in a 3.6 fold higher injuries when compared to an individual that is passively exposed. Also, an individual that is actively exposed to agrochemicals has 7.8 fold more injuries than an individual that is passively exposed. Thus, the presented results demonstrate that the frequency of MN inindividuals who are actively exposed is much higher than those who are passively exposed.

Patterns of Molecular Evolution in the Energetic Metabolism of Migratory Cetaceans

Michelle C. Rachid Ribeiro; Mariana F. Nery; Érica M. S. Souza; Elisa K. S. Ramos

Laboratório de Genômica Evolutiva - Departamento de Genética, Evolução, Microbiologia e Imunologia - Instituto de Biologia - Universidade Estadual de Campinas (UNICAMP)

mariananery@gmail.com

Key-Words: Cetaceans; Migration; Evolutionary Genomics.

Energetic homeostasis is the process responsible for balancing the energy rates ingested and expelled from our organism. In most mammals, there is a strict control of the homeostasis and satiety regulation, through anorexigenic and orexigenic peptides. However, some lineages of mammals have different regulation, being able to not feed during months and also going through periods of intense feeding, apparently without getting satisfaction. Notorious examples of this behavior are migrating whales that perform long migrations. This project aims to investigate the genetic basis of this distinct feeding behavior, that evolved independently in some lineages. Accordingly, we investigated the evolutionary rate in genes involved in the central control of the metabolism (*POMC, MC4R, NPY*) in mammals, focusing on cetacean lineage, to better understand their evolutionary histories. We performed DNA extraction, PCR and gene sequencing in tissue samples from humpback whale (*Megaptera novaeangliae),* blue whale (*Balaenoptera musculus)* and the common bottlenose dolphin (*Tursiops truncatus*). Also, we retrieved sequences from public databases, such a Ensembl and GeneBank, from a comprehensive set of tetrapod species, including representatives from as many mammalian orders as possible. After that, we performed selection analyses using different models implemented in PAML, and also used different algorithms in Datamonkey and Fitmodel. Positive or relaxing selection was not observed in NPY. For MC4R and POMC, omega value was significantly greater for the cetacean lineage compared to other lineages, indicating an acceleration on their evolutionary rate. Our preliminary results aid to draw a panorama on selection pressure on these genes along evolution and improve our understanding of molecular evolution of metabolic genes in mammalian history, suggesting that some of these genes underwent an accelerated evolution on specific lineages, such as on cetacean lineage.

Funding Agency: FAPESP.

DECONSTRUCTION OF REGULATORY COMPLEXITY IN ESCHERICHIA COLI THROUGH SYSTEMIC BIOLOGY APPROACHES

Bianca Naomi-Carvalho[1], Rafael Silva-Rocha[2]

[1]Systems and Synthetic Biology Lab, Cell and Molecular Biology Department, FMRP- USP (Avenida Bandeirantes 3900, Prédio Central, Sala 22, Ribeirão Preto – SP, Brazil)

bianca.naomi.carvalho@usp.br

Palavras-chave: architecture; cluster; expression.

Escherichia coli is the organism best investigated from the molecular point of view. For this organism, there are a large number of data referring to the mechanisms of regulation of gene expression that have been generated in the last decades. There are specialized databases that compile this information in a systematic way and manually cured, serving as a starting point for many studies in this organism. The objective, therefore, is to make use of this information through the use of computational approaches to map natural complex promoters of *E. coli* and to contrast the topological relationships of different promoters with the expression profiles of the target genes. For this, information on the regulatory interactions between transcription factors and target promoters in *E. coli* were extracted from the RegulonDB database version 9. That is, extraction of architectural information from promoters such as data about name of transcription factors and corresponding promoters, position of each promoter, site of interaction of transcription factors, as well as the effect activation or repression of these factors on target genes, make it possible to select naturally complex promoters of *E. coli*. In this sense, those promoters regulated by 5 or more transcription factors were selected to analyze the dynamics of gene expression with similar promoter architectures. We used python scripts to extract the information from the data collected from *E. coli*. it was possible to obtain a total of 227 complex promoters using the aforementioned criterion. The architectures of promoters regulated by 5 or more transcription factors were further analyzed and decoded using *ad hoc* scripts in the R platform. Thus, the list of selected promoterswas mapped and group with the expression profiles of the target genes extracted from the Colombos Commons gene expression database, version 3. The latter has more than 4,000 experimental conditions analyzed and normalized to the more than 4,500 *E. coli* genes based on microarray and RNAseq data. Thus, through the analysis of the correlation between the architecture of natural complex promoters and the final levels of expression of the target genes, it was possible to identify which promoters grouped by architecture control genes that are grouped by the levels of expression, as well as the cases where this rule is not obeyed. For those cases where promoter clusters do not result in clusters of gene expression, the potential mechanisms related to the lack of correlation will be investigated in more detail, thus allowing deconstructing the regulatory complexity in this model organism.

GENETIC DIVERSITY OF CEREUS INSULARIS (CACTACEAE), INFERRED BY MICROSATELLITE DNA

Felipe André Silva[1,2], Fernando Faria Franco[1]

[1]Centro de Ciências Humanas e Biológicas, Departamento de Biologia, Universidade Federal de São Carlos, Sorocaba, São Paulo, Brazil. [2]Instituto de Biologia, Departamento de Genética, Evolução, Microbiologia e Imunologia, Universidade Estadual de Campinas, Campinas, São Paulo, Brazil.

felipebio97@gmail.com

Key-words: Transferability; Microssatelite DNA; Biogeography

The species *Cereus insularis* and *Cereus fernambucensis* (Cactaceae) compose a monophyletic group of Pleistocenic origin. The first is endemic to the archipelago Fernando de Noronha, PE, and previous plastid DNA data suggest that *C. insularis* has peripatric origin from archaic populations of *C. fernambucensis*. To investigate the levels of population variation of *C. insularis*, as well as its relationship with *C. fernambucensis*, we sampled *C. insularis* specimens distributed into four localities and *20 C.fernambucensis* specimens from Rio Grande do Norte state, the location more geographically close to the archipelago. DNA was extracted from the root tissues and PCR (Polymerase Chain Reaction) were performed using 28 SSR loci described for other cactus species. Positive PCR amplifications were identified in 3% agarose gel. Genotyping were performed by capillary electrophoresis using labeled primers. The verification of these results as well as the confirmation of the sizes were made through the program GeneMarker 2.6.4 (SoftGenetics, State College, PA). The occurrence of null alleles in addition to possible genotyping errors were verified in the software Micro- Checker 2.2.3 and corrections in the allelic frequencies made in the software FreeNA. Standard diversity indexes were estimated from the data. The population structure was investigated by AMOVA and the Bayesian analysis implemented in STRUCTURE 2.3.4. Among the 28 primers tested, 20 presented positive amplification in at least one individual of *C. insularis,* a rate of 71.43% of transferability. The presence of null alleles was identified in all populations. Some loci showed heterozygote deficiency, most of them concentrated in the island populations. The K = 3 was recovered as the number of genetic clusters. However, results seems to be artificial, considering the absence of cohesive geographic groups. Overall, the data indicates a diversity in *C. insularis* equivalent to the continental populations. Further, we detect signal of admixture between *C. insularis* and *C. fernambucensis* populations. Transferability proved to be efficient for obtaining locos in Cereus, decreasing costs and time. The transferred locos were informative for the genetic diversity and investigation of the *Cereus insularis* genetic diversity and population structure.

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DYNAMICS OF VIRAL VARIANTS IN PATIENTS OF RIO DE JANEIRO WITH CHRONIC HEPATITIS C

Silveira, A. L. M.[1], Hoffmann, L.[2], Cabral, B. C. A.[1], Ramos, J.A. [2], Silva, A. L. R.[3], Ürményi, T. P.[1], Villela-Nogueira, C.A.[3], Silva, R.[1]

[1]Instituto de Biofísica Carlos Chagas Filho, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brasil. [2]Instituto Federal de Educação, Ciência e Tecnologia do Rio de Janeiro, Rio de Janeiro, Brasil. [3]Faculdade de Medicina, Hospital Universitário Clementino Fraga Filho, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brasil.

ama_laryssa@hotmail.com

Keywords: Hepatitis C virus, NGS, Viral Variants

It is estimated 3 million people in Brazil infected by Hepatitis C virus (HCV), being 200,000 in the state of RJ. Hepatitis C is a disease with a high rate of chronification and is responsible for 43% of the cases of cirrhosis in Brazil and may evolve to hepatocellular carcinoma. The HCV has a genome of ssRNA with positive polarity and 9,600 bases. An important characteristic of the HCV genome is that it exhibits significant heterogeneity as a result of the accumulation of mutations during replication. The high mutation rate is characteristic of RNA genome viruses due to inability to correct RNA-dependent RNA polymerase errors, resulting in quasi-species. This genetic heterogeneity has been associated with the prognosis and pathogenicity of the virus, as well as with response to antiviral therapy. New technologies such as massive parallel sequencing have been shown to be a highly effective method for identifying variant diversity, including low- frequency ones. The aim of this project is to identify viral variants in the HCV genome before, over and after conventional treatment (pegylated interferon + ribavirin) in patients in Rio de Janeiro with chronic hepatitis C. Of 100 patients, 3 non-responders, infected with HCV genotype 1a and with 10 years of medical follow-up were selected. Three samples were collected: (1) pre-treatment and (2) 7 days of conventional treatment, and (3) 7 years after treatment (equivalent to pre-treatment with triple therapy including a protease inhibitor to the conventional regimen). Viral RNA was isolated from 200 µL of serum according to the QIAmp® MiniElute® Virus Spin (Qiagen) kit protocol and quantified in Qubit fluorometer (Invitrogen). The reverse transcription reaction was performed using the SuperScriptTM VILO™ cDNA Synthesis (ThermoFisher) kit. A customized panel was developed, consisting of 2 multiplex reactions containing 90 and 92 pairs of primers using AmpliSeq On Demand technology. For the preparation of the library was used the Kit IonAmpliseq™ Library 2.0 (ThermoFisher), according to the manufacturer's protocol. The libraries were sequenced in IonProton (ThermoFisher). Approximately 747,937 sequences were obtained for each of the 9 samples, with a mean size of 93PB. Quality control has been done to eliminate sequences with a value of Phred < 20. The sequences were mapped using the HCV 1a reference (AF009606). We observed viral variants with synonyms and non-synonymous alterations, whose quantification and characterization are being made. However, the consensus sequences, using the CLC Genomics Workbench (Qiagen) software, were exported and submitted to the Geno2Pheno online platform to identify variants resistant to new drugs for treatment (DAA). These results may help to conduct the new treatment, increasing the chance of response, as well as understanding the distribution and emergence of variants along the HCV-1a infection.

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THE EPIGENETIC LANDSCAPE OF CHROMOSOME 21 IN THE CONTEXT OF GENOMIC IMPRINTING AND DOWN SYNDROME

Cristina dos Santos Ferreira[1*]; Juan Carlo Santo e Silva[1]; Douglas Terra Machado[1]; Amanda Pereira Vasconcelos[1]; Enrique Medina-Acosta[1]

[1]Núcleo de Diagnóstico e Investigação Molecular, Laboratório de Biotecnologia, Universidade Estadual do Norte Fluminense, Campos dos Goytacazes, RJ, Brazil.

*ferreira.cristina@gmail.com

Keywords: allelic-specific expression; Down syndrome; epigenome

In human trisomy 21 (T21, Down syndrome), there is a marked disparity in the occurrence of maternal (95%) versus paternal (5%) meiotic causative errors. We hypothesize that the disparity has an epigenetic basis in the form of a paternally imprinted gene component in chromosome 21 (HSA21). To date, only one imprinted locus, *DSCAM*, has been reported in HSA21, but it is maternally imprinted. We have analyzed the epigenetic landscape of HSA21 using large datasets of experimental information about deep next-generation sequencing (RNA-Seq, BS-Seq, and ChIP-Seq) aiming to identify novel candidate imprinted loci. We used in-house R-based scripts to investigate the allele-specific expression and allele-specific methylation profiles associated with 40 known imprinting genes to redefine investigative criteria about candidate imprinted genes. The experimental evidence for the epigenetic and epigenomic architecture about imprinted marks are the main components of our comprehensive computational analysis. The screen identified three genomic imprinting candidate loci, being one protein-coding (*UMODL1*) and two long noncoding RNAs (*C21orf128* and *LINC01423*), located in the Down Syndrome Critical Region (DSCR1) at 21q22.3. *UMODL1* and *C21orf128* overlap, but are expressed from opposite strands. The three genes are expressed monoallelically (≥ 3 informative SNPs) in at least five tissues from at least two donors in the Genotype-Tissue Expression project database. There are no allele-specific methylation CpGs, gametic or secondary differentially- methylated regions (DMRs) associated with the three loci. However, in ≥ 20 BS-Seq experiments, we found three novel DMRs in neighboring loci (*FTCD, COL6A2,* and *C21orf33*), in addition to the known DMRs at *LINC00319, WRB, PRMT2* genes, all within the DSCR1. The parental origin of the monoallelic expression of *UMODL1, C21orf128,* and *LINC01423* is under investigation. Several SNPs within UMODL1/C21orf128 genes showed statistically significant association with high myopia, which is a common clinical feature in Down syndrome. Deregulation of *C21orf128* has been reported as part of lncRNA signature used as a prognostic marker in human lung squamous cell carcinoma and breast cancer, pathologies not associated with Down syndrome.

Funding agency: CNPq; FAPERJ; CAPES

DDB2 knockdown increases migration and clonogenic capacity of GBM cells and promotes tumor growth *in vivo*

Luis Fernando Macedo Di Cristofaro[1]; Eliot Fletcher[2], Sandeep Burma[2]; Valeria Valente[1,3*]

[1]Department of Cellular and Molecular Biology and Pathogenic Bioagents, Ribeirao Preto Medical School, University of Sao Paulo, Ribeirao Preto/SP
[2]Department of Radiation Oncology, University of Texas Southwestern Medical Center, Dallas/Texas, USA.[3]Faculty of Pharmaceutical Sciences, São Paulo State University, UNESP, Araraquara Campus/SP, Brazil.

luischristovan@gmail.com; valenteval@gmail.com

Keywords: glioblastoma, DDB2, poor prognosis, clonogenic growth, tumor growth.

Background: Diffuse astrocytomas are the most common brain tumors in adults. They are classified according to aggressiveness into subtypes II, III and IV. Grade IV tumors, also known as glioblastoma (GBM), are the most frequent and lethal type, leading patients to death in approximately 14 months after diagnosis. Total surgical removal of GBM is impossible due to tumor invasiveness. In previous *in silico* studies, we have found strong correlation between DDB2 reduction and the poor prognosis of GBM patients, reflected in the increased Hazard Ratio (3.21) of individuals showing DDB2 decrease. **Objetives**: Here our goal was to evaluate the effects of DDB2 down regulation in the gain of characteristics associated with GBM aggressiveness. **Methods**: We performed DDB2 knockdown in two GBM cell lines (U118MG and GBM9) evaluated of proliferation, clonogenic growth, neurospheres formation and migration (transwells assays). *In vivo* experimental procedure was conducted with intracranial injections of GBM9 cells in nude mice, followed by tumor growth and overall survival evaluation. **Results:** Knockdown of DDB2 did not altered proliferation rates of U118MG cells or neurosphere formation in GBM9 cells. On the other hand, DDB2 down regulation promoted clogonenic capability in U118MG and increased migration of GBM9 cells *in vitro.* Furthermore, DDB2 knockdown increased tumor growth rate *in vivo*. **Conclusions:** Altogether, these data strengthen the correlation between DDB2 reduction and the poor prognosis of glioma patients. Additionally, we could suggest that DDB2 plays an important role in the development of migration and clonogenic growth competences of GBM cells, which contribute to the higher tumor growth rate observed *in vivo*.

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CARCINOMA PAPILLARY THYROID RELATED TO THE POLYMORPHISM XbaI OF THE GENE APO B

Antonio Avelino Ferreira Soares [1*]; Jessica Caroline C. Mendes[2]; Izabel Cristina Rodrigues da Silva[3]

[1]Universidade de Brasília. [2]Universidade de Brasília. [3]Universidade de Brasília.

clergun@gmail.com

Key-words: Apolipoprotein B; Papillary Thyroid Cancer; XbaI.

The apolipoprotein B gene (APOB) is located on chromosome 2 and is linked to the metabolism of LDL. The thyroid gland is responsible for the production of triiodothyronine and thyroxine which are related to lipid metabolism and studies indicate that higher serum cholesterol levels are associated with thyroid cancer risk. Among the polymorphisms already described, we analyzed XbaI, in which a cytosine nucleotide is replaced by a thymine in the third position of codon 2488, which encodes the same amino acid. However, the presence of thymine represents a restriction site for the XbaI enzyme. The study analyzes the presence of the three genotypes X+X+, X+X- and X-X- in exon 26, X+ being the allele with the polymorphism and X- the absence of it. The main inclusion factor is the presence of papillary thyroid cancer, which is one of the most common thyroid cancer, represents 80% of cases and develops in follicular cells, with slow growth, usually affects only one lobe, but can also be in the gland itself. The objective is to analyze the database for the association of single nucleotide polymorphism (SNP) of the XbaI gene of apolipoprotein B (APOB) with papillary thyroid cancer. Blood from 30 patients who had papillary thyroid cancer was used and the Invitrogen dna extraction kit was used. In the study 3 patients had a homozygous polymorphism, 2 male and 1 female, and 2 patients were heterozygous. The mean age and BMI of patients homozygous for the polymorphism was 48 and 27.67 respectively, which showed acceptable parameters for the patients, a defect of the study is that we do not have the serum LDL levels of the patients, a trump was statistically the association was possible.

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MOLECULAR DOCKING INTERACTION BETWEEN PIPLARTINE AND IMMUNE-RELATED PROTEINS AS THERAPEUTHIC STRATEGY FOR DOWN SYNDROME

Daniel Ramos de Oliveira Santos[1]; Marlon Fraga Mattos[1]; Lennon Pereira Caires[1], Tiago Henrique[2], Olívia Borghi Nascimento[1], Eny Maria Goloni-Bertollo[1],Eloiza Helena Tajara[2], Érika Cristina Pavarino[1]

[1]Genetics and Molecular Biology Research Unit - UPGEM, São José do Rio Preto Medical School- FAMERP – Brazil. [2]Laboratory of Molecular Markers and Bioinformatics, São José do Rio Preto Medical School FAMERP, SP – Brazil.

erika@famerp.br

Key words: Molecular docking, Immunological response, Down syndrome.

The Down syndrome (DS) is the most frequent chromosomal anomaly in the world, with an estimated prevalence of 1 in 850 live births. Immunological disorders like autoimmune diseases and high frequency of infections, especially in the respiratory tract, are more common in individuals with DS than in the general population. The etiology of the immunological deficiency in DS is not completely known. Differential expression of genes involved in immunological processes has been reported in individuals with DS. Piplartine, also known as Piperlongumine, is a biologically active component of Piper longun (Piper longum L. – Piperaceae), which has multiple pharmacological activities including a potential role in immunological response. Previously, we identified five immune-related genes, four genes (CD40, NOS2, ITGAM and ITGB1) down-regulated and one gene (IL-10) up-regulated in DS individuals. In the present study, we evaluated by bioinformatics methods the potential interaction between piplartine and proteins encoded by differentially expressed genes in our previous studies in DS. The protein structures of the molecular targets CD40, NOS2, ITGAM, ITGB1 and IL-10 were obtained on Protein Data Bank (PDB ID: 5DMI, 4NOS, 1NA5, 4WJK, 2H24, respectively) and used as the receptors for docking simulation and the three-dimensional structures were prepared with the Autodock tools. The interaction of the ligand piplartine with the molecular targets was performed using AutoDock Vina program. To ensure reliability of this interaction, the docking parameters were validated using the redocking approach. Molecular docking experiments showed a binding energy of -6.2kcal/mol for CD40 and ITGAM, - 6.3kcal/mol for IL-10, -7.3kcal/mol for NOS2, -7.6kcal/mol for ITGB1. ITGB1 presented the lowest energy, therefore, showed the most efficient ligation prediction with the piplartine. Although the physical interaction between this natural compost and ITGB1 was not tested in vitro, in previous study we demonstrated that the piplartine reduced the expression of ITGB1 in laryngeal tumor cell line. Therefore, in vitro studies in trisomic cells are necessary to evaluate the action of piplartine in these cells. In addition, this previous study also showed that CD40, NOS2, ITGAM genes are up- regulated by piplartine and could be promising targets to improve the immunological response. In conclusion, our results showed that the piplartine has potential binding site for the proteins evaluated in this study. It is important to conduct further studies both in vitro and in vivo on piplartine and their active principles to validate our in silico prediction.

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GLOBAL METHYLATION LEVELS IN ADHD AND COMORBIDITIES

Diana Müller[1,2]; Natasha Assis Figueira da Silveira[2,3]; Bruna Santos da Silva[3]; Eugenio Horácio Grevet[2,5]; Anelise Schneider[4]; Stefania Pigatto Teche[2,5]; Marcelo Moraes Victor[2,5]; Mariele Feiffer Charão[4]; Diego Luiz Rovaris[2]; Claiton Henrique Dotto Bau[1,2]

[1]Departamento de Genética, Universidade Federal do Rio Grande do Sul, Brazil. [2]Programa de Déficit de Atenção e Hiperatividade do Hospital de Clínicas de Porto Alegre – PRODAH – HCPA, Brazil. [3]Universidade do Vale do Rio dos Sinos, Brazil. [4]Laboratório de Análises Toxicológicas, Universidade Feevale, Brazil; [5]Departamento de Psiquiatria, Universidade Federal do Rio Grande do Sul, Brazil.

dianamuller14@gmail.com

Key-words: ADHD; global methylation; psychiatric genetics

Background: Genetic studies have identified possible variants associated with attention- deficit/hyperactivity disorder (ADHD) susceptibility; however, they explain only a small portion of the whole observed variability. In this sense, the evaluation of the broad environmental influence on the DNA background can complement the genetic studies and are useful to untangle the neurobiology of this disorder and related phenotypes. Epigenetic studies, especially the methylation ones, have emerged as an important approach to understand the interactions between environmental and genetic factors. However, most of the methylation studies published so far have focused solely on specific genes without measuring global methylation levels. Despite the few studies evaluating global 5-methylcytosine (5-mC) levels in psychiatric disorders have reported significant differences between patients and controls, none of such investigations focused on ADHD. Aim: To test the association between global 5-mC levels and diagnoses of ADHD and its comorbidities. Methods: Our sample comprised 443 adults diagnosed with ADHD (DSM-5) and 498 adults with negative screening for ADHD on the Adult Self-Rating Scale (ASRS-V1.1). All subjects were Brazilians of European Descent. Psychiatric comorbidities were assessed through Structural Clinical Interview (SCID-I). The DNA was extracted from whole blood and aliquots of 2 micrograms were obtained for methylation treatment protocol. Treated samples were submitted to separation in HPLC according to nucleotide differential elution times. Results: We detected a significant difference in the global methylation level between subjects with ADHD (mean=3.442 ± 0.376) and controls (3.533 ± 0.376), P<0.001). Subjects with comorbid Generalized Anxiety Disorder (GAD) presented lower 5-mC levels (P=0.004) while subjects with Antisocial Personality Disorder (ASPD) exhibited higher methylation (P= 0.036). In analysis separated by sex within subjects with ADHD, we observed that women presented lower methylation levels than men (mean=3.367 ± 0.383 vs 3.498 ± 0.358, P< 0.001). Discussion: Our findings on the association between 5-mC and ADHD, sex and comorbidities are compatible with previous associations of DNA methylation with sex and psychiatric disorders. Further studies should evaluate the contrasting results for GAD and ASPD in patients with ADHD since they may be reflecting either the difference in the prevalence of these disorders according to sex (GAD is more prevalent among women and ASPD in men) or a real biological effect related to these disorders. Conclusion: Our results reinforce global DNA methylation as a promising factor to consider in the complex pathophysiology of adulthood ADHD and its comorbidities. Additionally, we highlight the need to further evaluate the mechanisms underlying the methylation differences according to sex.

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Metatranscriptome analysis of the symbiotic interactions between lactic acid bacteria, acetic acid bacteria, and yeasts in Kefir

Diego L. Rios[1]; Patrícia S. Costa[1]; Francisco Sobrinho[1]; Analía G. Abraham[2]; Graciela Garrote[2]; Jacques R. Nicoli[3]; Elisabeth Neumann[3]; Gabriel da Rocha Fernandes[4]; Álvaro C. Nunes[1].

[1]LGMPP, Federal University of Minas Gerais; [2]CIDCA, National University of La Plata; [3]Department of Microbiology, Federal University of Minas Gerais; [4]René Rachou Institute – Fiocruz.

*lisboa.zootec@gmail.com

Keywords: Meta-transcriptome, microbiome, kefir.

Kefir grain possesses a microbiota formed by lactic acid bacteria and acetic acid bacteria in association with yeast in a polysaccharide matrix. In Brazil and Argentina, there are currently two types of kefir grains, one of them like the grain used worldwide to produce a traditional fermented milk. The other kind of grain is used to prepare a watery drink with brown sugar, usually in countries of Latin America. Thus, the objective of this work was to evaluate the transcriptionally active microbiome (TAM), and the metatranscriptome of milk and water kefir beverages. Microbial community of the kefir fermented milk and the kefir sugary water drinks were evaluated by reverse transcription of several housekeeping genes for bacteria typing or rRNA intergenic spacer regions for yeast typing. The main bacterial families were Leuconostocaceae, Lactobacillaceae, and Acetobacteraceae. The genus *Leuconostoc*, *Lactobacillus, Lactococcus*, with the species *L. mesenteroides, L. helveticus e L. lactis*, was most frequent in the microbiome of milk kefir respectively, while *Oenococcus* genus was most frequently seen in the water kefir, with the species *O. kitaharae*. The fungal families Saccharomycetaceae, in the water kefir, and Pichiaceae and Dipodascaceae, in milk kefir, were the most abundant. The genera Saccharomyces and Torulaspora, with the species *S. cerevisiae* and *T. delbrueckii*, were most recurrent in the microbiome of water kefir, while Pichia and Yarrowia were more abundant in milk kefir, with the species *P. fermentans* and *Y. lipolytica*. The metatranscriptome findings were somewhat different from that of the microbiome: in water kefir, Saccharomyces and Pichia were the main genera, with the species *S. cerevisiae* and *P. membranifaciens*, and an irrelevant role of *T. delbrueckii*; in milk kefir, an unknown Pichiaceae genus, *Pichia* and *Yarrowia*, with the species *P. kudriavzevii* and *Y. lipolytica* were the most important, and an insignificant role of *P. fermentans*. Regarding KEGG PATHWAY mapping of the KO functional orthologs, the six kefir bacterial libraries shared 37% of KO entries, 15% were exclusive to water kefir and 26% unique to milk kefir. KOs found in only one kefir library ranged from 3.8% to 12.6%; the six kefir fungal libraries shared 70% of KO entries, 8% exclusive to water kefir and 14% unique to milk kefir. KOs found in only one kefir bacterial library ranged from 3.8% to 12.6%, and unique to one fungal varied 1.5% to 4.8%. These findings corroborate the importance of punctual differences in unique features of beverages. Conclusively, was observed positive correlation between *galE* gene expression, viscosity and exopolysaccharide production in milk kefir. In water kefir beverages was found the expression of marker genes for nitrogen fixation, *nifKDH* operon. This work contributed with unpublished findings at several points for research related to kefir and microorganisms in foods as well.

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CERTIFICATION OF SAMPLES FOR SCIENTIFIC RESEARCH: GUARANTEEING THE APPLICABILITY OF RESULTS

Diego Martins[1]; Fernanda Prado[1]; Adriano Costa[2]; Diogo Hashimoto[3]; Fábio Porto-Foresti[1*]

[1]Universidade Estadual Paulista (Bauru). [2]Instituto Federal Goiâno. [3] Universidade Estadual (Jaboticabal).

* diego_g_martins@hotmail.com

Palavras-chave: hybrids; molecular markers; Serrasalmidae

Hybridization among fish species has become popular in Brazilian crops since the 1980s, being directly related to the increase in the frequency of hybrid organisms in wild populations. In addition to the ecological issues discussed within this theme, there is also a practical implication, which jeopardizes the applicability of results obtained in scientific research involving groups of fish of zootechnical interest, such as members of the Serrasalmidae family. The present work aimed at the identification of pure and hybrid strains of a Serrasalmids fishes stock used for research. For this, 88 samples previously identified morphologically as *Piaractus mesopotamicus*, *Piaractus brachypomus* and *Colossoma macropomum* were molecularly diagnosed by the application of nuclear markers α-tropomyosin (TROP), anti-enzyme inhibitor (AZIN) and Z-dependent protease inhibitor precursor (ZPI), using the PCR-Multiplex technique. The molecular diagnosis revealed the presence of 28 (31.81%) hybrids individuals, all classified as advanced hybrid strains. This result is relevant considering that scientific research can be compromised by the production of untrue data, which would harm not only the biased experiment itself but all those who use this false knowledge. In a more practical way, it is important to emphasize that by the genetic composition itself, the hybrid organisms are not representative in relation to the parental species, possessing behavioral, reproductive, physiological and genetic differences. Therefore, the present study draws attention to the need for the use of tools that allow a certification regarding the purity of the lineages used in research, especially when the studied groups have a history of hybridization. Thus, conferring reliability and applicability to the generated data.

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DISCOVERY AND CHARACTERIZATION OF CODING SMALL ORFs: AN EVOLUTIONARY APPROACH

Diego Guerra de Almeida[1*]; **Diogo A. Tschoeke**[2]; **Rodrigo Nunes da Fonseca**[1]

[1]Instituto de Biodiversidade e Sustentabilidade (NUPEM), Universidade Federal do Rio de Janeiro – Campus Macaé. [2]Instituto Alberto Luiz Coimbra de Pós-Graduação e Pesquisa de Engenharia (COPPE), Universidade Federal do Rio de Janeiro.

*diegoguerra.bio@hotmail.com

Keywords: small Open Reading Frames; *mille-pattes*; *Tribolium*

For many years considered junk DNA, Open Reading Frames smaller than 100 codons (smORFs) still have their coding potential discarded by gene prediction programs. Recently, dozens of genes composed of smORFs have been characterized as essential in several model systems, such as yeasts, fruit flies and humans. It has been speculated that peptides derived from smORFs may constitute, on average, about 0.1% of the entire protein content of tissues, ranging from 10 to 1,000 molecules per cell. Thus, smORFs can be considered an emerging frontier of modern biology. This work aimed to develop a new and accurate *in silico* methodology for coding smORFs prediction, by applying an evolutionary approach using the model beetle *Tribolium castaneum* as a reference taxon. Several open source bioinformatics tools were used for the analysis. First, the genome sequence of *T. castaneum* was obtained and its intergenic regions extracted using BEDtools and SAMtools packages. Second, the EMBOSS-GetORF software was used for a *de novo* detection of ORFs between 21 and 300 nucleotides. Third, these smORFs were subjected to a search for similarity (E- value <= $1x10^{-3}$) through BLASTp against computationally translated smORFs sequences from four other coleopteran genomes. Fourth, these coleopteran conserved smORFs sequences were subjected to a further validation process by their comparison with *T. castaneum* RefSeq transcripts by BLASTp (E-value <= $1x10^{-3}$). Fifth, these smORFs were submitted to searches by BLAST for possible orthologs expressed in distant groups. Finally, the genomic and evolutionary patterns of the predicted sequences were evaluated through various software packages, such as UCSC Jim Kent's Utilities, E-utilities, EMBOSS, BEDtools among others. 5,184 smORFs were predicted in sense and antisense transcripts of *T. castaneum*. New smORFs conserved between prokaryotes and eukaryotes were discovered and, six of them trace back to the genome of the Last Universal Common Ancestor (LUCA). Sequence similarity between smORFs and major ORFs have been identified, as well as a possible involvement of these small sequences in duplication events and high expression of potential paralogs. Further investigation of the well-known polycistronic gene containing smORFs *mille-pattes* (*mlpt*), indicates the existence of a new Hemiptera-specific smORF with a broad conservation pattern in the group. A new smORF classification proposal was developed based on the transcriptional characteristics described in the literature and our data. smORFs large conservation spectrum along the evolutionary tree of life, chromosomal specificities and considerable gene dynamics suggest that smORFs may constitute an important reservoir for the discovery of new genes and can no longer be dismissed.

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COMPARATIVE TRANSCRIPTOME ANALYSIS BETWEEN WORKERS OF THE STINGLESS BEE *SCAPTOTRIGONA POSTICA* (LATREILLE)

Danielle Luna-Lucena[1*]; Osvaldo Reis[2]; Zilá Luz Paulino Simões[1,3]

[1]Departamento de Genética, FMRP – USP, Brazil. [2]Departamento de Genética e Evolução, UNICAMP, Brazil. [3]Departamento de Biologia, FFCLRP – USP, Brazil.

*dlunalucena@usp.br

Keywords: Stingless bees, RNAseq, reproductive activity

The eusociality has in the division of reproductive labor as one of the most striking evolutionary traits. Two groups of bees are readily recognizable by their distinct social organization: the honey bees (Apini) and the stingless bees (Meliponini). The species *Apis mellifera* and *Scaptotrigona postica* are representatives of such societies. Despite some similarities concerning on the social organization, these groups are markedly distinct on their morphological structure of ovary and their reproductive behaviors. *A. mellifera* is the most studied bee species under several genetic and behavioral aspects. Conversely, little is known about the native species of Meliponini. This study aims to elucidate which genes are associated with the ovary activation in this group, by means of next generation sequencing (RNAseq). RNAseq libraries were generated for adult workers of *S. postica* using samples of ovary (OV) and fat-body (CG) tissues collected during three phases of development: 1) newly emerged (RE), 2) seven-day old (7D - nurse), and 3) forager (FOR). The choice for these phases was influenced by the physiological state of the ovaries that were characterized by means of a comparative morphology study of this structure. The morphology revealed that: newly emerged workers (RE) present ovaries at the beginning of activation, nurse workers (7D) are in the peak of reproductive capacity and last, forager workers (FOR) shown decline of reproductive capacity. The "*de novo*" method was used to assemble a reference transcriptome using software TRINITY v2.8.3. The analyses of sequences revealed 212,703 transcripts and 95,270 "genes". We used the software TRANSDECODER v5.5.0 and TRINOTATE v3.1.1 to functional annotation. The functional annotation returned 23,608 gene regions, with similarity matching with other insects. The differentially expressed genes (DEGs) were identified with DESeq2. In relation to the DEGs in the ovary libraries, we found a total of 6,531 genes in the pair comparisons using OV7D *vs* OVFOR; 7,998 between OV7D *vs* OVRE; and 5,408 between OVFOR *vs* OVRE. In the libraries of the fat-body, we found 2,295 in the comparisons between CG7D *vs* CGFOR; 4,623 between CG7D *vs* CGRE; and 4,825 between CGFOR *vs* CGRE. As preliminary result, we observed a larger number of genes highly expressed both in newly emerged workers and foragers libraries, suggesting that regulatory networks are more complex during start and final stages of the reproductive activity.

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Species delimitation suggests lower diversity of genus Elops (Elopiformes: Elopidae)

Carla Bessa-Brito[1]*; Rodrigo Petry Corrêa de Sousa[1]; Aurycéia Guimarães-Costa[2]; Marcelo Vallinoto [1,3]

[1]Laboratório de Evolução, Universidade Federal do Pará, Campus Bragança; [2]Laboratório de Genética Molecular, Universidade Federal do Pará, Campus Bragança. [3]CIBIO/INBIO, Universidade do Porto, Portugal.

*carlabessa3@gmail.br

Keywords: Cryptic species; taxonomy; mitochondrial DNA.

Elops is the only genus belonging to the Elopidae family, currently composed of seven valid species, which are widely distributed in estuaries and tropical and subtropical Atlantic, Pacific and Indian Oceans. As far as taxonomy is concerned, there are many uncertainties, mainly due to the cryptic character of these species, as well as due to the sympatric distributions. In this study, we used methods of species delimitation based on the mitochondrial gene Cytochrome C oxidase I (COI), in order to verify the genetic divergences among the species of *Elops*, being used five of the seven species of the genus. The analyses were based on three different widely used methods: Generalized Mixed Yule Coalescent (GMYC), Automatic Barcode Gap Discovery (ABGD) e Bayesian Poisson Tree Process (bPTP). All methods of species delimitation were unanimous in recovering only one cluster that includes all the species of *Elops* analyzed here. The values of interspecific divergence ranged from 0.8% (*Elops smithi vs. Elops sp*.) to 2.6% (*Elops affinis vs. Elops smithi*). It is emphasized that the 2% threshold of interspecific divergence is used to consider different species. Considering the high degree of morphological similarity between species, identification errors can lead to erroneous sequences being uploaded to public banks. Thus, although our database did not include all valid species of *Elops*, our evidence of molecular delimitation indicates that the diversity of the genus is smaller than what has been recorded in the literature.

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CYTOTOXICITY AND GENOTOXICITY EVALUATION OF PARAÍBA DO SUL RIVER WATER BY THE TEST SYSTEM *Allium cepa*

Emanoelly B. Sacramento[1]; Caroline de A. Azevedo[1]; Saulo T. Abreu[1]; Viviane;M. de Lima[1]; Hélcio R. Borba[1]

[1]Laboratório de Atividade Genotóxica de Plantas – Departamento de Biologia Animal (DBA), Instituto de Ciências Biológicas e da Saúde, Universidade Federal Rural do Rio de Janeiro (UFRRJ), Seropédica, Rio de Janeiro.

emanoellysacramento@hotmail.com

Key Words: *Allium cepa*; Genotoxicity; Cytotoxicity.

The Paraíba do Sul river is a water flow that pass by the states of São Paulo, Rio de Janeiro and Minas Gerais, being one of most important rivers of Rio de Janeiro state. Currently the river is in critical ecological condition, justified by the disposal of untreated domestic and industrial effluents, along with agricultural residues. As the Rio de Janeiro state is the user of the water coming downstream from the river, it becomes fully vunerable to the presence of pollutants in the aquatic environment. Therefore, this study aimed to analyze Paraíba do Sul river waters in the presence of pollutants with cytogenotoxicity potential, using the system test *Allium cepa*. Water samples were collected from four different spots, being them from downstream of transposition with Guandu river located in Barra do Piraí city. The collect were performed during the dry and rainy seasons, in random spots, equally distant from each other. The roots of the *A. cepa* were exposed to the water samples for 48 hours, with daily sample changes. In this study were used five onions per treatment, being them the samples of four different spots from the river, positive control (Ethyl methane sulfonate) and negative control (distilled water). The mutagenic effect was evaluated by analysis of chromosomal changes in meristematic cells of *A. cepa* by observing 1000 cells per onion, accounting 5000 cells per treatment. All of studying spots showed a certain toxicity index, two of them exhibit changes similar to those found in positive control, emphasizing cells with nuclear bud, nucleoli change, cells karyorrhexis-like, anaphases with chromosome delay and cells with citoplasmatic vacuole. There were numerical variations in cytogenotoxicity changes between dry and rainy seasons, indicating that mutagenic effects and cytotoxicity of pollutants present on water ocillates according to season, increased emission of contaminated effluents and transit of toxic compounds by watercourse. From the analysis of the results, it is possible to conclude that there was confirmation of the existence of cytotoxicity and genotoxicity substances at the analyzed sites, which compromises the water quality and affect the population's quality of life. Stands out the importance of biomonitoring studies that assist the management and regulation actions of discharges of potentially dangerous substances in the environment.

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EPSP synthase flexibility is determinant to its function: computational molecular dynamics study

Luís Fernando Saraiva Macedo Timmers[1]⌘, Antônio M. S. Neto[2], Rinaldo W. Montalvão[2], Luiz A. Basso[3], Diógenes S. Santos[3], Osmar Norberto de Souza[1]

[1]Laboratory for Bioinformatics, Modelling and Simulation of Biosystems (LABIO), Pontifical Catholic University of Rio Grande do Sul (PUCRS), Porto Alegre, RS, Brazil. [2]São Carlos Institute of Physics, University of São Paulo, São Carlos, SP, Brazil. [3]Research Centre of Molecular and Functional Biology (CPBMF), PUCRS, Porto Alegre, RS, Brazil. ⌘Present address: University of Taquari Valley - Univates, Lajeado, RS, Brazil.

luis.timmers@univates.br

Key-words: EPSP synthase, Molecular dynamics simulations, Protein flexibility

Flexibility is a phenomenon that acts as a trigger for many biological processes, such as protein-ligand and protein-protein interactions. A good understanding of molecular flexibility is therefore important in many fields of biophysics, as well as in drug design. 5-Enolpyruvylshikimate-3-phosphate (EPSP) synthase, the sixth enzyme in the shikimate (SK) biosynthetic pathway, must undergo a large conformational change to perform its function. Its dynamic motion was observed to be impeded when this protein is associated with strong inhibitors. However, although the structure of EPSP synthase has been determined, its plasticity has not been explored in depth. Therefore, one objective of this study is to explore how EPSP synthase transits between closed and open states, and to pinpoint regions of the protein that could be facilitating these motions. To evaluate the EPSP synthase's dynamics, we extensively examined the influence of the flexibility using classical and replica-exchange metadynamics simulations. We were able to identify five clusters of conformations for the apo EPSP synthase, ranging from closed to open states. These findings point to a classical model of conformational selection, where the apo EPSP synthase adopts a conformation that can bind strongly to a ligand before it actually binds to that ligand (i.e., the binding of the ligand does not induce the synthase to adopt the appropriate conformation for bind- ing). Taken together, the new findings presented here indicate how the hydrophobic regions modulate the flexibility of MtEPSP synthase, and they highlight the importance of considering these dynamic features in drug design projects employing this enzyme as a target.

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EVOLUTION AND KARYOTYPIC DIVERSIFICATION OF THE ELOPIFORMS (TELEOSTEI; ELOPOMORPHA)

Rodrigo Petry Corrêa de Sousa[1*], Auryceia Jaquelyne Guimarães Costa[2], Edivaldo Herculano Corrêa de Oliveira[3,4], Gláucia Caroline Silva de Oliveira[2], Marcelo Vallinoto[2]

[1]Universidade Federal do Pará; Instituto de Ciências Biológicas; Belém-PA; Brasil. [2]Universidade Federal do Pará; Instituto de Estudos Costeiros; Bragança-PA; Brasil. [3]Universidade Federal do Pará; Instituto de Ciências Exatas e Naturais; Belém-PA; Brasil. [4]Instituto Evandro Chagas; Seção de Meio Ambiente; Laboratório de Cultura de Tecidos; Ananindeua-PA; Brasil.

*rodrigopcsousa@gmail.com

Keywords: Cytogenetic; Heterochromatin; ITS

Elopomorpha is the first group to diversify from Teleostei, than includes the orders Elopiforms, Albuliforms, Notacantiforms, and Anguilliforms. Based on the data available for the Elopiforms, a systematic investigation of the extant species that represent the most basal lineages of the Teleosts may provide insights for the understanding of the chromosomal evolution within this group. The present study investigated the phylogeny and evolutionary dynamics of the chromosomal structure of Elopiforms. The specimens of *Elops smithi* and *Megalops atlanticus* were collected in Ajuruteua, Pará, Brazil. Cytogenetic techniques Ag-NOR, C-banding and Fluorescente *in situ* Hybridization with 18S and 5S rDNA probes, and telomeric sequences, were used in this study. In addition, Maximum Likelihood analyzes was performed from sequences of COI and Cytb from Holostei (brother group), and Halobatrachus didactylus (outgroup), available from GenBank and BoldSystem. The karyotype of *M. atlanticus* was composed of 2n=50A, and of *E. smithi* was 2n=50 (6M+4SM+40A). Heterochromatin was positive in the centromeres and signals interstitial were observed in pairs 3 and 9 of *M. atlanticus*, and in pairs 4, 5, 9, 19 and 22 of *E. smithi*. The NOR was observed in pair 14 in both species. The FISH with 18S rDNA and 5S rDNA probes were similar in both species, located in pairs 14 and 20, respectively. The telomeric sequences were positive in the telomeres of species, with interstitial telomeric sequences (ITS) in pair 3 in *M. atlanticus* and in acrocentric chromosomes of *E. smithi*. The mtDNA tree defined the Elopomorpha as a monophyletic group, the level of order, Elopiforms is brother group of the clade formed by Albuliforms, Nocantiforms, and Anguiliforms. The phylogenetic relationships associated with the chromosomal data on the Elopomorpha reflect a tendency of reduction in the diploid number, and the evidence found indicates that the reduction in the genome was related directly to events of centric fusion, in tandem and pericentric inversion. The interstitial heterochromatin in Elopiforms species provides insights from the karyotypic diversification of this group (fusions, fissions and chromosomal inversions). The combination of the NOR, 18S and 5S rDNA probes indicate conserved patterns shared by the order, which may represent plesiomorphies in Elopiforms. The ITS observed in *M. atlanticus* may have been the result of an *in* tandem fusion, while the presence of ITS in the acrocentric chromosomes of *E. smithi* indicates the insertion of telomeric DNA into unstable sites, or association of satellite DNA located in the centromeres. The results provide new perspectives on the chromosomal evolution of Elopiforms, as well as new insights into the karyotypic diversification of the Elopomorpha.

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MOLECULAR CHARACTERIZATION OF THE CIRCADIAN CLOCK ELEMENTS DURING THE DEVELOPMENT AND SENESCENCE OF *APIS MELLIFERA*

Fabiano Carlos Pinto de Abreu[1]; Flávia Cristina de Paula Freitas[3]; Camilla Valente Pires[4]; Joseana Vieira[3]; Thiago da Silva Depintor[2*]; Zilá Luz Paulino Simões[1]

[1]Departamento de Biologia, Universidade de São Paulo - Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto. [2]Deparamento de Genética, Universidade de São Paulo – Faculdade de Medicina de Ribeirão Preto. [3]Instituto de Ciências Biomédicas – Universidade Federal de Alfenas.[4]Centro de Pesquisa Rene Rechou - Fiocruz/Minas.

*fabiano.genetica@usp.br

Keywords: circadian clock, circadian rhythms, clock genes, miRNAs, *Apis mellifera*

The circadian clock is an advantageous adaptive system that enables organisms to anticipate and syncronize their biological activities during the daily environmental changes. The circadian clock acts through the ontogeny of circadian rhythms, which are generated by the cyclic expression of the clock genes in an autregulatory feedback loop. In insects, the circadian rhythms have important roles in the coordination of the developmental timing and behavior. In the last years, researchers revealed that the molecular clock of social insects is more similar to mammals than to insects. In particular, the social honeybee is an excellent model to investigate how the circadian rhythms are modulated accordingly to the social context, behavioral plasticity, and task-related activities. In this work, we characterized the expression patterns of the clock genes *period* (*per*), *cryptochrome mammalian-like* (*cry-m*), *clock* (*clk*), *cycle* (*cyc*), *timeout 2* (*tim2*), *par domain protein 1* (*pdp1*), *vrille* (*vri*) e *clockwork orange* (*cwo*) in the entire development of *Apis mellifera.* Our results revealed that the clock genes are expressed before the formation of the central nervous system in embryos and that their transcripts might be inherited maternally. The clock genes are diferentially modulated during the larval and pupal development and, except for *tim2* and *cwo,* all of them respond to the treatment with Juvenile Hormone (JH) in white-eyed pupae. The positive response to JH by *clk, cyc* and *pdp1* might be related to the involvement of these genes on the pathways of the JH signaling, interacting with *Kruppel (Kr-h1)* and *Methoprene-tolerant (MET)* genes. In the adult development, the clock genes *per* and *cry-m* are potential molecular markers of the behavioral plasticity and division of labor in a single-cohort colony, once they did not exhibit transcriptional oscillations in heads of young bees (3 and 7 days-old) during 24h, compared to the robust transcriptional oscillation in old bees (15 and 25 days-old). Additionally, we reconstructed protein-protein and miRNA-mRNA interaction networks and identified putative molecules involved in the post-transcriptional and translational regulation of the clock genes. Among those molecules, we validated interactions between the miR-34 and its binding sites in the 3`UTR of *cyc* and *cwo* by luciferase assay, showing that this miRNA is a negative regulator of both clock genes. We showed for the first time a broad analysis of the circadian clock elements in a social insect, and also identified news molecules with potential to act as modulators of the circadian rhythms. This work expands the knowledge about the biological roles of the circadian clock in honeybees and also contributes to highlight the importance of honeybees as an ideal model to uncover the molecular mechanisms that govern the circadian rhythms, not only in bees, but in other organisms, including mammals.

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ANALYSIS OF DIRS-37_XT RETROELEMENT IN *Xenopus* GENOMES

Johnny Sousa Ferreira[1*]; Camilla Borges Gazolla[1], Adriana Ludwig[2]; Shirlei Maria Recco-Pimentel[3]; Daniel Pacheco Bruschi[1]

[1]Genetics Departament at Universidade Federal do Paraná (UFPR). [2]Instituto Carlos Chagas - Fiocruz-PR. [3]University of Campinas (UNICAMP).

*johnny.sf@gmail.com

Keyword: Anura; comparative genomic; retrotransposon; DIRS.

The diversity and distribution of Transposable Elements (TEs) in amphibians is poorly studied. About 33% of the *Xenopus tropicalis* genome consists of transposable elements. The order DIRS, composed by unusual tyrosine recombinase (YR)-encoding elements, constitutes one of the major groups of retrotransposons. Currently, the order DIRS consists of three superfamilies: DIRS-like, Ngaro and VIPER. Only DIRS-like and Ngaro seems to be present in *Xenopus*, since *VIPER* is specific from trypanosomatids. Different from typical LTR, *DIRS-like* has non-identical inverted terminal repeats (TIRs) and a complementary internal region (ICR) presenting repeated sequences similar to the outer edges. We first analyzed the DIRS-37_XT sequence from Repbase and were able to classify it as a DIRS-*like* since we identified TIRs and the ICR, as well as the domains Gag (Lap2alpha), RT, RH and YR (DNA_BRE-C) with conserved ORFs. The amino acid sequence corresponding to RT was isolated and used in a BLASTn against the genomes of *X. tropicalis* (2n=20) and *X. laevis* (2n=36), two phylogenetically close species. *Xenopus laevis* species was originated by ancient allotetraploid event and evidences indicate distinct families of the TEs evolving independently in each subgenomes. After the tBLASTn, the ten most similar elements recovered were isolated and characterized using ORFfinder and CD-Search tools to verify the corresponding ORFs and conserved domains. The recovered sequences of *X. tropicalis* correspond to potentially encoding copies and few of them presented a DAM- methylase domain. The BLASTn against the Repbase DIRS database revealed that copies present a higher similarity with DIRS-37A_XT, as expected and with DIRS- 28_XT indicating a close relation of these two families. In *X. laevis*, most of the recovered copies also presented ORFs with conserved domains. After, the BLASTn against the Repbase DIRS database, showed that copies present higher similarity with DIRS-13_XL, DIRS-14_XL or DIRS-17_XL. This BLASTn search also indicates the similarity between DIRS-37A_XT and DIRS-28_XT. The problematic in TE nomenclature is clearly evidenced here (DIRS-37A_XT of *X. tropicalis* is probably the same element DIRS-13_XL in *X. laevis*), since the numbers refer to the order in which families were described. Also, we can observe that copies from other families are being recovered in our searches, indicating the close relationship among the *Xenopus* DIRS families reported in the Repbase. A phylogenetic analysis of the RT sequence of the elements will be done for a better understanding of the evolution and relationship of the elements in these genomes.

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CHARACTERIZATION OF SUGARCANE GENES CONTAINING PENTATRICOPEPTIDE REPEAT: THEIR GENOMIC CONTEXT AND DIFFERENTIAL EXPRESSION UNDER BIOTIC STRESS.

Marcelo Marques Zerillo[1]; Luiz Fernando Goda Zuleta[2]; Marie-Anne Van Sluys[1]

[1]Genomics and Transposable Elements Laboratory, Department of Botany, Institute of Biosciences, University of São Paulo. [2]Laboratory of Genetics and Molecular Cardiology - Heart Institute (InCor), University of São Paulo Medical School.

marcelo.zerillo@gmail.com & mavsluys@usp.br

Keywords: sugarcane genome; Pentatricopeptide Repeats; plant-pathogen interaction.

Proteins containing pentatricopeptide repeat (PPR) are ubiquitous in eukaryotes and are mainly involved in the regulation of RNA metabolism. The PPR sequence is composed of degenerated repeats of 35 amino acids, which fold into two antiparallel alpha-helices (helix-turn-helix motif). Each PPR-containing protein contains from 2 to 30 of these motifs and their consecutive arrangement results in a super-helix structure where the RNA molecules are bound. Many of these proteins are essential for photosynthesis and/or respiration and families of PPR-containing genes have greatly expanded among land plant genomes. In some species, the duplicated genomic regions containing PPR genes are considerably variable when compared to orthologous regions of phylogenetically related plants, and as for resistance-genes, PPR genes appear to be highly subjected to evolutionary selective pressure after duplication. We have analyzed 48 PPR-encoding genes from the sugarcane genome, as by: their classification into subfamilies; their differential expression when cane was infected with the fungus *Sporisorium scitamineum*; and their genomic context relativeto neighboring genes. Four distinct subfamilies of PPR-encoding genes were characterized based on their motif arrangement, but that distinction was not translated into distinct patterns of expression when transcriptomes were compared. In fact, we observed at least six different patterns of PPR-gene expression once sugarcane was infected with *S. scitamineum*, and the plant RNA was extracted after 5 and 200 days of the fungus inoculation. Comparative studies of PPR genes in sugarcane genome revealed that they are abundant, highly variable, differentially expressed under stressful conditions and located in dynamic genomic regions, such as in the vicinity of genes responsive to biotic or abiotic stresses, or genes targeting organelles.

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COMPARISON OF RUMEN MICROBIAL PROFILES IN TWO BRAZILIAN LOCALLY ADAPTED GOAT BREEDS.

Flavia Caroline Moreira Bezerra[1]; Joel Fonseca Nogueira[1,2,*]; Ana Paula Ribeiro Silva[3]; Aline Silva de Sant'ana[2]; Sheyla Priscila Oliveira do Nascimento[2]; Gisele Veneroni Gouveia[1,4]; Mateus Matiuzzi da Costa[4]; Daniel Ribeiro Menezes[5]; João José de Simoni Gouveia[1,4]

[1]Grupo de Pesquisa em Genética Animal Aplicada, Universidade Federal do Vale do São Francisco, Petrolina-PE. [2]Programa de Pós-Graduação em Ciências Veterinárias no Semiárido, Universidade Federal do Vale do São Francisco, Petrolina-PE, Brasil. [3]Programa de Pós-Graduação em Ciência Animal, Universidade Federal do Vale do São Francisco, Petrolina-PE, Brasil.[4]Colegiado Acadêmico de Zootecnia, Universidade Federal do Vale do São Francisco, Petrolina-PE, Brasil. [5]Colegiado Acadêmico de Medicina Veterinária, Universidade Federal do Vale do São Francisco, Petrolina-PE, Brasil.

* joel.fonseca@discente.univasf.edu.br

Keywords: *Capra hircus*; Ruminal Microbiome; Metagenome.

Goats are very important for economy in semi-arid region and the assessment its rumen microbiome can provide basis to better understand the aspects involved with health and performance of this specie. The aim of this study was to compare ruminal microbial communities of two locally adapted goat breeds from Northeastern Brazil (Canindé and Repartida). Six female individuals (3 from each breed) were maintained under the same conditions for 40 days and fed twice a day with a diet composed by elephant grass, milled corn, soybean meal and mineral salt. After this period, ruminal fluid was collected, and DNA was extracted for metagenome sequencing. Shotgun libraries were prepared with Nextera® DNA Library Prep Kit and sequenced with HiSeq 2500 from Illumina. Sequences generated were submitted to a quality control with Seqyclean, taxonomic analysis was performed with MetaPhlAn2 and statistical analysis (Fisher exact test) was performed using STAMP. The main groups found in both breeds were Firmicutes, Proteobacteria, Bacterioides, Fibrobacteria, Spirochaetes and Actinobacteria. A higher relative abundance of the families Methanobacteriaceae, Fibrobacteraceae and Ruminococcaceae were observed in the samples of Canindé in comparison to Repartida. Ruminococcaceae is a producer of butyrate and acetate, involved in the first step of microbiome-associated carbohydrate metabolism, already the Fibrobacteraceae family is essential for fiber digestion process. Methanobacteriaceae was the predominant methanogenic in the ruminal fluid and is important in interaction with the protozoa in rumen. Canindé individuals presented higher proportion of the genera Methanobrevibacter, Fibrobacter and Subdoligranulum when compared to the Repartida. The genus Methanobrevibacter is the dominant methanogen found in the digestive tract of ruminant livestock. The genus Fibrobacter is a fermenter of structural carbohydrates, related to the increase in the production of acetate in diets with soluble carbohydrates and starch. The genus Subdoligranulum is essential for butyrate production. Significant differences were observed in the pattern of ruminal microorganisms for the two locally adapted goat breeds, generating insights to the microbial composition and the impact of this relation to each breed.

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HAPLOTYPE NETWORK OF COI SEQUENCES FROM BRAZILIAM POPULATIONS OF *ZAPRIONUS INDIANUS* GUPTA 1970 (DIPTERA: DROSOPHILIDAE): PATTERNS AFTER 20 YEAR FROM AMERICA´S BIOINVASION

Luís Gustavo da Conceição Galego[1]; Claúdia Márcia Aparecida Carareto[2]

[1]Universidade Federal do Triângulo Mineiro (UFTM), Uberaba (MG). [2]Universidade Estadual Paulista "Júlio de Mesquita Filho" – *Campus* São José do Rio Preto (SP).

* luis.galego@uftm.edu.br

Palavras-chave: Brazilian Biomes; introduced Species; Founder Effect

Zaprionus indianus colonized Brazil in the end of 90´s, and quickly it was recorded inall regions of the country, and its current distribution goes from Canada to Uruguay, occupying different biomes along these regions, that suggests a niche generalist occupation, presenting an excellent model for the studies of biological invasions and their ecological and evolutionary unfolding. According to it, the aim of this work was to analyse genetic structuring and isolation of 17 Brazilian populations of *Z. indianus* from different biomes using haplotype network relationships constructed from a segment of the mitochondrial cytochrome c oxidase subunit I (COI) nucleotide sequences. The network haplotype showed two distinct clades, one of them composed by seven haplotypes, including the most frequent H1 (67.6%), detected in all populations, and another for two, including the H2 (29.0%), detected in all populations except in Fortaleza (Atlantic Forest-Caatinga ecotone). The other seven less frequent haplotypes were found only in populations from Brazilian Savanna and Atlantic Forest. Furthermore, the star shape of network haplotype indicate founder effect to genetic variance of COI haplotypes and the H1 as a founder haplotype in Brazilian populations of *Z. indianus.* Finally, a bottleneck effect could explain the distribution of less frequent COI haplotypes in populations from Brazilian Savanna and Atlantic Forest from the other ones.

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ANALYSIS OF LINE ORDER RETROELEMENTS IN *Pipa carvalhoi* (ANURA, PIPIDAE) GENOME WITH EMPHASIS IN REX ELEMENTS

Joana de Moura Gama[1]; Camilla Borges Gazolla[1]; Adriana Ludwig[2]; Shirlei Maria Recco-Pimentel[3] & Daniel Pacheco Bruschi[1]

1- Genetics Departament at Universidade Federal do Paraná (UFPR); 2- Instituto Carlos Chagas - Fiocruz-PR; 3- University of Campinas (UNICAMP).

* danielpachecobruschi@gmail.com

Keyword: Anura, comparative genomics

Pipa carvalhoi is an endemic Brazilian frog from Pipidae family, an ancient group in Anura phylogeny. This species occurs along the river basins Eastern Atlantic Northeast, São Francisco, East Atlantic and the basin Southeast Atlantic. Recently, *P.carvalhoi* genome was sequenced by NGS and revealed an expressive accumulation of transposable elements. Here, we investigated the presence of superfamily *LINE* elements in *P.carvalhoi* with an emphasis in the retroelements generically denominated *Rex*. The Rex elements (Rex1, Rex 3, Rex 5 and Rex6) represents different families isolated for the first time in teleosts fishes and are probably not closely phylogenetic related. However, historically these elements continued to be under this denomination which leads to misinterpretation about evolutionary relationships of these retrotransposons. The copies of all retroelements named as Rex1, Rex3, Rex5 and Rex6 from different species were recovered from Repbase and its reverse transcriptase (RT) amino acid sequences were used as a query in local tBLASTn searches against the *P.carvalhoi* genome. Contigs were retrieved and analyzed by the presence of ORFs in the ORFfinder and protein domains were predicted using the CD- Search tool. The RT domain of retrieved copies was used to phylogenetics inferences together with representative sequences from different LINE elements groups. The sequences were aligned by PSI-coffee and the evolutionary model LG+G was indicated in MegaX using AIC criteria. Bayesian inferences were performed in MrBayes software and the tree was root by the midpoint. We can observe that the LINE copies recovered from *P.carvalhoi* genome correspond to some of the major LINE groups already described: (a) Rex 1, (b) RTE, (c) L1 and (d) Rex5. Our data confirm that Rex families are not related and belong to distinct groups. The analysis of copies revealed a varying level of integrity, retrieving both putative degenerate copies and also several ones containing ORFs potentially encoding the expected enzymatic domains (Integrase and RT). Our results revealed the diversity of the retroelements from LINE order in *P.carvalhoi* and open new perspectives to the study of transposable elements in Anuran genomes.

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ANALYSIS OF DIRS RETROELEMENTS IN *Xenopus tropicalis* AND *Pipa carvalhoi* (ANURA, PIPIDAE) GENOMES

Camilla Borges Gazolla[1*]; Joana de Moura Gama[1];Johnny Sousa Ferreira[1]; Adriana Ludwig[2]; Shirlei Maria Recco-Pimentel[3]; Daniel Pacheco Bruschi[1]

1- Genetics Departament at Universidade Federal do Paraná (UFPR); 2- Instituto Carlos Chagas-Fiocruz-PR; 3-University of Campinas (UNICAMP)

*camilla.gazolla@hotmail.com

Keyword: Anura, comparative genomic; retrotransposon, *DIRS*

Amphibians have a large accumulation of transposable elements (TEs) in their genomes and there are some suggestions that TEs could contribute to the genomic evolution in this group. Retrotransposons from order DIRS encode a tyrosine recombinase (YR) as the key feature and contain some sort of terminal repeats. Currently, the order DIRS is composed of three superfamilies, *DIRS, Ngaro,* and *VIPER*. In this study, we first performed an analysis of all 75 *Xenopus tropicalis* TE families assigned as "DIRS" in the Repbase. From those, 38 families have all the main expected domains (RT-like, RNAseH, and DNA_BRE-C) preserved and could be classified either as *DIRS* (most of the elements) or Ngaro superfamilies (DIRS53_XT and DIRS54_XT) based on the reverse transcriptase (RT) tree. We then proceded a comparative analysis of DIRS54_XT homologous sequences in two amphibia species from Pipidae family: *X. tropicalis,* that is found in Sub-Sahara Africa and *P.carvalhoi,* found in Central and South America. We performed local BLASTn against the *X. tropicalis* reference genome and against a draft of *P.carvalhoi* genome (sequenced by our group), using the DNA sequence of DIRS54_XT from Repbase as a query. Copies were recovered from contigs together with 2-kb flanking regions and their identity was confirmed by Repeat Masking. The 10 most conserved copies were analyzed by the presence of ORFs and protein domains. Our data recovered copies with different level of integrity and a sequence tree showed species-specific clusters. In both species, we retrieved copies containing ORFs that potentially encode the expected enzymatic domains (RT, RNase, and YR). We also found a LAP2alpha domain in the predicted gag-like protein and an additional hydrolase domain, as found for some DIRS elements. The most conserved copies were recovered from the *X. tropicalis* genome. In contrast, in the *P.carvalhoi* genome, we found likely degenerated ORFs in a greater amount of copies. The presence of split direct repeats that is present in Ngaro superfamily elements is being investigated. We are now sequencing the *P.carvalhoi* genome with PacBio technology and the new assembly with long-read sequencing will certainly help to access the conservation of DIRS54_XT homologous sequences and other elements in *P.carvalhoi*. These results suggest that these two frog species (that have diverged at least 136 million years ago) share some DIRS elements that have diversified independently in each genome and offer new perspectives in the study of TEs in amphibians genomes.

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GENETIC VARIANTS IN BRAZILIAN MENNONITES REVEALED A FOUNDER EFFECT OF RHEUMATOID ARTHRITIS IN A WHOLE-EXOME EPIDEMIOLOGICAL STUDY

Denisson de Carvalho Santos[1]; Ismael Júnior Valério de Lima[1]; Caroline Grisbach Meissner[1]; Luana Caroline Oliveira[1]; Nathan Marostica Catolino[2]; Fabiana Leão Lopes[3]; Angelica Beate Winter Boldt[1]*.

[1]LaboratóriodeGenéticaMolecularHumana,DepartamentodeGenética,Universidade Federal do Paraná, Brasil. [2] Rheumatology, Hospital de Clínicas, Federal University of Paraná, Curitiba, Brazil. [3] Human Genetics Branch, National Institute of Mental Health, Bethesda, USA.

*angelicaboldt@gmail.com

Palavras-chave: Mennonites; Rheumatoid arthritis; Founder effect

Introduction: Mennonites belong to the Anabaptist population that originated in Central Europe about 500 years ago. They remained relatively isolated since and established their first communities in southern Brazil in 1930, suffering before, at least three bottleneck effects that reduced their genetic diversity. There are no studies in the Mennonite population addressing rheumatoid arthritis (RA), which has a strong genetic component. In this study we aimed to investigate the epidemiology of RA in the brazilian mennonites and if there are genetic variants predisposing to rheumatoid arthritis. Materials and methods: Information regarding the diagnosis of RA, family members with the disease and risk factors were collected from 469 Mennonites of three Southern Brazilian communities (109 from Curitiba-PR, 150 from Witmarsum-PR and 210 from Colônia Nova-RS), with a questionnaire adapted from the National Health Survey 2013 (approved by the local ethics committee CAAEE: 55297916.6.0000.0102). Whole-exome sequencing data, obtained with combinatorial probe-anchor ligation, was available for 98 participants (10 of those, with a history of rheumatoid arthritis). We selected the variants with Polyphen-2 score≥0.80 and CADD≥20, passing Hardy–Weinberg equilibrium ($p<10- 6$) and call rates >99%) and evaluated them with multivariate logistic regression (PLINK v1.09). Epidemiological data were also evaluated with logistic multivariate regression (STATA). Results: We found 41 cases of RA, of which 33 were women (80,5%, 8 female:2 male), with a prevalence of 8.74%. Affected relatives in the family increased the odds for being diagnosed to the disease (OR=4.09, $P<0.0001$), as well as age (OR=1.05, $P<0.0001$), whereas weekly practice of aerobic exercises were protective (OR=0.19, $P<0.0001$), being each factor, independent of each other. We found 12 variants associated with the disease, two of them, described for the first time in this study ($P<0.01$). These two and another eight polymorphisms were associated with a dominant susceptibility effect, increasing at least 10 times the odds for the disease. Another two were more common (minor allele frequency > 15%) and presented a recessive susceptibility effect. Of the 12 genes, six have been already associated with arthritis or autoimmune reactions in the literature or in genome-wide association studies (THADA, RGPD8, BST1, ADAMTS15, BICD1 and ZFC3H1). The role of the other six genes (if any) should be further investigated (PCDHB5, ALDH1B1, PTF1A, UTP14C, ZDHHC1 and CCDC105). Six polymorphisms were not previously identified in the Brazilian population (AbraOM database). Conclusion: The prevalence of rheumatoid arthritis in the Mennonite population is 8.7 times higher than the reported in Neo-Brazilians. This is possibly the result of a founder effect, which should be further investigated with increasing sample size and functional studies.

RESEQUENCING OF *Xanthomonas albilineans* GENOMES WITH THE MINION - OXFORD NANOPORE PLATFORM

Raquel Paulini Miranda[1]; Paula C. Gasperazzo Turrini[1]; Dora Takiya Bonadio[1]; Marie-Anne Van Sluys[1]

[1]Department of Botany, Institute of Biosciences, University of São Paulo – São Paulo, São Paulo, Brazil.

miranda.rp2008@gmail.com & mavsluys@usp.br

Key-words: *Xanthomonas albilineans;* Genomics; Bioinformatics

Xanthomonas albilineans is the pathogenic agent of leaf scald, a systemic disease that affects sugarcane. In public databases, only one fully sequenced genome is available, that of *X. albilineans* GPE PC73 (Access: FP565176). Aiming to compare the genome structure of Brazilian isolated strains with differing symptom disease phenotypes (Tardiani *et al.* 2014), we performed the resequencing of Xa04 and Xa21. The strains were sequenced with the MinIon platform (Oxford Nanopore) using the long reads sequencing protocol. In addition, the samples were complexed and applied in a single run. Processing of the .fast5 to .fastq sequences was performed with the Guppy software, using GPU processing. Then a *de novo* assembly of the genomes was performed using Canu (Koren *et al.* 2017) and base polishing of the sequence with Pilon (Walker *et al.* 2014). Finally, the assemblies were compared to existing genomic drafts through global and local alignments. The total was generated 740,909 reads with average size of 13,634 bp. Of these reads, 310,818 consisted of Xa04 sequences, 400,830 Xa21 sequences and the remainder were classified as non-barcode readings. The assembly of both genomes resulted in single circular chromosomes with sizes of 3,843,056 bp (Xa04) and 3,956,332 bp (Xa21). Initial comparative analyses have shown that, despite the high global similarity, the genomes present unique regions that differentiate the strains.

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QUANTIFICATION OF THE TRANSCRIPTS OF THE GENE EUKAROTIC STRETCH FACTOR 1A (eEF1A) IN SAMPLES OF PATIENTS AND PROSTATIC CELLULAR LINES

Flávia Mirelle Silva[1]; Dayanne Silva Borges[1]; Isabella Castro Martins[1]; Sara Teixeira Soares Mota[1,2]; Adriana Freitas Neves[3]; Luiz Ricardo Goulaut[2,4]; Thais Gonçalves de Araújo[1,2]

[1]Federal University of Uberlandia, Institute of Biotechnology, Laboratory of Genetics and Biotechnology, Patos de Minas, MG, Brazil. [2]Federal University of Uberlandia, Institute of Biotechnology, Laboratory of Nanobiotechnology, Uberlandia, MG, Brazil. [3]Federal University of Goiás, Institute of Biotechonology, Laboratory of Molecular Biology,Goiás, MG, Brazil. [4]University of California Davis, Dept. of Medical Microbiology and Immunology, Davis, CA, USA.

*E-mail: flaviamirelle05@gmail.com

Keywords: Prostate Cancer, Gene eEF1A, qPCR.

Prostate cancer (PCa) is currently the second non-cutaneous malignant tumor in men, accounting for about 29% of the new diagnoses. Different molecular markers have been described for PCa, although still lacking specificity. The eukaryotic elongation factor 1A gene (eEF1A) is considered to be very promising since it is involved in the regulation of translation elongation. However, the role of this gene in PCa has not yet been fully elucidated. For this reason, we evaluated the transcriptional levels of eEF1A insamples from patients with PCa benign prostatic hyperplasia (BPH) and in prostatic tumor cell lines by qPCR. mRNA levels were correlated with the diagnosis of patients and with cell subtypes. For this, total RNAs were extracted from blood samples, prostatic tissues and from PC3 (castration resistant), LNCaP (hormone-dependent) and RWPE-1 (non- neoplastic) cell lines. The comparative Cq method was used and data were normalized with the reference gene B-2-microglobulin (B2M). The relative expression of eEF1A mRNA in tissue samples was significantly higher in PCa (1.43 fold) compared to BPH ($p<0.05$). The cut-off of 0.71 was established for the transcripts based on the ROC curve, with sensitivity of 73.68% and specificity of 60.0%. After categorization, patients with relative mRNA levels higher than 0.71 presented 4.2-fold higher chance to develop PCa (CI: 1.19-14.81; $p <0.05$). The accuracy of the test was 70% (AUC = 0.70, $p = 0.02$). No correlation was found between the transcriptional levels of eEF1A in tissue and the PSA, Gleason and tumor invasion data. The results for the blood samples showed no difference between groups and did not correlate with clinicopathological data. No statistically significant difference was found among the tumor lines. Our data suggest that eEF1A is an essential marker for malignant transformation of prostatic cells and is not involved in its progression. However, functional studies are needed to validate our hypothesis.

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ACTIVATION OF RNA INTERFERENCE MECHANISM (RNAi) IN SHRIMP *Litopenaeus vannamei* VIA PROBIOTIC *Bacillus subtilis* MANIPULATED BY CRISPR/Cas9

Laura Dall'Agno[1]; Jade Riet[1]; Kamila O. Santos[1]; João C. Filho[2]; Dariano Krummenauer[1]; Wilson Wasielesky[1]; Luis F. Marins[1]*

[1]Federal University of Rio Grande – FURG, Rio Grande, RS, Brazil. [2]University of São Paulo – USP, São Paulo, SP, Brazil.

*lauradallagno@hotmail.com

Palavras-chave: Shrimp farming; CRISPR/Cas9; dsRNA.

RNA interference (RNAi) is a biological mechanism that inhibits gene expression at the transcriptional level by degrading a specific mRNA from related double-stranded RNA (dsRNA). It is considered an important tool in genetic studies, so that several investigations in the field of shrimp farming have been using this technique for the degradation of viral mRNAs. The production of such dsRNAs can be carried out through a strain of *Escherichia coli* (HT115) or commercial kits that are costly. In view of this, probiotic strains are an alternative for the production of dsRNAs, which in addition to being harmless and beneficial to the host, are easily genetically manipulated. Thus, the objective of this work was to test the activation of the RNAi mechanism of *Litopenaeus vannamei* shrimp through the probiotic *Bacillus subtilis* genetically engineered with CRISPR/Cas9 for the production of dsRNAs. For this, juvenile shrimp were allocated in three experimental groups: Group 1: shrimp fed commercial feed; Group 2: shrimp fed with feed supplemented with *B. subtilis* KM0 strain (not producing dsRNAs); and Group 3: shrimp fed with feed supplemented with B. subtilis JJBs3 strain (genetically engineered for dsRNA production). After 15 and 30 days, hemolymph was collected to evaluate the expression of genes (qPCR) related to the activation of the RNAi system (*sid1*, *dicer2*, *argonaute2*) in the hemocytes. In both analyzed times there was a significant increase in the expression of the genes *sid1* and *argonaute2* in the group fed with the strain of *B. subtilis* genetically manipulated, when compared with the other experimental groups. Similar results have been reported for the microalgae *Chlamydomonas reinhardtii*. However, unlike *B. subtilis*, this microalgae is not able to colonize the intestinal tract of the shrimp. These results demonstrate that the probiotic was able to release the dsRNAs into the intestinal tract, which were absorbed by the enterocytes (probably through the Sid1 transporter), released into the hemolymph, reaching into the hemocytes and finally activating the RNAi-related genes. To our knowledge, this study is pioneer in the use of a probiotic for the purpose of activating the RNAi mechanism of *L. vannamei* shrimp. The results obtained here indicate that a probiotic strain genetically manipulated to produce dsRNAs may be effective in the activation of RNAi against shrimp viruses and for other biotechnological applications.

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COMPARATIVE ANALYSIS OF SUGARCANE *Xa21 locus IN POACEAE* FAMILY

Diana Martinez Corcino[1*], Geovani Tolfo Raganin[1], Marie-Anne Van Sluys[1*]

[1.]Genomics and Transposable Elements Laboratory, Department of Botany, Bioscience Institute, University of São Paulo.

*diana.martinez.c@usp.br & mavsluys@usp.br

Keywords: Poaceae, sugarcane, Xa21

Saccharum spp. (sugarcane) is a commercially important crop and a major source of sugar. Leaf scald is an important disease of sugarcane caused by *Xanthomonas albilineans* and the mechanism of resistance to this disease are not still understood. Such as rice, plants in the *Poaceae* family, has at least one analog gene Xa21, which confers resistance against the bacterium *Xanthomonas oryzae*. In view of that *Xanthomonas oryzae* preferentially colonizes the xylem vessels, as well as *Xanthomonas albilineans*, we explored the sugarcane orthologous region for evidences that Xa21 homologs can be involved in the process of resistance to leaf scald in sugarcane. A comparative genomic approach was undertaken in the *Poaceae* of Xa21 analogues initially identified in sugarcane. In the present study, a comparative analysis of a 100 kb region flanking of the Xa21 locus was performed with Oryza sativa, Sorghum bicolor, *Zea mays* and *Setaria viridica* and *Saccharum* spp. (SP80-3280, R570 and the wild cultivar *Saccharum spontaneaum*), to understand the structure and divergence of Xa21 locus in the family *Poaceae* based on 21 sugarcane sequenced BACs. Phylogenetic analysis of Xa21 in *Saccharum* spp. and their counterparts such as *Oryza* provide new insight in the evolution of the *Saccharum* spp.

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Small synthetic RNAs: a new tool for metabolic engineering in *Bacillus subtilis*

Milca Rachel da Costa Ribeiro Lins[1*]; Graciely Gomes Corrêa[1]; Laura Araujo da Silva Amorim[1]; Gabriela Barbosa de Paiva[1]; Danielle Biscaro Pedrolli[1]

[1]Universidade Estadual Paulista "Júlio de Mesquita Filho"-Unesp, Araraquara-São Paulo.

*milca.biomed@gmail.com

Keywords: Small RNAs; Synthetic Biology; Synthetic RNAs.

Optimization of industrial bacterial strains aims at improving the bioproduct titers and strain robustness. *Bacillus subtilis* is widely used as an industrial chassis because it is easily manipulated and produces high bioproduct yields in large fermentation scale. The most commonly used techniques for metabolic engineering of producing strains are deletion and/or insertion of regulatory sequences or genes in the bacterial chromosome. However, these approaches are disadvantageous, as they permanently change the metabolic pathway disturbing the metabolic balance during the *lag* phase of growth, decreasing cell viability. The aim of this work was to modify the regulation of the purine metabolism in *B. subtilis* through synthetic small RNAs (sRNAs) to create a strain with an enhanced intracellular pool of purines. Clonning was performed using Biobrick standard restriction enzymes *Eco*RI, *Xba*I, *Spe*I and *Pst*I. Synthetic sRNAs were designed using the Ribomaker software, targeting the *purE* riboswitch sequence. Two sequences were constructed: sRNA(*purE*), to interfere in the *purE* riboswitch sequence, and sRNA(*purE*) fused to RiboJ, a ribozyme with stabilizing activity on RNA. The sRNAs were cloned between the constitutive promoter P*grac* and the T500 terminator. After strains cultivation and purine quantification by HPLC, it was observed that both RiboJ- sRNA(*purE*) and sRNA(*purE*) reprogrammed bacteria presented increased intracellular guanine concentrations by 60% compared to the control without sRNA. The sRNA with or without RiboJ neutralized the riboregulatory effect of the *purE* riboswitch during the exponential growth phase. Therefore, the rational design of regulatory sRNAs is a real possibility, aiming at neutralizing the regulatory effect of riboswitches or other riboregulation systems, causing redirection in the metabolic flow. In addition, the expression of synthetic sRNAs did not case metabolic stress for the strains. The advantage of this innovative control concept is that gene expression can be either switched ON or OFF as needed and it is an alternative to gene deletion widely used in metabolic engineering. This tool is widely applicable in different strains, being an innovative element in the portfolio of synthetic biology.

Funding: Fapesp; Capes; CNPq

Molecular and functional characterization of variants found in DNA repair genes in a Brazilian cohort with Hereditary Breast and Ovarian Cancer

Simone da Costa e Silva Carvalho [1,2,3], Danielle Barbosa Brotto [1,3], Luiza Ferreira de Araujo [1,3], Lorena Alves Texeira [4], Jéssica Rodrigues Plaça [3], Kamila Chagas Peronni [3], Greice Andreotti Molfetta [1,2], Victor Evangelista de Faria Ferraz [1,2,4], Wilson Araujo da Silva Junior [1,2,3].

[1] Department of Genetics, Ribeirão Preto Medical School, University of São Paulo, Ribeirão Preto, SP, Brazilк [2] Center for Medical Genomics at General Hospital of the Ribeirão Preto Medical School, University of São Paulo, Ribeirão Preto, SP, Brazilк [3] Center for Cell-Based Therapy (CEPID/FAPESP); National institute of Science and Technology in Stem Cell and Cell Therapy (INCTC/CNPq), and Regional Blood Center of Ribeirão Preto, Riberão Preto, Brazil; [4] Department of Medical Genetics of University Hospital of Ribeirão Preto Medical School, São Paulo University, Ribeirão Preto, São Paulo.

Palavras-chave: HBOC; molecular diagnosis; DNA repair genes.

The Hereditary Breast and Ovarian Cancer Syndrome (HBOC) is characterized by families with a history of breast/ovarian cancer inherited in an autosomal dominant manner. This syndrome covers about 10% to 15% of all cases of breast and ovarian cancer, respectively. The *BRCA1* and *BRCA2* genes are high penetrance genes associated with an increased risk of up to 20x for breast and ovarian cancer. However, only 20-30% of HBOC cases present pathogenic mutations in those genes and other DNA repair genes have emerged as increasing the risk for HBOC. In the Brazilian population, mutations in genes such as *ATM, ATR, CHEK2, MLH1, MSH2, MSH6, POLQ, PTEN*, and *TP53* have been reported in up to 7.35% of the studied cases. The aim of this study was to investigate and characterize mutations in 21 HBOC-related genes (*ATM, ATR, BARD1, BRCA1, BRCA1, BRIP1, CDH1, CHEK2, FAM175A, MLH1, MRE11A, MSH2, MSH6, NBN, PALB2, PMS2, PTEN, RAD50, RAD51, TP53* and *UIMC1*) in 95 individuals clinically diagnosed with HBOC in Southeastern Brazil. With the multi-gene panel analysis, we identified 78 pathogenic mutations and variants of uncertain significance (VUS) with conflicting data on pathogenicity for 19 of the 21 genes analyzed. About 23.4% of the patients presented pathogenic mutations in *BRCA1, BRCA2,* and *TP53*, a frequency higher than that identified by other studies in the Brazilian population. However, 76.6% of patients carry variants that are of uncertain significance. Two VUS in *FAM175A* and *UIMC1* genes presented high frequency in the studied cases and when in double heterozygosity, presented deficiency in the repair of DNA damage after irradiation. The association analysis showed 13 VUS in 8 genes, as significantly associated with increased risk for HBOC in about 16% of patients. The functional evaluation of variants associated with risk in *BRIP1, CHEK2*, and *PALB2* and three variants in *BRIP1* and *CHK2* seen to be affecting the protein function. Data such as this present epidemiologic and translational importance, and also emphasizes how important is the characterization of VUS for estimating the risks and thus enabling adequate clinical follow-up and genetic counseling for patients clinically diagnosed with HBOC.

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SEARCHING FOR THE MUTATION ASSOCIATED WITH INHERITED RICKETS IN SANTA INÊS SHEE BREED

Ane de Souza Novaes[1]*, Nara Nagle Vieira Matos Martins[1], Jean Victor Nunes Hissette[1], Keren E. Dittmer[2], João José de Simoni Gouveia[1,3]

[1]Grupo de Pesquisa em Genética Animal Aplicada, Universidade Federal do Vale do São Francisco, Petrolina-PE, Brasil.[2]School of Veterinary Science, Massey University, Palmerston North, New Zealand. [3]Colegiado Acadêmico de Zootecnia, Universidade Federal do Vale do São Francisco, Petrolina-PE, Brasil.

*anedesouza10@gmail.com

Keywords: ARMS-PCR; genetic diseases; *Ovis aries*

Recent studies have shown the presence of mutations associated to genetic diseases segregating in Brazilian sheep flocks resulting in the emergence of some case reports identifying affected individuals for recessive diseases such as dermatosparaxis and spider lamb syndrome. Despite of this, studies aiming to identify these mutations in Brazilian locally adapted sheep breeds are incipient. Hereditary rickets is an autosomal recessive disease characterized by decreased growth rate, thoracic lordosis and deformities in the angular limbs and was first described in New Zealand Corriedale sheep. It is caused by the c.250C> T transition in exon 6 of the DMP1 gene, which leads to the introduction of a premature stop-codon at amino acid 145 of the polypeptide chain. The objective of this study was to screen the Santa Inês sheep breed for the c.250C> T transition in exon 6 of the DMP1 gene. For this, 150 blood samples were collected from flocks located in Bahia and Pernambuco states, and the DNA was extracted using a salting out protocol. A pool containing a wild-type individual (White Dorper CC homozygote) and a confirmed affected individual (Corriedale TT homozygote) was used as a control. Genotyping was performed using an ARMS-PCR approach consisting in 2mM MgCl2; 0.2 mM dNTP; 0.1µM DMP1_O_R; 0.2µM DMP1_O_F; 1.5µM DMP1_I_R; 1.0µM DMP1_I_F; 1U Taq DNA Polymerase; buffer for 1x PCR and 80ng DNA, in a final reaction of 25µl. Amplification steps were: 1) 94 ° C, 5min; 2) 94 ° C, 60s; 3) 60.4 ° C, 60s; 4) 72 ° C, 60s; 5); repeat steps 2-4 for 30x; 6) 72° C, 10min. Genotype confirmation was performed after agarose gel electrophoresis (2%), at 80v for 2h and visualized on ultraviolet light. All genotyped samples were confirmed as CC homozygote, suggesting that there is a low risk of the c.250C> T transition in exon 6 of the DMP1 gene to segregate in the population analyzed. The search for mutations associated to genetic diseases in breeds different from that breed on which the disease was originally described can contribute to the better understanding of the distribution of these mutations and can prevent the dissemination of the deleterious allele, avoiding economic losses caused by the presence of affected individuals.

GENOTYPING OF HUMAN NOROVIRUS IN WATER DESTINED FOR CONSUMPTION IN THE CITY OF BELÉM, PARÁ, BRAZIL, 2016 TO 2018.

Isis Souza[1]; Dielle Teixeira[1]; Lena Morais[2]; Liete Silva[2]; Edivaldo Sousa Junior[1]; Hugo Resque[1]; Yvone Gabbay[1]

[1]Seção de Virologia, Instituto Evandro Chagas (IEC). [2]Seção de Meio Ambiente, Instituto Evandro Chagas (IEC).

*isiss5040@gmail.com

Key-words: norovirus; water; Belém.

Introduction: Microbiological safety of drinking water is necessary, since many pathogens are associated with water-related diseases. Norovirus (NoV) is one of the main causes of gastroenteritis outbreaks worldwide. They are divided into seven genogroups (GI-GVII) with GI, GII and GIV infecting humans. **Objectives**: Genotyping positive GI and GII NoV detected in water samples, targeting two different partial NoV genome regions: ORF1 (RNA dependent RNA polymerase - RdRp) and ORF2 (capsid). **Methodology**: Water samples were monthly collected (January 2016 to December 2018) in two lakes and a Water Treatment Plant in the city of Belém. Two liters of water were concentrated by adsorption-elution method and reconcentrated by centrifugation in Amicon units, obtaining a final volume of 2 ml. Viral nucleic acid was extracted by commercial kit. ORF2 NoV molecular detection was performed by semi- nested (GII) or nested (GI) RT-PCR. The semi-nested RT-PCR was used to amplify GI and GII by partial ORF1 gene. The GI/GII positive amplicons were purified and sequenced in automatic sequencer. The nucleotide sequences were edited, assembled and aligned using the Geneious software, CAP3 and MAFFT program, respectively. The phylogenetic reconstruction was based on the GTR model, maximum likelihood method with bootstrap of 1000 replicates. The phylogenetic trees were generated in the FastTree software and edited in the online tool Evolview. NoV GI and GII prototypes were obtained from NCBI and NoV genotyping tool. **Results and Discussion**: Of 108 water samples analyzed, 9.25% (10/108) were successfully amplified and sequenced: 30% (3/10) by capsid and 70% (7/10) by RdRp. Three different genotypes were identified by capsid: GI.1, GI.3 and GII.4. The nucleotides identity in relation to the prototypes was 87.33 to 89.33% for GI.1; 84.7 to 92.35% for GI.3; 88.64 to 97.73 for GII.4. Only the GII.Pe genotype was found for the RdRp region, with nucleotide divergence ranging from 0.03 to 0.13, regarding prototypes. NoV are genetically distinct, especially genotype GII.4, in which different variants emerges every two to three years with rapid spread across continents and with great epidemic potential. This study identified the GII (GII.4 and GII.Pe) and GI (GI.1 and GI.3) circulation. The GI.1 genotype was previously detected in sewage samples from Belém. It has been reported an increase in outbreaks associated with NoV GII.Pe since 2012. Studies show that the GII.Pe genotype (RdRp) is generally associated with the GII.4 variant Sydney 2012 (Capsid). In this study it was not possible to sequence the capsid region in our GII.Pe samples. therefore, new tests must be performed to obtain this sequences. **Conclusions**: The results obtained demonstrated the existence of a fecal contamination source in the water destined to the public supply, a fact that should be better investigated considering the NoV implication as cause of gastroenteritis.

BRCA1 phosphorylation modulates PALB2 association

Thiago Torres Gomes[1*]; Thales da Costa Nepomuceno[1]; Guilherme Suarez-Kurtz[1], Alvaro N. Monteiro[2], Marcelo Alex de Carvalho[1*]

[1]Instituto Nacional de Câncer; [2]H. Lee Moffitt Cancer Center;

*thiago.torres.456@gmail.com; *marcelo.carvalho@ifrj.edu.br

Key words: Cancer; DNA Damage Response; BRCA1.

Germline mutations in the tumor suppressor gene *BRCA1* correspond to the vast majority of hereditary breast and ovarian cancers. BRCA1 protein plays a key role in the maintenance of genomic integrity through the homologous recombination (HR)- mediated DNA damage response (DDR) signaling. Mechanistically, BRCA1 is recruited to the DNA double-strand break sites by RAP80/BARD1-histone recognition and promotes HR by stimulating CtIP endonuclease activity and 5' DNA end resection. In addition, BRCA1 interacts directly with PALB2 to mediate BRCA2 recruitment to the DNA damaged sites, followed by RPA replacement by RAD51 filaments in single- strand exposed DNA. Recently, the association between BRCA1 and PALB2 was shown to be orchestrated in a cell cycle and DNA damage-dependent manner by post- translational modifications in PALB2, such as ATM/ATR and CDKs-mediated phosphorylations. The mechanisms are not fully understood and, interestingly, ATM/ATR and CDKs phosphorylation sites predicted in BRCA1 are linked to the PALB2-interaction region. Those sites are associated with DDR, but it is not clear whether they modulate BRCA1/PALB2 interaction. By site-directed mutagenesis, we generated BRCA1-phosphomimetic mutants where serine residues target by ATM/ATR and CDKs were replaced by alanine or glutamic acid (mimicking a dominant negative mutant or a constitutive phosphorylated one, respectively). Using a GST pulldown approach, we evaluated BRCA1/PALB2 association in HEK293FT cells overexpressing mutant proteins. Preliminary data suggest that BRCA1/PALB2 interaction is enhanced by ATM/ATR-constitutive phosphorylated mutant in a PALB2 independent phosphorylation status. The understanding of the molecular mechanisms underlying BRCA1/PALB2 association is fundamental to DDR knowledge and, therefore, genomic instability control.

FABC4 EPITOPE MAPPING IN BREAST CANCER

Douglas Alexsander Alves[1,2], Helen Soares Valença Ferreira[1], Sara Teixeira Soares Mota[1,2], Lara Vecchi[2], Galber Rodrigues Araújo[2], Emília Rezende Vaz[2], Mariana Alves Pereira Zóia[2], Matheus Alves Ribeiro[1], Fabrícia Matos Oliveira[3], Yara Cristina de Paiva Maia[2], Luiz Ricardo Goulart[2,4], Thaise Gonçalves Araújo[1,2]

[1]Federal University of Uberlandia, Institute of Biotechnology, Laboratory of Genetics and Biotechnology, Patos de Minas, MG, Brazil. [2]Federal University of Uberlandia, Institute of Biotechnology, Laboratory of Nanobiotechnology, Uberlandia, MG, Brazil. [3]Federal University of Uberlandia, Faculty of Mathematics, Patos de Minas, MG, Brazil. [4]University of California Davis, Dept. of Medical Microbiology and Immunology, Davis, CA, USA.

*douglasalexsanderptu@hotmail.com

Keywords: Breast cancer; Molecular Docking; Epitope-mapping.

Epidemiological data on death rates and incidence of breast cancer (BC) are still alarming, highlighting the need for new strategies for diagnosis and therapy. BC is considered the most common type of tumor in women. It accounts for 25% of all cancers affecting females and the fifth largest cause of death by tumors, worldwide. The complexity of BC relies on the accumulation of numerous molecular changes that lead to the progressive and metastatic malignant phenotype. More studies are needed in order to draw a more complete scenario in BC, especially in triple-negative BC (TNBC). Our group previously described a new antibody in Fab format, FabC4, for diagnostics, and staging of BC. The FabC4 was also a prognosis marker for TNBC. However, further characterization of its target is essential for the clinical management of this neoplasm. The aim of this work was to select FabC4 epitopes through Phage Display-based assay. After biopannig against FabC4, four peptides (pA5, pA7, pC4 and pD6) were selected and molecular modeling, docking and cell culture experiments were performed to characterize them as FabC4 ligands. The peptides and the FabC4 were tridimensional modelled using *de novo*, *ab initio* and homology-based prediction through bioinformatics simulation. Molecular docking analyzes confirmed the interaction between FabC4 and peptides, with better coverage and identity to pC4. This peptide also mimicked both regions of Annexin A2 (ANXA2) and Cytokeratin 10 (CK10) proteins. We showed, for the first time, by immunofluorescence, the cytoplasmic co- localization of ANXA2 and CK10 in MDA-MB-231 cell line, suggesting a new molecular behavior of these proteins on TNBC. Therefore, our strategy resulted on a successful mapping of FabC4 epitopes, opening new perspectives to better understand TNBC.

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STUDY OF ARISING OF PHO-CONSTITUTIVE MUTANTS IN *Escherichia coli*

Henrique Iglesias Neves[1], Beny Spira[1]

[1]Departamento de Microbiologia, Instituto de Ciências Biomédicas, Universidade de São Paulo, São Paulo, Brasil.

* iglesias.henrique@gmail.com

Keywords: PHO Regulon, Growth Inhition, Glycerol-2-Phosphate

Introduction and Objectives: PHO-constitutive mutants lost the ability to repress the transcription of the PHO regulon genes, which are involved in the uptake and assimilation of phosphate compounds. These mutants can be isolated in minimal media containing Glycerol-2-Phosphate (G2P) as the only carbon source. PHO- constitutive mutant colonies start appearing on the selective plate after the third day of incubation. However, upon restreaking on a fresh medium, they form colonies in less than 48 h. hitherto isolated grow colonies on G2P in less than 48 h. We sought to investigate this phenomenon. **Material and Methods:** Cultures of non PHO-constitutive strains of *Escherichia coli* were plated together with a countable number of PHO-constitutive mutants on minimal media containning G2P as the only carbon source. Plates were incubated for two days at 37ºC and the growth of PHO-constitutive mutants was followed. **Results:** Growth of the PHO-constitutive mutants was inhibited by the presence of wild type bacteria. This inhibition was abolished if other carbon sources besides G2P were present on the plate. The growth inhibition was directly proportional to the concentration of wild type bacteria on the plate. A phenotypic screening of a mutant library (Keio collection) showed that the inhibition is associated with the ability of the wild type bacteria to grow on glycerol, showing that the inhibition is associated with the uptake of glycerol produced through the hydrolysis of G2P. When the PHO-constitutive bacteria are let to do few replications, the bacteria can escape the inhibition. Probably, within a micro-colony, mutant bacteria can inter-feed sufficient glycerol to allow the growth. **Conclusion:** The growth of spontaneous PHO- constitutive mutants is prevented by the surround wild type bacteria. The probable mechanism of this inhibition is by the wild-type bacteria uptaking the glycerol produced by the PHO-constitutive bacteria before the mutants can use it as a carbon source for growth.

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INTEGRATION OF RETICULOENDOTHELIOSIS VIRUS INTO THE FOWL POXVIRUS GENOME ASSOCIATED WITH MORTALITY IN VACCINATED PULLET CHICKENS.

Ruy D. Chacón Villanueva[1]; Claudete S. Astolfi-Ferreira[1]; David I. De la Torre[1]; Ana P. Arellano[2]; Antonio Piantino Ferreira[1*]

[1]Department of Pathology, School of Veterinary Medicine, University of São Paulo, São Paulo 05508-270, Brazil. [2]Food Research Center, University of São Paulo, São Paulo 05508-000, Brazil.

* ajpferr@usp.br

Key-words: reticuloendotheliosis virus; retroviral integration; molecular characterization.

Fowlpox virus (FPV) is a pathogen of double-stranded DNA, common in poultry farming and usually controlled by vaccination. Reticuloendotheliosis virus (REV) is an avian retrovirus unusual detected and is associated with immunosuppression and neoplasia. The aim of this study was to evaluate a Fowlpox outbreak, to characterize the Reticuloendotheliosis virus and to assess its integration into the Fowl Poxvirus genome. The outbreak with clinical sings corresponding to fowlpox disease was localized in a pullet chickens farm, with 11-weeks old, vaccinated with a commercial FP vaccine, at São Paulo State in 2019 and where caused 6% of mortality. Nodular lesions obtained from the legs of the birds were harvested and evaluated. After nucleic acids extraction, PCRs were performed to detect FPV, REV as well as other viral oncogenic and immunosuppressive agents. Afterwards, the partial sequencing of the polymerase (pol) and core (P4b) genes for FPV genotyping, and the complete sequencing of the functional genes of REV (gag, pol and env) was carried out to characterize these viruses by phylogenetic analysis and similarity according to other world strains. Additionally, PCRs designed to amplify the integration site of REV into FPV as well as heterologous PCRs which amplify the FPV-REV chimeric genome at 5´and 3´ends of the REV was carried out to evaluate the integration status of this virus. FPV corresponds to the subclade A1 of the genus Avipoxvirus and REV belongs to subtype 3 of REV, the prevalent ones in poultry. Results of PCR and sequencing reveals the co-existence of two types of chimeric FPV-REV genomes, with partial and total REV genomes integrated into FPV. The presence of both strains is likely associated to homologous recombination events occurring during FPV replication or during REV infective activation. According to these results, the outbreak, notwithstanding FPV vaccination, is probably caused for the immunosuppressive action of REV which permits the pathological manifestations. This study remarks the importance of a complete and appropriate diagnosis of REV in Brazilian poultry.

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INTRANASAL IN VIVO GENE EDITING IN MPS II MICE

Luisa Natalia Pimentel Vera[1,2], Roselena Silvestri Schuh[1,4], Ângela Maria Tavares[1,3], Esteban Gonzalez[1,2], Paola Barcelos[1,5] Graziella Rodrigues[1], Édina Poletto[1,2], Helder Teixeira[4] , Roberto Giugliani[2], Guilherme Baldo[1,2,3].

[1] Gene Therapy Center, Hospital de Clínicas, Porto Alegre, Brazil. [2] Post-Graduate Program in Genetics and Molecular Biology, UFRGS, Porto Alegre, Brazil. [3] Post- Graduation Program in Physiology, UFRGS, Porto Alegre, Brazil [4.]Postgraduate Program in Pharmaceutical Sciences, UFRGS, Porto Alegre, Brazil. [5.] Graduate Program in Biology, Porto Alegre, Brazil.

lvera@hcpa.edu.br

Palavras-chave: MPS II, IDS, CRISPR-CAS/9

Mucopolysaccharidosis type II (MPS II) known as Hunter syndrome is a lysosomal storage disease caused by mutations in the IDS gene that results in a deficient production or function of the Iduronate 2- sulfatase enzyme. This enzyme is involved in the breakdown of glycosaminoglycans (GAGs) heparan (HS) and dermatan sulfate (DS) in lysosomes. Progressive accumulation of these substrates causes the loss of lysosomal function, that implies a series of multisystemic symptoms as neurological impairment, skeletal abnormalities, cardiovascular and respiratory problems, leading to death at an early age. By now, therapies are not fully effective or present risks that make it difficult to use. For that, the search for new approaches as gene therapy is justified. The CRISPR-Cas9 system allows precise gene editing, being a potential new gene therapy strategy to treat lysosomal disorders. In this study we treated MPS II mice with a liposomal vector carrying the CRISPR / Cas9 system and IDS gene donor plasmid by intranasal administration. Young adult MPS II mice (n=8) received the complex for 30 days and where sacrificed at 6 month of age to evaluate IDS activity and HS and DS accumulation in tissues, blood and urine. As control groups, we compared the results with wild-type (normal) and untreated MPS II mice. During the first 15 and 30 days the enzymatic activity of the serum IDS was monitored, showing an increase between day 15 and day 30 of 3-4% of normal activity (wt mice), however, the following months after treatment, serum activity decreased to 0.5- 1.5%. A significant increase in IDS activity was found in the heart and lung. In the brain, IDS was elevated in the olfactory bulb and frontal cortex, but this increase was only around 1% relative to a normal mice. GAGs were quantified in serum, urine and all the tissues, and we found that levels of HS and DS where reduced in serum. For GAGs in tissues a significant reduction of HS was observed in the lungs and the liver, although this last one did not show an increase in IDS activity. The results suggest that the intranasal administration of the system leads to improvements in tissues such as the lung, but also to an increase in some brain areas, despite in low levels. Since small increased in IDS activity is found in the brains of patients with non-neurological MPS II, this treatment could still benefit MPS II patients.

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Investigating genetic factors contributing to penetrance and expressivity in carriers of class I 17p13.3 microduplications

Giovanna Cantini Tolezano[1], Marília de Oliveira Scliar[1], Silvia Souza da Costa[1], Walter Luiz Magalhães Fernandes[2], Paulo Alberto Otto[3], Débora Romeo Bertola[4], Carla Rosenberg[1], Angela M. Vianna-Morgante[1], Ana Cristina Victorino Krepischi[1]

[1]Human Genome and Stem-Cell Research Center, Department of Genetics and Evolutionary Biology, University of São Paulo – Institute of Biosciences, São Paulo, Brazil. [2]Department of Pediatrics, College of Medicine of Pouso Alegre, Pouso Alegre, Brazil. [3]Department of Genetics and Evolutionary Biology, University of São Paulo - Institute of Biosciences, São Paulo, Brazil. [4]Instituto da Criança, Hospital das Clínicas, University of São Paulo Medical School, São Paulo, Brazil.

giovannact@usp.br

Key-words: 17p13.3, microduplication, intellectual disability, neurodevelopmental disorder, penetrance, expressivity

The human chromosome 17 is particularly susceptible to submicroscopic rearrangements due to its genomic architecture, resulting in several recurrent different microdeletions and microduplications. Recently, 17p13.3 microduplications encompassing an adjacent region to the deletion of Miller-Dieker syndrome have been documented in association with a variable and not fully penetrant phenotype without pathognomonic signs; however, facial dysmorphisms, neuropsychomotor developmental delay, intellectual disability and/or autism spectrum disorders are commonly seen in affected carriers of these 17p13.3 microduplications. Typically, the presence of a CNV already described as pathogenic is assumed to be the main cause of the clinical phenotype, even when incomplete penetrance and variable expressivity are known to occur. Therefore, alternative genetic factors contributing to the clinical manifestations or even constituting its main cause are not usually investigated. Our study aimed at investigating additional pathogenic variants, by whole-exome sequencing (WES), in two families in which overlapping 17p13.3 microduplications had been identified to segregate with variable expressivity and incomplete penetrance. Family A included three affected maternal half-siblings, two of the boys presenting moderate intellectual disability, strabismus and joint hyperextensibility, and the third one with joint hyperextensibility, speech delay and autism. In Family B, the two affected siblings had microcephaly and speech impairment; the boy also presented autism, while his older sister had congenital glaucoma and learning difficulties. Microarray chromosomal analysis (CMA) was performed in a 180K Agilent platform; genomic libraries for whole exome sequencing (WES) were constructed using Agilent SureSelect Human All Exons V6 for siblings carrying the 17p13.3 microduplications. WES data filtered for rare coding variants that could be deleterious and related to their phenotypes. In Family A, the 17p13.3 microduplication encompassed 730 Kb and was detected in the two half-brothers with intellectual disability, but not in their mother's peripheral blood, thus evidencing germline mosaicism; the third half-brother with autism did not carry the microduplication. Both affected siblings from Family B carried a 450 Kb microduplication at 17p13.3, inherited from an apparently normal mother. WES analysis did not detect any pathogenic or likely pathogenic variant in genes previously associated with intellectual disability, developmental delay or microcephaly (OMIM and Deciphering Developmental Disorders project). A variant of uncertain significance (VUS) in the *SHANK2* gene was detected in the boy with autism from Family B, but not in his sister, who was found to carry a single pathogenic variant in the *CYP1B1* gene, which is related to a recessive congenital form of glaucoma. In conclusion, we did not detect additional pathogenic variants in the half-brothers of the family A. However, likely pathogenic variants in genes already associated with autism and congenital glaucoma were present in Family B, probably adding to the sibling's phenotype.

Funding Agency: CNPq, FAPESP

INVESTIGATION OF A POLYMORPHISM IN THE *D-LOOP* REGION AND THE RISK OF BREAST CANCER IN THE PARÁ STATE POPULATION

Anna Carolina Lima Rodrigues[1], Caio Dantas Alves [1,2], Brenda Suellen Jardim de Oliveira [1], Deyse Dayanne Borges [1,2], Ana Carolina Pinheiro [1,2], Mariana Diniz Araújo[1], Danilo do Rosário Pinheiro [1,2], Rommel Rodrígu Burbano[3], Bárbara do Nascimento Borges[1].

[1]Laboratório de Biologia Molecular, Universidade Federal do Pará - Belém, PA. [2]Universidade da Amazônia - Belém, PA. [3] Hospital Ofir Loyola

Correspondence: Laboratório de Biologia Molecular, Instituto de Ciências Biológicas, Universidade Federal do Pará. R. Augusto Correa, 01. Guamá, Belém, Pará.

rodriguesannacl@gmail.com

Keywords: breast cancer, mitochondrial DNA, susceptibility

The *D-loop* corresponds to a non-coding region of the mitochondrial DNA (mtDNA), with 1,122 base pairs. This region is one of the major mutational hotspots of the mtDNA leading to genomic instability and, consequently, metabolic dysregulation, one of the cancer cell markers. In breast cancer, the influence of mutations in this region is of relative importance due to its functional consequences. The aim of this study was to associate the presence of polymorphisms in the *D-loop* region with the risk of develop breast cancer. Thirty-six samples of patients from Ofir Loyola Hospital and 26 samples from women with no history of cancer, for the control group, were analyzed. All procedures were approved by the Ethics Committee of the Federal University of Pará (043/2008- CEP/NMT) and Ophir Loyola Hospital (184,445/2013). The samples were extracted by the phenol-chloroform method, amplified with standard PCR and sequenced by the Sanger method. Statistical analyses were performed using the BioEstat software. A polymorphism in the 160-170 region was identified and analyzed in the cases and control groups. In all analyses the C5TC4 was considered the wild-type allele. Besides the wild-type allele, other three alleles were found in the control group. When considering the tumoral group, nine alleles plus the wild-type were found. The presence of those uncommon alleles was not correlated with the risk of breast cancer in the population, nor with none of the clinical and histological features of the patients. Our results suggest that the polymorphism in the 160-170 region was not associated with breast cancer in the analyzed population.

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THE *ASPERGILLUS FUMIGATUS* MUCIN MSBA REGULATES THE CELL WALL INTEGRITY PATHWAY AND CONTROLS RECOGNITION OF THE FUNGUS BY THE IMMUNE SYSTEM

Isabella Luísa da Silva Gurgel[1]; Karina Talita de Oliveira Santana Jorge[1]; Nathália Luísa Sousa de Oliveira Malacco[1]; Jéssica Amanda Marques Souza[1]; Marina Campos Rocha[2]; Marina Faria Fernandes[1]; Flávia Rayssa Braga Martins[1]; Iran Malavazi[2]; Mauro Martins Teixeira[3]; Frederico Marianetti Soriani[1]

[1]Centro de Pesquisa e Desenvolvimento de Fármacos, Instituto de Ciências Biológicas, Departamento de Genética, Ecologia e Evolução, Universidade Federal de Minas Gerais, Belo Horizonte, Brazil. [2]Departamento de Genética e Evolução, Centro de Ciências Biológicas e da Saúde, Universidade Federal de São Carlos, São Carlos, São Paulo, Brazil. [3]Centro de Pesquisa e Desenvolvimento de Fármacos, Departamento de Bioquímica e Imunologia, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, Belo Horizonte, Brazil.

*bellaluisa.gurgel@gmail.com

Key-words: *Aspergillus fumigatus;* msbA; immune system

Aspergillus fumigatus is a filamentous fungus, which causes invasive pulmonar aspergillosis in immunocompromised individuals. In fungi, cell signaling and cell wall plasticity are crucial for maintaining physiologic processes. In this context, Msb2 is an important signaling mucin responsible for activation of a variety of mitogen-activated protein kinase (MAPK)-dependent signaling pathways that regulate cell growth in several organisms, such as the cell wall integrity (CWI) pathway. Here, we aimed to characterize the MSB2 homologue in *A. fumigatus*. Our results showed that MsbA plays a role in the vegetative and reproductive development of the fungus, in stress adaptation, and in resistance to antifungal drugs by modulating the CWI pathway gene expression. Importantly, cell wall composition is also responsible for activation of diverse receptors of the host immune system, thus leading to a proper immune response. In a model of acute *Aspergillus* pulmonary infection, results demonstrate that the Δ*msbA* mutant strain induced less inflammation with diminished cell influx into the lungs and lower cytokine production, culminating in increased lethality rate. These results characterize for the first time the role of the signaling mucin MsbA in the pathogen *A. fumigatus*, as a core sensor for cell wall morphogenesis and an important regulator of virulence.

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DUSP3 knockdown contributes to gamma radiation resistance by accelerating myeloid leukemic cells differentiation and polarization

Jessica O. Farias[1*]; Lilian C. Russo[1]; Fábio Luís Forti[1]

[1]Instituto de Química - USP.

*jessicafarias@usp.br.

Palavras-chave: Gamma radiation resistance; myeloid leukemia cells ; DUSP3.

DUSP3, a dual-specificity phosphatase, has been implicated in the maintenance of genomic stability, cell proliferation and differentiation. Our group previously reported the physical interaction of DUSP3 with the protein nucleophosmin (NPM), although the precise mechanisms that mediate these cellular responses have not been fully clarified. NPM is a protein responsible for the indirect regulation of p53 activity, and both have a broad description of mutations related to a diverse set of leukemias. Within this scenario, this work aimed to evaluate the differentiation and polarization of DUSP3- silenced myeloid acute leukemia cells (HL-60 and THP-1) as well as DNA damage/repair following gamma radiation exposure. The differentiation and polarization of THP-1 and HL-60 in macrophages by PMA (9 ng/mL and 100 ng/mL, respectively) were monitored by changes in cell morphology (adhesion), proliferation restrain and surface markers (CD11b, CD14, CD68, CD80, CD206, CD86, HLA-DR, CD163). On the other hand, the DNA damage/repair was quantified by alkaline comet assay and hystone phospo-H2AX after exposure to gamma radiation (15Gy). The obtained results demonstrated higher levels of DUSP3 expression in THP-1 cells compared to HL-60 cells under basal conditions, whereas the latter presented a decrease in DUSP3 protein expression after differentiation. The p53 protein expression was not detected in both cell lines, whereas NPM expression levels dramatically decreased after the differentiation. Therefore, the permanent DUSP3 knockdown contributed to THP-1 and HL-60 differentiation and polarization into M2-macrophages after PMA exposure as well as turned them more resistant to gamma radiation. With that, we provide evidences that DUSP3 is an essential regulator of differentiation and polarization of these immune cells. Moreover, these findings should be further explored in the context of genomic stability, leading to a better understanding of the mechanisms involved in Leukemia's disease prognosis and resistance to chemotherapy.

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blaZ GENE and β-LACTAMIC RESISTANCE IN ISOLATES OF *Staphylococcus aureus*

Gabriela Dias Rocha[1]*; Marion Venâncio Gomes dos Santos [2]; Joanna Adrielly Boaventura[3]; Joel Fonseca Nogueira[4], João José de Simoni Gouveia[5]; Mateus Matiuzzi da Costa[5]; Gisele Veneroni Gouveia[5]

[1]Ciências Biológicas, Universidade Federal do Vale do São Francisco, Petrolina-PE, Brasil; [2]Programa de Pós-graduação em Ciência Animal, Universidade Federal do Vale do São Francisco, Petrolina-PE, Brasil; [3]Medicina Veterinária, Universidade Federal do Vale do São Francisco, Petrolina-PE, Brasil; [4]Programa de Pós-Graduação deCiências Veterinárias no Semiárido, Petrolina-PE, Brasil; [5]Colegiado Acadêmico de Zootecnia, Universidade Federal do Vale do São Francisco, Petrolina-PE, Brasil.

gabdr2511@gmail.com

Key-words: Mastitis; Bacterial resistance; SNP

Bovine mastitis is a pathology that affects not only the herd involved but also the country's economy, and antimicrobial resistance is one of the greatest challenges today. The literature points the blaZ gene as a marker of β-lactam resistance, however, many studies have found sensitivity in blaZ positive strains. The objectives of the present study were to analyze in *Staphylococcus aureus* the relationship of resistance to β-lactam with the presence of the blaZ gene, to identify polymorphisms in the blaZ gene and to verify if the identified mutations can influence the structure and function of the encoded proteins. Sixteen samples of *Staphylococcus* isolates were used. These isolates were obtained from the collection of milk present in the bovine ceiling. *Staphylococcus aureus* were confirmed by PCR of the nuc gene. The positive samples for the gene were used in PCR to verify the presence of the blaZ gene. Positive isolates for nuc and blaZgenes were tested for resistance / sensitivity to beta-lactam, oxacillin, through Minimum Bactericidal Concentration (MBC) and Minimum Inhibitory Concentration (MIC). Three sensitive samples to oxacillin and two resistant samples were chosen for analysis of the presence of polymorphisms in the blaZ gene. For this, the blaZ gene was completely sequenced. Contigs were assembled with the CAP3 program and aligned with CLUSTAR W on MEGA6. Impacts on the structure of the protein encoded by the blaZ gene due to the detected polymorphisms were evaluated with the Provean program and the protein modeling was performed with Swissmodel. The quality of the model generated for each modeled protein was evaluated with the PROCHECK, WHATIF and VERIFY3D programs. The similarity of the dimensional structures of the reference protein was compared to -dimensional structures of the proteins encoded by the TM- align software. Thus, 15 isolates were confirmed as belonging to *Staphylococcus aureus* species, among them 10 samples were positive for the blaZ gene. Only 20% of *S. aureus* blaZ positive isolates were phenotypically resistant to the β-lactam tested. The presence of the blaZ gene in *Staphylococcus aureus* isolates obtained from cases of bovine mastitis was not proportional to the occurrence of phenotypic resistance to the tested β- lactam. Fourteen polymorphisms were detected in the blaZ gene, of which 10 resulted in amino acid changes in the proteins. Four mutations were classified as deleterious by PROVEAN, two exclusive mutations of one oxacillin sensitive isolate and two exclusive mutations of an oxacillin resistant isolate. However, TM-align results indicated that the mutated proteins have practically the same conformation of the reference protein. *In vitro* studies of β-lactamase function need to be performed and, analyzes of the blaZ regulators may aid in the elucidation of this mechanism of resistance.

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Alternative splicing and modulation in HSPs transcripts as a cellular adaptation mechanism in *Trichophyton rubrum*

João Neves-da-Rocha*; Tamires A. Bitencourt; Vanderci M. de Oliveira; Pablo R. Sanches; Antônio Rossi; Nilce M. Martinez-Rossi

University of São Paulo, Ribeirão Preto Medical School, Department of Genetics – Ribeirão Preto-SP, Brazil.

* joão.neves.fonseca@usp.br

Key-words: *Trichophyton rubrum*; heat shock proteins; cellular adaptation.

Trichophyton rubrum is one of the leading species causing dermatophytosis worldwide. In dermatophytes, heat shock proteins have been shown as fundamental for several aspects of their biology: from invasion into host tissues to their degree of resistance and pathogenicity. RNA-Seq analysis of *T. rubrum* exposed to the antifungal agent undecanoic acid (UDA) suggested the occurrence of intron retention events in HSPs genes. Since HSPs are modulated in response to various stimuli and alternative splicing may determine a broad phenotypic diversity in the proteome of eukaryotic cells, our objective was to validate the referred alternative splicing events, as well as to investigate its consequences and extent. Furthermore, we searched to determine: (1) the importance of hsp90 regulation for *T. rubrum* keratinolytic potential and (2) the expression profile of representative HSPs genes in an infection-like scenario. RT and RT-qPCR analyses comparing the presence/absence of UDA and terbinafine (TRB) at different exposure times managed to confirm the occurrence of two isoforms in the transcripts of the *hsp7-like* gene, a member of the Hsp70 superfamily. The frequency of retention events (resulting in mRNAs with premature stop codons), compared to the total gene expression, exhibited quite distinct patterns in response to UDA and TRB, what indicates the existence of a regulatory pathway for the processing of these transcripts according to cellular conditions. Molecular inhibition of hsp90 by 17-AAG at 37°C resulted in a significant decrease in *T. rubrum* keratinolytic potential; withal, the HSPs expression profile revealed two upregulated, two downregulated and four not significantly modulated genes under the infection-like scenario (*T. rubrum* co-cultured with human keratinocyte cell line HaCaT). Associated, these results demonstrate the importance of HSP-mediated stress response pathways for cellular adaptation and other aspects of the biology of dermatophytes, indicating such proteins as potential drug molecular targets for antifungal therapy.

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FROM PARASITES TO GENOME GUARDIANS: B CHROMOSOME CONTAINS piRNA CLUSTERS THAT PROTECT THE GENOME AGAINST TRANSPOSABLE ELEMENTS INSERTIONS

Jordana I. N. Oliveira[1]; **Cesar Martins**[1]

[1]Departament of Morphology, Institute of Biosciences at Botucatu, São Paulo State University (UNESP).

*jordana.oliveira@unesp.br

Keywords: mobile elements; small non-coding RNA; supernumerary chromosomes.

B chromosomes (Bs) are supernumerary elements and their constitution, morphology and number vary among organisms. The B chromosome in *Astatotilapia latifasciata* (Cichlidae) is present in both sexes and is composed of pseudogenes, long-non coding RNAs, coding genes and transposable elements (TEs). In gonads, TEs are controlled by PIWI-interacting RNA (piRNA), a small non-coding RNA transcribed from clusters originated from TEs "junkyards". PiRNAs ensure the genome integrity against new TE insertions. Therefore, the aim of this study was to investigate the piRNAs role under the B chromosome presence, since this chromosome is enriched with TEs. The *A. latifasciata* piRNome was constructed using proTRAC.pl pipeline based on small RNAseq, RepeatMasker, transcriptome and genome annotation data. Looking for piRNAs, B+ genomic regions (B blocks – B+ genomic regions with higher coverage of sequencing reads comparing to the B- genome) were subject to prediction of piRNA clusters. Further, a B coding gene list was investigated to identify piRNA related genes. The B genes were validated by PCR using primers with B specific mutations. The piRNA clusters and gene expression were validated by qRT-PCR using RNA from B- or B+ gonads. Three clusters located into B blocks were identified; these clusters were named *biwi1*, *biwi2*, *biwi3* and are enriched by degenerated TEs. *Biwi1* and *biwi3* are expressed only in B+ samples (males and females), and *biwi2* is upregulated in B+ samples. *Biwi1* has high similarity with BEL32-I retrotransposon. Previous studies reported this element enriched in the B chromosome, but its transcription was not detected, suggesting that *biwi1* from the B could be controlling the BEL32-I element. Additionally, a piRNA biogenesis gene with B specific mutations was found on the B gene list. This gene, "mitochondrial cardiolipin hidrolase" (*pld6*), is an endonuclease that cleaves the piRNA precursor transcripts. The *pld6* expression is also upregulated in the B+ samples. Our data suggest that the accumulation of B+ TEs seems to be co- evolving with piRNA control, also under the control of B gene copy *pld6* in the *A. latifasciata*. Thus, the B could be working to benefit the genome integrity against TEs mobilization, as a genome guardian.

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TRANSCRIPTOME INVESTIGATION OF RNA EDITING IN MONOZYGOTIC TWINS DISCORDANT FOR DOWN SYNDROME

Juan Carlo Santos e Silva[1*]; Cristina dos Santos Ferreira[1]; Ronaldo da Silva Francisco Junior[2]; Douglas Terra Machado[1]; Amanda Pereira Vasconcelos[1]; Ana Beatriz Garcia[1]; Enrique Medina-Acosta[1]

[1]Núcleo de Diagnóstico e Investigação Molecular, Laboratório de Biotecnologia, Universidade Estadual do Norte Fluminense, Campos dos Goytacazes, RJ, Brazil. [2]Laboratório Nacional de Computação Científica, Petrópolis, RJ, Brazil.

*juancss99@gmail.com

Keywords: Allele-specific expression; Down syndrome; RNA editing

A-to-I(G) RNA editing, a sophisticated mechanism of regulation of gene expression, alters allele-specific expression (ASE) that contributes to the phenotype of complex diseases. The objectives of this study were to determine the extent of RNA editing associated with Down syndrome and the impact of editing on phenotypes. We determined ASE profiles using RNA-Seq public experiments from heterokaryotypic monozygotic twins discordant for trisomy 21 (fetal fibroblasts and derived induced pluripotent stem cells, iPSC) and homokaryotypic monozygotic twins (cultured B-cells). ASE differences between pairs were established by comparative transcriptomics. Modular gene co-expression network analysis was used to investigate functional gene sets. Expression single nucleotide variants (eSNVs) were cross-referenced with RNA editing sites from the Rigorously Annotated Database of A-to-I(G) RNA editing, and RNA editing levels were estimated. We found different co-expressed gene modules among the trisomic (T1DS) and euploid twin (T2N). Overall, there were 3955 editing sites in heterokaryotypic twin pair in 615 genes and 1796±413 editing sites in homokaryotypic twin pairs in 397±99 genes. About 5% of all sites exhibited discordant RNA editing levels higher than 25% in either hetero- or homokaryotypic co-twins. In the heterokaryotypic co-twins, the *CYP20A1* gene in fibroblasts exhibited the most substantial difference in the extent of editing (81.25% in the T1DS and 33.33% in T2N). Three known imprinted genes (*SNURF*, *SNHG14*, *ZNF264*) were biallelically expressed (supported by ≥10 eSNVs with biallelic imbalance, with at least four RNA editing sites per gene) in iPSC. In the heterokaryotypic co-twins, nine RNA editing sites will result in non-synonymous mutations. For example, two editing sites in the *CDK13* gene will change lysine (Lys; chr7_39950928) and glycine (Gly; chr7_39950949) to arginine. In the homokaryotypic twin pairs, 0.4% of editing sites will lead to non-synonymous mutations. None of RNA editing sites in either hetero- or homokaryotic create stop codons. Our genome-wide transcriptome scan identified an unprecedented disparity between RNA editing sites in monozygotic co-twins. Moreover, RNA editing may contribute to the biallelic expression of imprinted genes in iPSC.

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ASSOCIATION STUDY OF rs75932628 *TREM2* IN ALZHEIMER'S DISEASE

Jucimara Ferreira Figueiredo Almeida[1,2]; Maira Trancozo[1,2]; Flavia de Paula[1,2].

[1]Núcleo de Genética Humana e Molecular, Departamento de Ciências Biológicas, Centro de Ciências Humanas e Naturais, Universidade Federal do Espirito Santo, Vitória, ES, Brazil; [2]Programa de Pós-Graduação em Biotecnologia, Universidade Federal do Espirito Santo, Vitória, ES, Brazil;

jucimarabiology@gmail.com

Key-words: LOAD; PCR; case-control study.

Alzheimer's disease (AD) is a neurodegenerative disorder, one of the most frequent causes of age-related dementia. The most of cases appeared after 65 years old (late- onset Alzheimer's disease or LOAD). LOAD has about 80% of heritability with a multifactorial inheritance. The ε4 allele in *Apolipoprotein E* (*ApoE*) was the only established susceptibility marker for LOAD. Recently, many groups reported the association of more variants for risk of LOAD and those studies can contribute for establishes biomarkers for the disease. Recently, the C/T rs75932628 polymorphism in the *Triggering receptor expressed on myeloid cells 2 protein* (*TREM2*) gene was identified as a risk factor in Genome-Wide-Association-Studies (GWAS). However, given the conflicting roles of this rare genetic polymorphism in LOAD, it would be of interest to validate it in distinct populations. The present case-control study aimed to analyze the association of rs75932628 polymorphism in *TREM2* gene with LOAD in population from Vitória-ES. The study has a sample of 275 individuals, including 109 patients (mean age 81.5 ± 7.05 years) diagnosed with probable AD according to the NINCDS-ADRDA criteria and 166 controls (mean age of 81.2 ± 7.0 years) matched by sex and age. The polymorphism was genotyped by Real-Time Polymerase Chain Reaction usingTaqMan SNP Genotyping Assay. PCR cycling was performed on 7500 Fast Real-Time PCR System. Statistical analysis was performed by SPSS v23.0 software for Windows and analyzed by Mann-whitney test and Chi-square test. The $p \leq 0.05$ was considered statistically significant. As result, all individuals in our study had the allele C of rs75932628 *TREM2*. The T allele of rs75932628 was not detected in our sample. All patients and controls in our study belong to *TREM2* rs75932628 CC genotype and none showed TT or CT genotypes. Functional studies suggest that levels of TREM2 glycoprotein increased in both plasma and brain in patients with AD. The TREM2 protein activate an interaction of T cells and microglia in brains, can be related in regulating the immune system. In addition, the TREM2 protein could might act to phagocyte amyloid deposit, resulting damage of neurons. The T allele of rs75932628 *TREM2* was identified as a risk allele in Europeans population. However, in this study, the rs75932628 *TREM2* is not associated with LOAD for Grande Vitória-ES population. Our results were corroborated by other studies, such as in Japanese and Chinese population. It can be explained by different genetic risk for LOAD in ethnicities distinct.

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Pharmacogenomic screening at Instituto Nacional de Câncer: *TPMT* and *NUDT15* genotyping for thiopurine chemotherapy.

Karolyne Wolch[1], Anna Beatriz Ribeiro Elias[1], Thais Ferraz Aguiar[2], Lavínia Lustosa Bergier[3], Thais Alcantara Bonilha[2], Eduardo Cerello Chapchap[4], Elaine Sobral Costa[5], Jane Almeida Dobbin[6], Patrícia Guimarães Gonçalves[7], Maura Rosane Valerio Ikoma[8], Marcelo Land[5], Mecneide Mendes Lins[9], Marcia Trindade Schramm[3,6], Mariana Emerenciano[1], Guilherme Suarez-Kurtz[1, 10], Marcela B. Mansur[1]

[1]Coordenação de Pesquisa, Instituto Nacional de Câncer – INCA, RJ; [2]HEMORIO, RJ; [3]Prontobaby Hospital da Criança, RJ; [4]Hospital Albert Einstein, SP; [5]IPPMG - UFRJ, RJ; [6]Hospital do Câncer I – INCA, RJ; [7]Hospital Fundação do Câncer, RJ; [8]Hospital Amaral Carvalho, SP; [9]IMIP, PE; [10]Rede Nacional de Farmacogenética, RJ, Brazil.

karolyne.wolch@inca.gov.br

Keywords: *TPMT*; *NUDT15*; pharmacogenomics.

Thiopurine drugs have a unique role in the treatment of several malignancies, including acute lymphoblastic leukemia (ALL). It has been known for decades that *TPMT* polymorphism is a major determinant of thiopurine adverse effects. More recently, *NUDT15* polymorphism was also linked to thiopurine cytotoxicity, and the 2019 updated revision of the CPIC (Clinical Pharmacogenetics Implementation Consortium) guidelines recommend genotyping for both *TPMT* and *NUDT15* polymorphisms in candidates to receive thiopurine chemotherapy. The Instituto Nacional de Câncer (INCA, RJ) is currently implementing pharmacogenetic screening for oncologic drugs covered by the CPIC guidelines. We report here results obtained up to this point for *TPMT* and *NUDT15* screening in 71 pediatric and adult patients diagnosed with either B-ALL (n = 63) or T- ALL (n = 8). Patient samples and information were obtained through the cooperative efforts of eight Brazilian centers and the project was conducted in accordance with the declaration of Helsinki (Ethical accession number #33709814.7.1001.5274). The data will be updated for the presentation at the SBG congress. Genomic DNA is isolated from bone marrow and/or peripheral blood samples at diagnosis. We performed allele discrimination of commonly reported *TPMT* (rs1421345, rs1800460 and rs1800462) and *NUDT15* (rs116855232) polymorphisms using specific Taqman probes for each SNP. TPMT haplotypes *2, *3A - *3C are derived from the variant alleles, and TPMT metabolic phenotypes are inferred from the corresponding haplotypes. NUDT15 phenotypes are inferred from the presence of the variant rs116855232 T allele. All SNPs were successfully genotyped in 67 patients; poor quality of DNA samples accounted for failure of pharmacogenetic screening in 4 patients. The *NUDT15* rs116855232 variant T allele was not detected in the 67 samples, which is consistent with the rarity or absence of rs116855232 T in populations of European and/or African ancestry. The *TPMT**2 and *3B haplotypes were also absent, in agreement with their rarity in our control cohort in healthy Brazilians. Haplotypes *TPMT**3A and *3C were detected with frequency of 4.5% and 3.0%, respectively. On the basis of the inferred compound phenotypes (comprising both TPMT and NUDT15 phenotypes), CPIC recommendations to consider a reduced starting dose of thiopurine apply to 5 patients (7.5%), whereas the recommendation to start thiopurine therapy with drastically reduced doses is not applicable to the patients analyzed so far.

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DEVELOPMENT OF SPECIES-SPECIFIC MICROSATELLITE MARKERS FOR *Trachurus trecae* USING 454 PYROSEQUENCING

Kenneth Gabriel Mota[1*]; Jussara Oliveira Vaini[1]; Alexandre Wagner Silva Hilsdorf[1]

[1]University of Mogi das Cruzes, UMC, Mogi das Cruzes, SP.

*mota.kenneth2@gmail.com

Keywords: Marine Research; Conservation; Short Tandem Repeat

The species *Trachurus trecae* is a marine fish occurring on the coast of the Eastern Atlantic Ocean between Morocco and Northern Namibia. It is considered an economically important fishing resource for the countries where it occurs. However, overfishing associated with the lack of fishing management has contributed to the decline of this species. Thus, it is necessary to evaluate the genetic diversity of this species, where microsatellite markers are the most frequently used tool in marine fish. This study presents the first set of species-specific microsatellite loci for *T. trecae* to be used in future population studies for sustained management and conservation of this genetic resource. For the development of the panel, a DNA pool of 8 individuals of *T. trecae* was forwarded to GenoScreen/France for commercial microsatellite library production. Enriched microsatellite library was carried out by 454 GS-FLX Titanium pyrosequencing. The identification of microsatellite from the raw sequences was performed using the software QDD. A total of 6,591 sequences were recovered having microsatellite motifs, and 939 sequences presented simple and perfect repetitions with a minimum of ten repeat motifs. Primers sets were designed from the microsatellite flanking regions using software QDD. Twenty-three loci were initially selected for polymorphism assessment, and 12 of these loci amplified reliably and showed evidence of polymorphism. The number of alleles per locus varied from 02 (TTR22) to 12 (TTR09), and observed and expected heterozygosities from 0.17 (TTR22) to 1.00 (TTR01 and TTR11) and 0.41 (TTR14) to 0.98 (TTR22) respectively. Two loci (TTR13 and TTR23) showed significant deviations for the Hardy-Weinberg Equilibrium. This study was the first to develop a panel of species-specific microsatellite markers for *T. trecae*. This tool can auxiliary future population studies, programs of management and conservation of this important genetic resource.

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DECIPHERING NOVEL DUAL REGULONS IN BREAST CANCER

Leandro E. Garcia[1]; Carolina Mathias[1]; Sheyla Trefflich[2]; Mauro A. A. Castro[2]; Daniela F. Gradia[1]; Enilze M. S. F. Ribeiro[1]; Jaqueline C. Oliveira[1]

[1]UFPR, Departamento de Genética. [2]UFPR, Departamento de Bioinformática.

*leandrogarcia20504@gmail.com

Palavras-chave: Breast cancer; ceRNA; ncRNAs

Breast cancer (BC) is the most common cancer in women worldwide, with nearly 60.000 new cases estimated in Brazil in 2018-2019. With the advance of molecular diagnosis, genomic information has been used more often to diagnose and classify breast cancer tumors. BC patients are classified according to molecular characteristics in four main subtypes: basal-like, HER2 enriched, luminal A and luminal B. Aberrant expression of coding genes and non-coding RNAs, such as microRNAs and long non- coding RNAs (lncRNAs) are associated with several aspects of tumorigenesis in different types of cancer, including BC. LncRNAs can interact with miRNAs acting as sponges and with mRNA sharing microRNA response elements, forming a competitive endogenous network (ceRNA). ceRNA interaction is a post-transcriptional regulatory network, in with different types of RNAs competing for shared miRNAs, this process can attenuated the ability of miRNA targeting a specific gene, playing an interference role in gene regulation. In the present study, we evaluated potential lncRNA-miRNA-mRNA interactions using breast cancer TCGA cohort expression data. We utilized the RTN duals R package to calculate common mutual information (CMI) in order to evaluate competing relationships between lncRNA and miRNAs as regulator of coding genes. In our analysis, we found 615 dual regulons (lncRNA and miRNA) with more than 1000 targets (mRNA) involved with regulatory patterns. After statistical analysis, with cutoff adjust p-value <0.001, 141 regulons were considered more significantly. Here we chose the first fifteen more significantly regulons for analysis. In the top 15 networks, we found 10 lncRNA (AC013726.1, LINC02637, AP000251.1 AC100810.1, AC080037.1, AL121760.1, AL136366.1, LINC02321, AC015712.5 and AC008514.1) interacting with 5 miRNAs (hsa-miR-18a-5p, hsa-miR-130b-3p, hsa-miR-937-3p hsa-miR-9-5p hsa-miR- 942-5p) as dual regulons, that have shared coding genes with predicted competitive endogenous potential. For example, a dual regulon identified here, AC013726.1~ hsa- miR-18a-5p have 17 shared targets between the patient samples. AC013726.1 have higher expression in luminal A samples compared to other types and miR-18a-5p have lower expression in luminal A compared to basal-like samples, demonstrating that these two regulators are competing influencing targets in the opposite directions. Betweenthe shared targets in these regulons, we highlight target genes *CDC20, CCNB2, FANCA* are differentially expressed in luminal A compared to basal-like and are also related with poor prognosis. Our results suggest that expression of a fraction of breast cancer genes have the potential to be regulated by ceRNA interactions.

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DUSP3-NPM-P53 AXIS: A KEY REGULATOR OF GENOMIC STABILITY OF XERODERMA PIGMENTOSUM CELLS UNDER UV-INDUCED DAMAGE

Lilian C. Russo; Fábio L. Forti

Laboratory of Signaling in Biomolecular Systems, Department of Biochemistry, Institute of Chemistry, University of São Paulo – SP – Brazil.

lilianrusso@gmail.com

Key-words: DNA repair; UV radiation; Xeroderma Pigmentosum

The dual-specificity phosphatase 3 (DUSP3) is an atypical tyrosine phosphatase that dephosphorylates residues of p-Tyrosine and p-Threonine in protein substrates. DUSP3 is overexpressed in some human tumors, where it regulates cell cycle progression and has preferably nuclear localization. DUSP3 contributes to cellular genomic stability though mechanisms that are beginning to be clarified here. Our group used biochemical, bioinformatics, proteomics and interactome approaches to identify and validate nuclear proteins interacting with DUSP3 under cellular conditions of genotoxic stress promoted by UV-radiation. One of these targets is the nucleophosmin (NPM) protein, which plays key roles in nucleotide excision repair (NER). Surface Plasmon Resonance analyses showed a very strong physical bimolecular interaction between NPM and DUSP3. In order to verify the involvement of this phosphatase in DNA repair, DUSP3 knocking down was carried out in MRC-5 (normal fibroblasts) or XPA cell line (NER pathway deficient XPA protein). Through alkaline comet assay we figure it out DUSP3 knockdown caused delay in DNA repair. In spite of XPA cells do not repair specifically damage caused by UV-radiation, the silencing of DUSP3 got it worse. This effect was supported by immuno-slot-blot assay to test specifically the distortions of CPD and 6,4-photoproducts caused by UV-radiation. Once again, MRC-5 cells exhibited delay in the repair of these lesions under DUSP3 silencing. XPA cells were not able to repair them even after 24 h of the UVC exposure, but the accumulation of CPD and 6,4-photoproduct lesions was bigger under DUSP3 knocking down. Next we verify if NPM was a substrate of DUSP3 at upstream signaling to DNA repair. Immunoprecipitation showed NPM is tyrosine-phosphorylated, and with an increase in the DUSP3 knocked-down cells. Using phospho-specific antibodies designed against the 4 different tyrosine residues of NPM, immunoblotings revealed that DUSP3 specifically dephosphorylates 29, 67 and 271 tyrosine residues of NPM and its sub-nuclear localization after UV radiation. Furthermore immunoblotting and imunofluorescence revealed an unexpected increase in phospho-p53-Ser15 in DUSP3 deficient cells submitted to UV, which can be correlated to nuclear NPM functions in the repair of UV-promoted DNA lesions. Through Immunofluorescence, we observed that knockdown of DUSP3 also causes an earlier nucleolus-nucleoplasm translocation of NPM. This phenomenon is followed by an earlier ARF translocation and co- localization of both proteins with HDM2 and p53. This leads to HDM2-p53 disrupting, non-degradation and earlier phosphorylation of p53. Additionally, DUSP3 knockdown accumulates HDM2 at nucleolus. Interestingly, using cycloheximide to inhibit protein synthesis, we confirmed that the half-life of p53 and NPM is longer in the DUSP3- deficient cells. Altogether our data point out DUSP3 unknown roles in different signaling modules regulating biological processes involved in genomic stability through an unusual target, the NPM protein.

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GENETIC DIVERSITY OF *Bombus dahlbomii* (HYMENOPTERA: APIDAE): ANALYSIS OF HISTORICAL AND CONTEMPORARY COLLECTIONS

Larissa Nunes do Prado[1*]; Paulo Cseri Ricardo[1]; Víctor Hugo Monzón Godoy[2]; Maria Cristina Arias[1]

[1]Departamento de Genética e Biologia Evolutiva, Universidade de São Paulo – Instituto de Biociências, São Paulo, Brazil. [2]Departamento de Biología y Química, Universidad Católica del Maule, Talca, Chile.

*larissa.nunes1996@hotmail.com

Keywords: Chile; pollinator; mitochondrial DNA.

In the last decades, the decline of many natural populations of bumble bee (*Bombus*) species has been documented worldwide. This is the case of *Bombus dahlbomii*, a native bumble bee species of South America, which was historically distributed from central to the south of Chile and Argentina. However, the introduction in South America of exotic bumble bee species and their pathogens has led to a decrease on *B. dahlbomii* distributional ranges. Using pinned museum specimens of *B. dahlbomii*, we were able to evaluate changes in the genetic diversity through time. Initially we tested one mitochondrial DNA region (*CO*I gene) seeking for polymorphic and informative markers to enable comparisons on the levels of genetic diversity between *B. dahlbomii* samples collected before (1964 to 1993) and after (2008) the introduction of exotic *Bombus* species. Samples were obtained from 11 locations in Chile. Surprisingly only one haplotype for the *CO*I region was verified. It is likely that *B. dahlbomii* always had a low genetic diversity for this molecular marker even before the introduction of the exotic species. The low genetic diversity of *B. dahlbomii* may have contributed to increase the species susceptible to threats, e.g., parasites carried by the exotic bumble bee species, thus reducing the viability of their colonies and leading populations to decline. However, other molecular markers should be tested to support this assumption. We hope our data could be helpful to guide future conservation projects of this important pollinator.

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BEEPITRANSCRIPTOME: RNA-EDITING MACHINERY RESPONSIVE TO AGING, NUTRITION AND TISSUES IN HONEYBEES

Luana Bataglia[1*]; Marcela A. F. B. Laure[1]; Zilá L. P. Simões[2]; Francis M. F. Nunes[3]

[1]Departamento de Genética, FMRP – USP. [2]Departamento de Biologia, FFCLRP – USP. [3]Departamento de Genética e Evolução, CCBS – UFSCar.

*luanabataglia@usp.br

Key-words: RNA methylation; aging; diet; *Apis mellifera*.

The adult life of honeybee (*Apis mellifera*) workers is pronounced by behavioral and nutritional transitions of which young bees perform in-hive tasks and consume a protein- rich diet while older bees eat a carbohydrate-rich diet and go outside the hive to forage. These transitions are accompanied by physiological and gene expression changes in many tissues, mostly in the brain and fat body. This scenario of phenotypic plasticity suggests crosstalks between tissues supported by biological circuits involving gene regulatory networks and metabolism. In this context, the post transcriptional RNA editing events are reversible modifications and potential candidates to modulate appropriately this developmental (phenotypic) flexibility in bees. Two types of RNA methylation, the m^6A (N-6 methyladenosine) and m^5C (5-methylcytosine) have been in the spotlight in literature since they are responsive to environmental changes, including feeding, and participate in the regulation of several biological processes such as development and longevity. Our goals were to verify the presence of genic machinery related to m^6A and m^5C methylation in the *Apis mellifera* genome and investigate the expression of these genes in the brain and fat body of bees with differential ages (8 and 29 days) and bees of the same age (8 days) feeding on protein-rich or carbohydrate-rich diets. By using sequence alignments against known m^6A and m^5C methylation genes (writers, readers and erasers) of humans and flies, we found 19 orthologs in the *Apis mellifera* genome. RT-qPCR assays revealed that all of them are expressed in brains and fat bodies of adult workers. In addition, differential gene expression was tested (test T, $p<0.05$). Age and diet do not affect the expression of m^6A genes, but both contexts affect the expression of m^5C genes. Expression of m5C-Mtases increase with aging and a protein-rich diet in brains, but opposite patterns were observed in fat bodies. Global m^6A methylation was measured using a specific-antibody in a colorimetric assay and no significant differences were found between age or diet contexts, reinforcing the expression data. However, patterns of global m^6A methylation were distinct between tissues (test T, $p=0.003$), being more frequent in the fat body-derived transcriptome. The results are the first identification of functional epitranscriptome machinery in *Apis mellifera* and suggest that m^5C modification is more plastic through transitions experienced by adult workers, while m^6A modification seems to be more stable and involved in the maintenance of basic cellular mechanisms.

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ANTI-TUMORIGENIC EFFECT OF THE DICHLOROMETHAN AND HEXANE FRACTION OF THE *Piper nigrum* EXTRACT IN THE CERVICAL CANCER

Luana Pereira Cardoso[1*]; Mayra Carolina da Silva Ferreira[1]; Amanda Regina Acerbi[1]; Leilane Bernardes Freitas[1]; Stefanie Oliveira de Sousa[2] Rosangela da Silva de Laurentiz[3]; Flávia Cristina Rodrigues-Lisoni[1]

[1]São Paulo State University (UNESP), Faculty of Engineering (FEIS), Campus Ilha Solteira, Department of Biology and Animal Science. [2]São Paulo State University (UNESP), Institute of Biosciences, Letters and Exact Sciences (IBILCE), Campus São José do Rio Preto, Department of Biology. [3]São Paulo State University (UNESP), Faculty of Engineering (FEIS), Campus Ilha Solteira, Department of physics and chemistry

*luanacardosobio@gmail.com

Keywords: phytotherapy; cell culture; pepper.

Cervical cancer is the fourth leading cause of cancer deaths in women, it is directly associated with human papillomavirus (HPV) and estrogen. Phytoestrogens act to inhibit the action of estrogen and consequently inhibit tumor proliferation. Among the phytoestrogens are the lignans present in *Piper nigrum* (black pepper), but have not yet been studied very well in the leaves. The research focuses on the metabolites present in the fruit and seeds of this pepper, demonstrating different pharmacological activities, among them the antitumorigenic. The antiproliferative activity of the *Piper nigrum* leaf extract on the uterine cervix was evidenced by our research group, but it is not known which portion of the extract is attributed to this role. Faced with this situation, this research aimed to investigate among the apolares fraction (dichloromethane and hexane), to which fraction is attributed to antiproliferative activity and whether these fractions are toxic and reduce cell viability. Then, the uterine cervix carcinoma (SiHa) cell line was cultivated and treated with dichloromethane and hexane fraction, in concentrations of 10, 50 and 100µg/mL, both extracted from *Piper nigrum* leaf extract, for 4, 24, 48, and 72 hours. The results showed a significant reduction of cell proliferation at all times and concentrations after the treatment, both in hexane and dichloromethane fraction. The data also showed that there was no statistically significant reduction in cell viability and no cytotoxicity from treatment with the hexane fraction. However, the dichloromethane fraction was cytotoxic and reduced cell viability. As there was no reduction of cell viability in the hexane fraction, the mechanism that led to the antiproliferative effect was not cell death but another mechanism that probably inhibits cell division. Therefore, the antiproliferative activity was attributed to two apolar fractions of the leaf extract of *Piper nigrum*, then new studies must be considered and this phytoterapic can be a therapeutic alternative on cervical cancer in the future.

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CHARACTERIZATION OF PHO-CONSTITUTIVE MUTATIONS IN LABORATORY AND NATURAL *E. coli* ISOLATES

Luisa A. V. da Fonseca[1]; Henrique I. Neves; Beny Spira[2]

[1]Departamento de Microbiologia, Universidade de São Paulo – Instituto de Ciências Biomédicas.

* luisavf@usp.br

Palavras-chave: *E. coli*, PHO-regulon, bacterial genetics

Introduction: Mutations are rare changes that occur in the sequence of nucleotides in the genome of an organism. Since the classical demonstration from Luria-Delbrück it is believed that mutations are random, not targeted to any specific loci or in response to specific selective pressures. Since the work of Cairns et al, there is a second category of bacterial mutations that appear to be targeted in response of non-lethal selective pressure, the so-called adaptive mutations. The nature of adaptive mutations is controversial and is still the subject of intense debate. PHO-constitutive mutations have characteristics in common with the adaptive mutations described in literature: they arise only after contact with the selective pressure (glycerol-2-phosphate – G2P, as the only carbon source), new mutants emerge on the selective media throughout the days of incubation and this accumulation presents in a sigmoid curve of accumulation. Nevertheless, recent findings in our laboratory suggest that the origin of these mutants are not adaptive. We believe that the characteristics described above can be explained by a new phenomenon: growth inhibition of the mutants in the selective medium. In order to better understand the nature of growth inhibition, this work aims to characterize precocious PHO-constitutive mutants, comparing them to late mutants. **Material and Methods:** To obtain PHO-constitutive mutants, bacteria from the laboratorial strain MG1655 will be plated on minimal media with G2P as carbon source, in several concentrations. Early mutants are expected to arise around 48h when less than 10 bacterial cells are plated after incubation; while late mutants arise after this interval. Both early and late mutants will be isolated for later tests. Natural E. coli isolates will be collected from the Pirajuçara stream (23°33'53.31"S; 46°42'49.86"W) and PHO-constitutive mutations will be isolated on G2P minimal medium. All mutants, both from MG1655 and from natural isolates will be tested for alkaline phosphatase (AP) activity, complementation essays with plasmids containing combinations of the *pst* operon genes and tested for growth inhibition when plated in the presence of different MG1655 strain concentrations. **Perspectives:** Based on previous results, we expect large variation in the AP levels between the PHO- constitutive mutants isolated from MG1655 and from the natural isolated, regardless of their day of emergence. The hypothesis of inhibition will be strengthened if the we do not observe any significant difference of distribution of locus of mutation concerning the day of emergence and if the same happens for the AP levels between the mutants.

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Expression of longer genes is downregulated in Cockayne syndrome cells after oxidative DNA damage

Maira Rodrigues de Camargo Neves[1], Livia Luz Souza Nascimento[1,2], Alexandre Teixeira Vessoni[3], Carlos Frederico Martins Menck[1]

[1]Department of Microbiology, Institute of Biomedical Sciences, University of Sao Paulo, Sao Paulo/SP, Brazil. [2]Department of Cellular and Molecular Medicine, UC San Diego, La Jolla/CA, USA. [3]Department of Medicine, Washington University in Saint Louis, Saint Louis/MO, USA.

maira.rn@gmail.com

Key-words: Transcriptomics, Cockayne syndrome, transcription-coupled repair, iPS cells.

Cockayne syndrome (CS) is an autosomal recessive monogenic disease caused by mutations in genes coding for proteins CSA and CSB, involved in the DNA repair pathway TC-NER (transcription-coupled nucleotide excision repair). TC-NER is a sub pathway of the nucleotide excision repair, in which lesions that occur in the transcribed strand of actively transcribed genes in the cell are recognized by CSA and CSB when RNA-polymerase is stalled at these lesion sites. CS is characterized by RNA synthesis arrest after UV exposure in affected cells, and its main symptoms include severe neurological and neurodevelopmental problems, as well as premature ageing. This disease symptoms are paradoxical, because while the molecular defect seems to affect all the transcribed portion of the genome, the symptoms appear to be mostly involving nervous tissues. Also, while TC-NER is believed to be responsible for the removal of UV-caused bulky lesions in the DNA, the neurological tissues, most affected by this disease, are not exposed to UV light. In the present work, we are investigating the effects of oxidative lesions in gene expression of CS affected cells. RNAseq data were gathered from control and CS-affected induced pluripotent stem cells (iPSC) and neural precursor cells (NPC) 24 hours after $KBrO_3$ treatment, used to induce oxidative stress. We have identified 3189 differentially expressed (DE) genes in CS NPC after oxidative stress, while only 3 DE genes were found in control NPC, showing that CS cells are much more sensitive to oxidative stress than control cells. The same was observed in iPSC, but not as prominently: 109 DE genes in CS iPSC, while only 1 DE gene was found in control iPSC. PCA analysis revealed that larger genes are more effective in capturing the variance between gene expression in the different conditions than smaller genes, both in iPSC and NPC. This shows that larger genes are more affected not only by oxidative stress but also in CS cells compared with control cells. When analysing the gene length of up and downregulated genes, we found that only CS cells show an enrichment of longer gene lengths in the downregulated gene population. With that, we suggest that CS cells are more sensitive to oxidative stress, and that the expression of larger genes is most altered in these cells. Also, we propose that gene length is contributing to the outcome of expression levels in CS cells.

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Regulatory effect of the pH-responsive *pac-3* transcription factor in the growth and development of *Neurospora crassa*

Maíra P. Martins*; Nilce M. Martinez-Rossi; Pablo R. Sanches; Antonio Rossi

Genetics Department, Ribeirão Preto Medical School, University of São Paulo, Ribeirão Preto, SP, Brazil.

*mairapompeu@gmail.com

Keywords: RNA-Sequencing; Inorganic orthophosphate; pH.

In fungi, the extracellular pH influences gene expression, affecting a range of cellular processes, including growth, differentiation, and development. The zinc finger transcription factor PAC-3/RIM101/PacC has a defined role in the secretion of enzymes and proteins in response to ambient pH, and also contributes to the virulence of species. As previously reported in *Neurospora crassa*, the absence of PAC-3 affects the fungal development and phenotype directly, reducing aerial growth, conidiation, and inducing high accumulation of the pigment melanin. Here, we demonstrate through global transcriptome analyses, that *N. crassa* modulates a range of conidiation and hyphal development-related genes through the PAC-3 transcription factor. In our RNA- sequencing results, conducted under inorganic orthophosphate (Pi) variant conditions, including limited and sufficient Pi availability, we evaluated the association between phosphorus acquisition and pH regulation in *N. crassa* development and survival. We observed that the absence of gene *pac-3* leads the fungi to reorganize gene transcription, aiming to sense and interact with the environment, to resist the gene deletion and ambient conditions, seeking to survive and reproduce. The observed reprogramming transcription can be exclusively coupled to the *pac-3* absence or can be both influenced by the deletion and the associated Pi condition. We highlight this transcription factor as involved mainly in fundamental biological processes, evidencing remarkable regulatory functions of PAC-3.

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Point mutation investigation on soluble *S. mansoni* recombinant *Sm*KI-1 Kunitz-domain protein for functional and structural analysis

Fábio Mambelli S.[1,2*]; Bruno de P. O. Santos[1,2]; Henrico G. T. Gimenez[1]; Sergio Costa Oliveira[1], Mariana T. Q. de Magalhães[2]

[1]Laboratory of Immunology of Infectious Diseases, UFMG. [2]Laboratory of animal Venoms and Toxins, UFMG.

*fabio_mambelli@yahoo.com.br.

Keywords: Kunitz; mutation; *Schistosoma*.

The *Schistosoma mansoni Sm*KI-1 protein, present in both parasite tegument and gut, is intrinsically associated to the parasite survival. Its primary structure presents two domains: (1) a Kunitz-type serine protease inhibitor motif and (2) a disordered C- terminus domain with no homology outside the genera. The Kunitz-domain (KD) has been proved to play an essential role in neutrophil elastase binding blockage, in neutrophil migration/activation impairment and to be of elevated anti-coagulant and anti- inflammatory biotechnological properties. The KD presents in its tertiary structure an κ- helix and double antiparallel κ-sheets, being stabilized by six highly conserved cysteine residues connected in three arrangements of disulfide-bridges, two of them crucial for maintaining native structure conformation. The other disulfide-bridge stabilizes the inhibitory domain and the reactive site (P1), the later one being central for its serine protease inhibitory activity due to an arginine residue (^{18}Arg). Therefore, this study aimed to produce the *Sm*KI-1 KD domain in its functional form for point mutations for serine protease inhibition assays and structural analyses based on previous *in silico* investigation. The domain (here termed KD) was cloned and expressed in a specific system developed in our lab in order to recover a functional and soluble protein after cell lyses. After several strategies used for correctly assembled protein recovery, the functional and folded KD was obtained. Structural integrity conformation was checked through circular dichroism and 1D-NMR experiments. *In silico* analyses were performed for selection of key aminoacids for point mutation studies. The ^{18}Arg residue on the reactive site (P1) or ^{14}Glu due to its close interaction with this residue seem to be key residues for enhancement of its inhibitory activity, generating powerful biotechnological products. *In silico* point mutations on the inhibitory site were assessed to predict its structural changes on *Chimera* software. Two mutations were chosen: R18L, changing charge and adding hydrophobicity to the system, and R18G, changing significantly the size of the residue on the P1 site. R18L point mutation seemed to disrupt the formerly strong interaction between ^{18}Arg and ^{14}Glu on the inhibitory loops. This mutation has been associated to enhancement of elastase affinity towards another Kunitz-containing molecule. On the other hand, R18G seemed to compromise the P1 stability, showing the importance of this residue for the inhibitory activity of this molecule. *In vitro* experiments are being carried out for validation and structural analyses. In summary, our results described not only an optimized system from the expression of a functional disulfide-rich protein but also gave us insights on important KD residues allowing us to design new molecules for biotechnological applications.

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TOOLS FOR MOLECULAR IDENTIFICATION OF SHARKS AND RAYS (ELASMOBRANCHII) PRODUCTS AND THEIR IMPLICATIONS FOR THE CONSERVATION OF BIODIVERSITY

Marcela Alvarenga de Almeida Simões[1]; Daniela Ferreira dos Santos de Souza[1]; Rafaela Guilherme Soares[1]; Antonio Mateo Sole-Cava[1]; Frederico Henning[1*]

[1]Departamento de Genética, Instituto de Biologia, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brasil

Palavras-chave: conservation genetics, elasmobranchii, NGS

The exploitation of natural fish resources is an increasing activity, leading to overfishing, depletion of natural genetic variation and loss of biodiversity. Seafood mislabeling is widespread, which is harmful to the economy, consumer rights, public health and species conservation. Sharks and rays (Elasmobranchii) have 31% of its species at the IUCN Red List of Threatened Species, which highlights the need for an increase in the efforts for the conservation of this subclass. In Brazil fishing and marketing activities of threatened marine species are forbidden. However, inspection is poor due to the difficulty of morphological identification (post-processing landing) and the paucity of DNA sequences, which impairs the molecular identification. Here, we report the expansion of the molecular database and the development of PCR-based assays for species identification. We identified threatened species of shark and ray sold as "cação" (generic term for shark meat) and guitarfish in Rio de Janeiro, Brazil. DNA sequences of species from Brazil coast, including confection of 8 mitochondrial genomes were newly obtained by targeted-enrichment using RNA baits in a single MiSeq run, resulting in fully circular annotated mitogenome sequences. A PCR multiplex approach and a series of species- specific primers were designed to allow time- and cost-efficient identification. 106 samples distributed as "cação", 72 distributed as guitarfish and 99 entire fish were used to compose the RENIMP (Rede Nacional de Identificação Molecular do Pescado) database. DNA was extracted followed by PCR amplification of the mitochondrial genes Cytochrome B (CytB) and Cytochrome Oxidase I (COI) and sequencing using a capillary system (ABI3500). The sequences were compared to sequences available on Genbank using BLAST (Basic Local Alignment Search Tool) and used for phylogenetic reconstruction, made for both genes by maximum likelihood. The PCR multiplex approach involved the design of specific primers based on the previously available as well as new DNA sequences. We employed a hierarchical multiplexing approach where first identification was performed on the order level (Carcharhiniformes, Lamniformes, Squatiniformes and for the superorder Batoidea) followed by specie-specific multiplex PCRs. We detected 32.5% and 20% of mislabeling and 32.5% and 80% of threatened species distributed as "cação" and guitarfishes, respectively. Including teleost fish in the mislabeling, as *Xiphias gladius* (12%) and species critically endangered, as *Pseudobatos horkelii* (8.6%) and *Squatina occulta* (1.7%). Only 2 species that can be legally sold (least concern and near threatened) were detected, *Prionace glauca* (32.7%) and *Pseudobatos percellens* (3.5%). We conclude that despite legislation, endangered elasmobranchs are commercialized in high numbers and the rate of mislabeling is high. This work highlights the need for the development of molecular tools to inform legislators and foster regulations that are more effective for the conservation of the elasmobranchs.

TRANSLESION SYNTHESIS DNA POLYMERASES FOR TEMOZOLOMIDE INDUCED DNA DAMAGE

Marcela Latancia[1]; André Uchimura Bastos[1]; Natália C. Moreno[1]; Clarissa RR Rocha[1]; Carlos FM Menck[1]

[1]Institute of Biomedical Sciences, University of Sao Paulo (USP), Sao Paulo, SP , Brazil

marcela.latancia@usp.br

Keywords: Temozolomide; Translesion DNA polymerase; CRISPR/Cas9

Translesion DNA polymerases are capable of replicating damaged DNA, without removing the lesions, performing translesion synthesis (TLS), a mechanism of DNA damage tolerance. Tumor cells use this mechanism in order to survive lesions caused by chemotherapy and, therefore, this may be a strategy that tumor cells use to resist treatments. Moreover, this process is error-prone and can lead to mutagenesis increasing resistance potential of tumor cells. Little is known about the role of TLS in in tumor therapy with temozolomide (TMZ). This drug is an alkylating agent that damage DNA. Our aim is to investigate how TMZ affects TLS mutated cells, to understand how these polymerases are related to cells tolerance. Hence, we firstly treated primary fibroblasts cells lacking TLS mechanisms (XP-V, deficient on DNA pol eta) and checked for cell survival (XTT) and apoptosis (Sub-G1 by flow cytometry). We observed that TMZ diminishes cell viability in XP-V cells, indicating that pol eta is important to overcome TMZ-induced DNA damage. We also used TCGA data to analyze TLS expression in glioblastoma patients. Curiously, in recurrently patients treated with TMZ, POLK (another TLS polymerase) expression was significantly increased. Therefore, we performed by CRISPR/CAS9 knockout (KO) of glioblastoma cell lines, U138 and U251, to the following genes of TLS polymerases: REV1, Pol iota and Pol kappa. After clone selection, KO cells were validated using Sanger sequencing. We observed that cell viability after TMZ treatment was impaired in KO cells. In summary, as a partial conclusion, we can infer that TLS enzymes play important roles overcoming resistance to TMZ. As perspective, we intend to observe how cell cycle is affected in these KO cells treated with TMZ by PI-staining using Flow Cytometry and measure DNA strand breaks by Comet Assay.

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GENOMIC SEQUENCING AND BIOINFORMATICS ANALYZES IN *PROCERATOPHRYS* (AMPHIBIA, ANURA) REVEALS A GREAT ABUNDANCE OF SATELLITE DNAS

Marcelo J. Silva[1*]; Thiago Gazoni[1]; Célio F.B. Haddad[2]; Patrícia P.P. Maltempi[1]

1Departament of Biology, Bioscience Institute, UNESP, Rio Claro, São Paulo, Brazil. 2Departament of Zoology, Bioscience Institute, UNESP, Rio Claro, São Paulo, Brazil.

* marcellojoao010@gmail.com

Key-Words: Cytogenetics; Centromere; RepeatExplorer.

Satellite DNA are characterized by sequences of monomers and thousands of these monomers can construct homogeneous and megabase-long genomic segments. Normally, any DNA sequence accumulates mutations after time, and contrary to what can be expected, the divergence in the nucleotide sequence between monomers within satellite DNA arrays is generally quite low, not exceeding a few percentages. Because they are repeated tandem sequences, satDNAs comprise long matrices located in a highly compacted heterochromatin, usually in the centromeres and telomeres. In this sense, the species *Proceratophrys boiei* (2n = 22) is an interesting target for the search of repetitive sequences, since it has a karyotype with large heterochromatic blocks in their centromeres and a ZZ:ZW sex system with a large amount of heterochromatin related to these chromosomes. Therefore, the study of these sequences can contribute to the understanding of the evolution and diversity of repetitive DNAs, besides the understanding of genome organization. Illumina® sequencing data were used to be analysed in the RepeatExplorer software, it was found that 41% of the species genome corresponds to repetitive sequences (218 clusters), including satDNAs (28 clusters; 15%) and other repetitive elements. The size of the sequences ranged from 31 to 304 bp (mean 91 bp), the A+T amount was 47.8% to 72.7% (mean 55.4%) and the ratio of the reads in the genome was 0, 0210 to 8%, according to the analyzes performed. The most abundant satDNAs were named from PboSat1-176, PboSat2-173 and PboSat3- 189 and mapped by FISH on *P. boiei* chromosomes. The satellite DNAs studied here hybridized in the centromeric and pericentromeric region of the chromosomes, suggesting a possible involvement of these sequences in centromeric function. The centromere is a complex and specialized locus, crucial for proper chromosomal segregation in the cell divisions. Functional centromeres are usually positioned in large domains of satellite DNAs. In addition, an interesting feature of many centromeric satellite DNAs is the similarity in the length of the monomer, which in turn corresponds to the length of the nucleosomal unit, thus supporting a structural role of those sequences. The impact of rapidly evolving satellite DNAs can be seen in the context of species radiation. One form of tandem repeat sequences can be evolutionarily favored in (peri) centromeric regions because long matrices exhibit double characteristics: maintaining sequence homogeneity crucial for centromere stability while at the same time being a source of extremely rapid changes.

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CHANGES IN MITOCHONDRIAL MORPHOLOGY AND DOWN- REGULATION OF ESTROGEN-RELATED RECEPTOR GENE EXPRESSION ARE IMMEDIATE RESPONSES TO JUVENILE HORMONE IN HONEY BEE CASTE DEVELOPMENT

Matheus Schefer[3], Douglas E. Santos[1], Luciane C. Alberici[2], Klaus Hartfelder[1]

[1]Departamento de Biologia Celular e Molecular e Bioagentes Patogênicos, Faculdade de Medicina de Ribeirão Preto, Universidade de São Paulo, Ribeirão Preto, SP. [2]Departmento de Física e Química, Faculdade de Ciências Farmacéuticas de Ribeirão Preto, Universidade de São Paulo, Ribeirão Preto, SP. [2]Departmento de Genética, Faculdade de Ciências Farmacéuticas de Ribeirão Preto, Universidade de São Paulo, Ribeirão Preto, SP.

scheffer.94@usp.br

Keywords: *Apis mellifera*; juvenile hormone; mitochondrial function.

Caste differentiation division in the honey bee *Apis mellifera* is regulated by the interaction of juvenile hormone (JH) with nutrient sensing signaling pathways (IIS, TOR and EGFR) and, through hypoxia signaling, also with caste-specific mitochondrial dynamics. For instance, queen larvae have higher JH titers and higher respiration rates than worker larvae. Nonetheless, we do not know how these factors are functionally connected. Here we investigated how exogenous JH directly affects the expression of genes in the fat body of worker larvae. Fourth (L4) and early fifth instar worker larvae (L5F2) were collected from brood frames of honey bee hives and transferred to an artificial rearing system that sustains worker development in the laboratory. Three groups were defined consisting of 5 larvae each. In the first group, the larvae received a topical application of 10 µg of synthetic JH-III in 2 ĸL acetone, which is a dose that knowingly can induce queen development. The second group received the same volume of solvent, and the third group consisted of untreated larvae. After 1, 6, and 24 hours, the fat body of these larvae was dissected for RNA extraction and cDNA synthesis for transcript quantification by RT-qPCR. The efficiency of the treatment was assessed from the expression of *kr-h1*, an early-response gene to JH, and the JH response was tested for several candidate genes, such as the hypoxia pathway genes *sima*, *tango,* and *fatiga*, the mitochondrial biogenesis genes *TFB1*, *TFB2,* and *ERR*, genes involved in antioxidant system functions (*catalase*, *MnSOD*, *CuZnSOD*), and *FOXO* as a general downstream readout of the nutrient signaling pathways. For *kr-h1* we found a very rapid, transient increase in expression in both larval stages of the JH treatment group, as expected for an early response gene, but for the other genes we found a significant change in transcript levels only for *ERR,* which encodes an estrogen-related receptor (*ERR*). Likewise, the high resolution respirometry analysis did not reveal any difference in oxygen consumption as a direct response to JH. However, we observed a very rapid response to JH when looking at the mitochondrial morphology, especially so in L5F2 larvae, as already within one hour after the JH treatment we observed a cleft in the inner mitochondrial membrane indicating a switch from a longitudinal, extended morphology to a cup-shaped one, which is typically observed in the fat body of queen but not in worker larvae. We conclude that, although JH is not directly connected to mitochondrial efficiency (oxygen consumption) in a general sense, it lowers the expression of *ERR*, a gene that is predicted to connect mitochondrial function with metabolism, and it can quickly alter the shape of the mitochondria from a worker to a queen morphology.

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AIRE GENE CONTROLS mRNAs AS WELL AS lncRNAs IN MEDULLARY THYMIC EPITHELIAL CELLS AS EVIDENTIATED BY GENOME EDITING WITH CRISPR/CAS9

Max Jordan Duarte* [1], Amanda Freire Assis [1], Cesar Speck-Hernandez [1], Pedro Paranhos Tanaka[1], Geraldo Aleixo Passos* [1,2]

[1]Molecular Immunogenetics Group, Department of Genetics, Ribeirão Preto Medical School, USP, Ribeirão Preto, SP. [2]Discipline of Genetics and Molecular Biology, Department of Basic and Oral Biology, School of Dentistry of Ribeirão Preto, USP, SP.

*maxjordan@usp.br, *passos@usp.br

Keywords: Aire; lncRNAs; CRISPR/Cas9.

Introduction. The Autoimmune regulator (Aire) gene is a non-classical transcription factor of medullary thymic epithelial cells (mTECs) whose encoded protein releases chromatin-anchored RNA Pol II and regulates the expression of self-peripheral tissue antigens (PTAs) mRNAs in the thymus gland. The biological function of this gene is linked to central immune tolerance in the thymus preventing the onset of autoimmune diseases. Induction of immune tolerance is a result of negative selection of thymocytes (T-cell precursors) that strongly recognize PTAs presented by mTECs. Co-cultures of mTECs with thymocytes represent an adequate in vitro model-system to approach the cell interaction that occurs within the thymus. Previous results from our laboratory demonstrated that besides the control of PTAs by mRNAs, Aire also participates in the modulation of miRNAs in mTECs, since this RNA species is transcribed by RNA Pol II. Considering that RNA Pol II also transcribes long non-coding RNAs (lncRNAs), we raised the **hypothesis** that Aire might modulate the expression of this RNA species. Thus, the **objective** of this study is to evaluate whether Aire could be linked to the expression of lncRNAs in mTEC cells. **Material and Methods.** To test this, we use microarray hybridization data to explore the expression of mRNAs as well lncRNAs of wild-type mTECs and an Aire $^{-/-}$ KO clone previously obtained in our laboratory by Crispr/Cas9 mutagenesis in the exon 3 of the Aire gene (clone mTEC 3.10E6 Aire KO $^{-/-}$). Microarray data were analyzed using the R platform to trace expression profiling and hierarchical clustering of mRNAs and lncRNAs (heat-maps) for comparing wild-type mTECs vs clone mTEC 3.10E6 co-cultured with thymocytes during 12 or 36 hours. The differentially expressed mRNAs were functionally annotated according to their biological function using the DAVID annotation tool and mRNA-lncRNA interaction force was calculated using the LncTAR tool. Moreover, we reconstructed an mRNA-lncRNA interaction network using cytoscape software. **Results and Discussion.** The mTEC 3.10E6 clone co-cultured with thymocytes during of 12h modulates 38 lncRNAs (10 up- and 28 down- regulated) and during 36h modulates 58 lncRNAs (16 up- and 42 down-regulated), which establish interaction with mRNAs. The set of modulated lncRNAs were categorized as lincRNAs, antisense RNAs or bidirectional RNAs. Among the differentially expressed mRNAs were found those involved with modulation of the transcriptional activator for the RNA pol II promoter, phosphoprotein, and positive regulation of gene expression. These results demonstrate that Aire may act as an upstream transcriptional modulator in mTEC cells controlling both coding and non-coding RNAs.

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EVALUATION OF ALCALOID PIPLARTIN ACTIVITY IN UTERINE SQUAMOUS CELLS

Corrêa Beltrame Mariana[1]; Soares Pienna Christiane[1].

[1]Faculdade de Ciências Farmacêuticas, Unesp, Araraquara.

marianacorrea16@gmail.com

Key words: Piplartin, Cervical cancer, *Piper longum L*, HPV, HPV16.

Cervical or cervical cancer is responsible for approximately 15.000 cases in Brazil. The most common histological type of this cancer is squamous cell carcinoma, accounting for about 85% to 90% of cases, caused in majority of patients by Human Papillomavirus (HPV) infection. In virus infection, the peak of incidence occurs between 20 and 25 years old, followed by a decline that reaches a plateau around the age of 35. The second peak of incidence occurs around 45 and 50 years. Although the vaccines are a global reality, the approach is to young girls without the first sexual intercourse. Moreover, at this moment, the therapeutic vaccines have not present good results. Therefore, the present study purposes a new therapeutic strategy against HPV 16 (the more prevalent type). Previous studies of our laboratory demonstrated that alkaloids are good class of chemical compounds to treatment of cervical cancer associated to HPV16. This way, the alkaloid piplartin (PL), isolated from *Piper longum L.* (long pepper), seems to be a good candidate to therapy to cervical cancer related to HPV. Thus, the aim of the present study was to evaluate the cytotoxicity of PL in cervical cancer cell lines positive to HPV16 (SiHa), not positive to the virus (C-33A) and spontaneous immortalized human keratinocytes (HaCaT). We evaluated apoptosis and/or necrosis by the Annexin V, as well as, by Hoechst and Propidium Iodide cytomorphological assay in order to differentiate early and late apoptosis, as well as, necrosis. Moreover, crystal violate method was performed to verify cell proliferation in the all three-cell lines. According to the scientific literature, PL has been demonstrated low toxicity and induction of cell death, causing as consequence an inhibition or reduction of cell proliferation. In our results, with cytotoxicity assay (Sulforodamine B), the concentrations of PL confirmed its low toxicity. Through of cytotoxicity, we determined the IC_{50} and all treatment concentrations (5, 10, 12,5, 15, and 20 μM) to all cell lines. To apoptosis assay with annexin V and cytomorphological method, using Hoechst, propidium iodide and fluorescein diacetate, we verified late apoptosis and necrosis in tumor and non-tumoral cervical cancer cell lines. In crystal violet method, we observed less cell proliferation in cervical cancer cells lines HPV 16 positive. In conclusion, Piplartin seems to be able to induce apoptosis and reduce proliferation of tumor cells and its do not cause cytotoxicity in non-tumor cells.

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LUDIC-LEARNING ACTIVITIES IN TEACHING AND LEARNING CONCEPTS OF GENETICS WITHIN THE DISCIPLINE OF BIOLOGY IN HIGH SCHOOL

Mirian Vieira Teixeira[1*]; Kleber Santiago Freitas e Silva[2]

[1] Secretaria de Estado da Educação de Goiás. [2]Universidade Federal de Goiás.

*biomvite@gmail.com

Keyword: Teaching of Genetics; Ludic; High school

In high school, it is common for Biology teachers to report difficulty in teaching Genetic contents. Most concepts are reasonably abstract, which often causes students to have difficulty visualizing those concepts, leading to loss of interest and impairing learning. It is necessary to use alternative models and practices that make lessons more attractive, interesting and enjoyable. The objective of the present work was to apply and evaluate two different activities involving basic concepts of Genetics. One is based on crosswords and the second a per se game for high school students. The obtained data suggest that the proposed leisure activities facilitated the understanding of the contents, and were adequate for the students' learning. The use of active learning methods, such as game-based learning, enable better a engagement and a good assimilation of the Genetic contents. Thus, the use of playful activities in the teaching and learning of Genetic within the Biology area, instigate students' ability to relate to themselves, to colleagues and to the surrounding environment, promoting the interaction between the events in the classroom and stimulating the students' will of learning.

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UNRAVELING MIRTRONS KNOWLEDGE WITH DATA MINING AND BIOINFORMATICS METHODS

Bruno Henrique Ribeiro da Fonseca[1]; Douglas Silva Domingues[2]; Alexandre Rossi Paschoal[1*]

[1] Departamento de Computação, Programa de Pós-Graduação em Bioinformática (PPGBIOINFO), Universidade Tecnológica Federal do Paraná, UTFPR, Cornélio Procópio - PR. [2] Departamento de Botânica, Instituto de Biociências, Universidade Estadual Paulista, UNESP, Rio Claro - SP.

* paschoal@utfpr.edu.br

Keywords: mirtrons; biological data; data analysis.

Mirtrons arise from short introns with atypical cleavage by using the splicing mechanism, and it can act in mRNA regulation processes in animals and plants. In the current literature, there is no repository centralizing and organizing the data available to the public. To fill this gap, we developed mirtronDB, the first knowledge database dedicated to mirtrons, and it is available at http://mirtrondb.cp.utfpr.edu.br/. MirtronDB currently contains a total of 1,407 precursors and 2,426 mature sequences in 18 species (chordates, invertebrates, and plants). This repository is a specialized and unique resource to explore mirtrons data and its regulations. The mirtron data available provided initial mirtrons characterization and novel discoveries. In our mirtron bioinformatics and data mining analysis, we highlighted that that most studies on mirtrons were focused on *H. sapiens* and *M. musculus*. Consequently, we identified more similarity results among chordates than in the other groups. We also highlight that chordates have mature sequences with the highest GC content and, consequently, greater molecule stability. In plants presented few available mirtron precursors, and in this group, we noticed that average mature mirtron size and other characteristics are dissimilar from mammals. Using mature mirtron size characterization by organism group, we identified that, in most cases, mature mirtrons in chordates and invertebrates present 22 nucleotides (nt) in size, and in plants, 21 nt. It is well known that in plants, miRNA mature size is predominantly 21nt. Despite this, our results showed also a high amount of 24 nt mature mirtrons. When characterizing mirtrons acting as targets, we could identify mirtrons presenting the potential to play regulatory roles in mRNA and act as competing endogenous RNAs (ceRNAs) in plants such as *A. thaliana*, *M. truncatula*, and *O. sativa*. We identified that 966 mature mirtrons are available in miRBase, reinforcing the novelty provided by mirtronDB in better characterizing existing data. Particularly, only 2% of plant mirtrons are available in miRBase. The analyses performed in this study provide initial mirtron characterization and can be used as a guide about mirtrons potential as ceRNAs and gene expression regulators. The database could also provide novel standardized approaches to predict and identify mirtrons in organisms that do not have them described. Suplementarly, it can be used as a reference to cross-validation with other databases.

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Distribution analysis of the F1534C and V1016I *kdr* mutations in *Aedes aegypti* in west region of Brazilian northeast

Larissa Paola Rodrigues Venancio[1,2*]; Ilana Luize Rocha Santana[1,2]; Mara Rúbia Santos e Silva[1,2]; Paloma Oliveira Vidal[2,3] Jaime Henrique Amorim Santos[1,2]

[1]Centro das Ciências Biológicas e da Saúde – CCBS, Campus Reitor Edgard Santos, Universidade Federal do Oeste da Bahia, UFOB, Barrerias, Bahia, Brazil.[2]Laboratório de Agentes Infecciosos e Vetores (LAIVE), Núcleo de Estudos em Agentes Infecciosos e Vetores (NAIVE), Universidade Federal do Oeste da Bahia (UFOB), Barreiras, Bahia. [3]Faculdade São Francisco de Barreiras, FASB, Barreira, Bahia, Brazil.

*larissa.venancio@ufob.edu.br

Key-word: insecticide resistance; *kdr* mutation; selection

Aedes aegypti is an anthropophilic mosquito species with a worldwide distribution in tropical, subtropical and temperate climate zones. It is the principal vector of yellow fever, dengue fever, chikungunya, and zika virus. Control of the viruses transmitted by this mosquito relies on deployment of insecticides to suppress mosquito populations, but the evolution of insecticide resistance threatens the success of vector control programs. Insecticides, such as pyrethroids, target the transmembrane voltage gated sodium channel (Na_v) from the insect nervous system, triggering a phenomenon known as *knockdown* effect. Although several mutations have been identified in natural populations at *Ae. aegypti* Na_v, only the V1016I and F1534C substitutions were clearly related to the loss of pyrethroid susceptibility. In the northeast of Brazil, the western region of Bahia state frame within the area of municipalities with an important incidence in this state for the three arboviruses. In this study, we aim to evaluate the simultaneous occurrence of the V1016I and F1534C mutations in the western region of Bahia, in order to evaluate the resistance/susceptibility status to pyrethroids insecticides. Pupae and larvae were collected during the rainy season in 2017 and 2018. Genomic DNA was extracted from 47 adult mosquitoes, and for genotyping, allele-specific PCR reactions were performed and visualized by 12% polyacrylamide gel stained. It is known that 1016 and 1534 sites are linked, then, genotypic and allelic frequencies were taken as a single locus (1016V+1534F – wild type or Na_v^S; 1016V+1534C – 1534 *kdr* or Na_v^{R1}; 1016I+1534C – 1016 *kdr* + 1534 *kdr* or NA_v^{R2}). Then, the genotypes were defined such as SS, SR1, SR2, R1R1, R1R2 and R2R2. Hardy-Weinberg equilibrium (HWE) was evaluated by the classical equation, being the null hypothesis of equilibrium checked by a chi-square test with three degrees of freedom (six genotypes). We identified the three alleles with the following frequencies: Na_v^S – 0.011; Na_v^{R1} – 0.330; Na_v^{R2} – 0.660. The observed frequency of the six genotypes was: SS – 0.0; SR1 – 0.022; SR2 – 0.0; R1R1 – 0.0; R1R2 – 0.64; R2R2 – 0.34. The HWE assumption was rejected ($\chi 2 = 12.57$; $p<0.01$). The 1534C mutation is widespread in Brazilian territory. In the west of the northeast region, the homozygous profile to 1534F was not identified, suggesting possible fixation of the mutant allele. The Na_v^{R2} is the more frequent *kdr* allele, indicating that this allele would be more advantageous for pyrethroid resistance. However, the higher frequency of the R1R2 genotype is probably due to the Na R2 homozygous, highly resistant to pyrethroids, exhibited a fitness cost in a series of life-trait parameters, as already identified in the literature. Highly-level pyrethroid resistante is common in northeast of Brazil in *Ae. aegypti*. This suggest that the resistance monitoring and management is recommended in light of the potential for arbovirus outbreaks in region.

Funging Agency: FAPESB (research grant - LPRV), CNPq (PIBIC/UFOB).

EVOLUTIVE LINEAGES OF THE TAXONOMIC SPECIES *DROSOPHILA SERIDO*

Ana Beatriz Zichinelli[1]; Maura Helena Manfrin[1,2]

[1]Departamento de Genética, Universidade de São Paulo – Faculdade de Medicina de Ribeirão Preto, Ribeirão Preto, São Paulo, Brasil. [2]Departamento de Biologia, Universidade de São Paulo – Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto, Ribeirão Preto, São Paulo, Brasil.

anab_zichinelli@usp.br

Key words: delimitation of species; nuclear DNA; mitochondrial DNA.

One of the basic comparative units in scientific studies is the species. Despite its importance, there is no consensus on how to define it as a unit or limit it in the field, considering its populations. However, analytical methodologies using sequences of several genes have been promising in the delimitation of species in nature. The "cluster" *Drosophila buzzatii* is formed by seven endemic species from South America and have mandatory ecological relationships with tissues of necrotized cacti, which are used for the development of their larvae. Among them, *Drosophila serido* has a broad geographic distribution. Previous studies on the basis of inversions and mitochondrial DNA sequences indicated the existence of two population groupings in this species: coastal populations and northeastern populations. In this study, using sequences of the nuclear genes GstD1, KL5 and α Esterase-5 and mitochondrial COI, we tested the hypothesis that the taxonomic species *D. serido* is composed of more than one evolutionary lineage, through analysis of population structure, population grouping, haplotypical network and phylogeny. The results for the analysis of population structure indicate that, when we analyze the genes separately, we can have up to two groupings for the species, while when we analyze the genes together, we have only one grouping. For the analysis of population grouping based on genetic distance, we analyzed that some coastal populations are distasteing from the rest of the coastal and northeast populations, but we still have the two populations of the two hypothetical groupings next. For the analysis of haplotypical networks, the only gene that completely separates the two clusters is the α Esterase-5 gene. For the remainder of the markers, all still point to a large mixture among the populations. And for the analysis of phylogeny, we analyze that the coastal populations are forming a new grouping, but still do not differentiate completely from the rest of the populations. We conclude that, despite the geographic variations that are differentiating the populations, we have only one evolutionary lineage for *D. serido*.

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Brachypodium distachyon as a genetic model to examine Nitrogen Use Efficiency (NUE) in Sugarcane

Luís Henrique Damasceno Serezino[1]; Antonio Figueira[1]; Eric Lam[2]

[1]Centro de Energia Nuclear na Agricultura, Universidade de São Paulo, USP – São Paulo/Brasil. [2] Rutgers University – New Jersey/USA

lhdserezino@usp.br

Palavras-chave: NUE; post-transcriptional regulation; negative feedback.

Growing concern about energy security and global warming has stimulated the replacement of fossil fuel with renewable resources. Brazil has a worldwide recognition due to the cultivation of sugarcane (*Saccharum* spp.) for the production of bioethanol fuel. Sugarcane response to nitrogen fertilizers is limited compared to other crops, and losses of N applied to the soil can reach 50%. Compared with sorghum and maize, sugarcane shows the lowest nitrate uptake efficiency and limited storage capacity, partially explaining the large N losses from the cultivated soil and the low nitrogen use efficiency (NUE). Therefore, the physiological and molecular characterization of the nitrate acquisition process is fundamental for the improvement of sugarcane NUE. In previous studies, we identified a post-transcriptional regulation mechanism of High- Affinity Transporter genes (NRT2/NRT3 subfamilies – *NITRATE TRANSPORTERS*) in sugarcane roots submitted to nitrate resupply. This mechanism may be one of the major processes responsible for the low NUE in sugarcane roots when compared to other grasses. Due to the genomic complexity of sugarcane, *Brachypodium distachyon* stands out as a potential model system for gene function studies of grasses. Despite the exponential growth in research using *B. distachyon* as a model plant, little has been explored in relation to investigating the N uptake, and particularly NUE. Experiments were carried out to identify the presence of post-transcriptional regulation in response to nitrate provision in *B. distachyon*. Plants grown in N sufficient solution (2 mM NH_4NO_3) were subjected to N restriction for 7 d. Then, plants were cultivated in the presence of nitrate (10 mM KNO_3) or ammonium nitrate (5 mM NH_4NO_3), and root samples were collected 0, 2, 4, 24 and 72 h after treatments. ^{15}N-nitrate uptake analysis revealed a negative feedback regulation of ^{15}N-nitrate uptake by ammonium nitrate resupply.Gene expression analysis by RT-qPCR indicated *BdNRT*2.1 and *BdNRT*2.2 as the main *B. distachyon* genes responsible for nitrate acquisition. *BdNRT3.1* expression was inducible, revealing the co-expression of members from both subfamilies in response to N resupply. There was no correlation between ^{15}N-nitrate influx and transcript accumulation of members of the two-component complex (NRT2/ NRT3) after ammonium nitrate resupply, corroborating the occurrence of a post-transcriptional regulation of HATS in *B. distachyon*. Thus, even ammonium being a preferential N inorganic source in *B. distachyon*, HATS is repressed by ammonium, as described for other species.

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In silico evaluation of HIF1α inhibitory drugs as a therapeutic strategy for head and neck cancer

Bianca Barbério Bogdan Tedeschi[1], Lennon Pereira Caires[2], Tiago Henrique[3], Vilson Serafim Junior[2], Érika Cristina Pavarino[2], Juliana Garcia de Oliveira Cucolo[2], Eny Maria Goloni-Bertollo[2]

[1]UNESP - São Paulo State University, [2]Genetics and Molecular Biology Research Unit – UPGEM, FAMERP- São José do Rio Preto Medical School. [3]Laboratory of Molecular Markers and Bioinformatics, FAMERP – São José do Rio Preto - Brazil.

biancabogdan04@hotmail.com

Keywords: Head and neck; HIF1ĸ; Bevacizumab

Head and neck cancer (HNC) affects anatomic sites such as oral cavity, larynx and pharynx. Angiogenesis is a process that accounts for the tumoral maintenance. Bevacizumab, na antiangiogenic drug, is used to inhibit the interaction between vascular endotelial growth fator (VEGF) and its receptor (VEGFR). The Hypoxia- inducible factor 1-alpha (HIF1α) can be responsible for Bevacizumab resistance since this fator contributes to angiogenesis by means of VEGF independent pathways. The aim of this study was to asses in silico drugs that inhibit HIF1α that can be used as therapeutic strategy associated with Bevacizumab in the HNC treatment. The three-dimensional structure of HIF1α protein was obtained on Protein Data Bank (PDB ID: 1MZE) and used as receptor in molecular docking. The drugs used were Topotecan, Bisfenol A, Vorinostat and Acriflavine. The protein and ligands were prepared on Autodock Tools Sofware and the docking between ligands and HIF1α was accomplished using Autodock Vina. The hydrogen bond was the greater interactions obtained in the molecular docking experiment, therefore the best results were -7.3 and -8,5 kcal/mol for Acriflavine and Topotecan, respectively. These results showed that theses drugs have a higher potential to inhibit HIF1α gene. Despite advances in HNC treatment, the survival rate is still low doe to tumor recurrence and metastasis. Characteristics of this subpopulation may be associated with activity of the HIF1α gene that can promote a stem-like phenotype. For this reason the development of therapeutic methods that can inhibit the action is important to block the process of angiogenesis independente of VEGF. In conclusion, the use of HIF1α inhibitor drugs in combination with Bevacizumab may me a promising new strategy in blocking angiogenesis for the treatment of HNC. Thus, in vitro studies are relevant to validate our findings.

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Modulation of the proliferation by anti-inflammatory protein Annexin A1 in normal and cervical tumorigenic cells

Tháyssi Fernanda de Oliveira Rios[1] Stefanie Oliveira de Sousa[2]; Barbara Maria Frigieri[2], Flávia Cristina Rodrigues Lisoni[1]

[1] State University (UNESP), Faculty of Engineering Ilha Solteira (FEIS), Department of Biology and Animal Science, Ilha Solteira, SP, Brazil. [2] São Paulo State University (UNESP), Institute of Bioscience, Humanities and Exact Science (IBILCE), Department of Biology, São José do Rio Preto, SP, Brazil.

thayssi.rios18@gmail.com

Keywords: cell culture; alternative therapy.

In Brazil, cervical cancer is the third malignant neoplasm that affects women, only surpassed by non-melanoma skin cancers and breast cancer. Cervical carcinogenesis is related to genetic alterations, human papillomavirus (HPV) infection, angiogenesis and inflammatory processes. The idea that inflammation is involved in tumorigenesis is supported by the observation that cancer often arises in areas of chronic inflammation. On the other hand, the inflammatory response is controlled by the action of anti- inflammatory mediators, which act to maintain the homeostasis of the immunological response and prevent tissue damage. Among these mediators, we highlight the annexin- A1 (ANXA1), which is expressed by tumor cells and acts as a modulator of the inflammatory process. Studies suggest the involvement of this protein family in cancer by means of signalling cascades that include genes related to the cell cycle, differentiation and apoptosis. The present work aimed to investigate the influence of this anti-inflammatory on cervical tumorigenic cells and compare with normal cells. For this, the peptide ANXA1Ac2-26 was used at the concentration of 10 µg/mL for 2, 4, 24, 48 and 72 hours on SiHa (cervical squamous carcinoma) and HaCat (normal keratinocytes) cell lines. Morphology, proliferation and migration cell were evaluated. Both cell lines showed no alteration in cellular morphology. The peptide treatment in the SiHa cells reduced cell proliferation progressively, in relation to the control, with 72 hours being the most significant. While, in HaCat cells, the treatment only reduced cell proliferation in relation to the control, after 2 and 24 hours. In the cell migration, there was a significant reduction in the SiHa cells (after 48 and 72 hours), but there was no change in the HaCat cells. The results point to the peptide ANXA1Ac2-26 as a potential target of the further studies in genetic activities, being possible its indication in innovative therapies in the treatment of cervical carcinomas for its efficient antiproliferative action.

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MOLECULAR KARYOTYPING IN 900 SPONTANEOUS ABORTIONS BY SEQUENTIAL ANALYSIS with QF-PCR, HGQ-PCR AND SNP-ARRAY

Cassiana S. de Sousa[1]; Heloisa B. Pena[1]; Juliane Rocha[1]; Betânia M.A. Pena[1]; Sérgio D.J. Pena[1*]

[1]Laboratório GENE - Núcleo de Genética Médica. Belo Horizonte, MG, Brazil

*spena@gene.com.br

Key-words: molecular cytogenetics; QF-PCR/HGQ-PCR; SNP array

Spontaneous abortions, which occur in 15-20% of all pregnancies, constitute the most common human genetic disease, since the majority of miscarriages are caused by chromosomal anomalies. There is evidence that scientific explanations for the spontaneous abortion are useful for the restoration of the emotional homeostasis of the parents and also for orienting the medical conduct. The use of conventional karyotyping for the study of miscarriages fails in 30-40% of instances because of bacterial contamination and/or not successful culture. Molecular karyotyping is the ideal solution, since it does not depend of availability of live fetal tissues. In our laboratory, we have devised a protocol for sequential use of Quantitative Fluorescent PCR (QF-PCR), Homologous Gene Quantitative PCR (HGQ-PCR) and SNP-Arrays that allows the examination of numerical and structural analyses of all chromosomes in spontaneous abortions at reasonable costs. We wish to describe our results with 900 samples of fetal tissues collected after pregnancy losses. Fetal placental tissues spontaneously expelled, obtained by curettage or collected by intra-uterine aspiration were dissected to eliminate maternal decidua using an inverted microscope. This was followed by DNA extraction and rapid initial analysis with QF-PCR and HGQ-PCR, which together allowed the detection of numerical abnormalities of chromosomes 2, 3, 4, 5, 7, 8, 9, 15, 13, 16, 18, 20, 21, 22, X and Y. Positive signals with QF-PCR and HGQ-PCR were always confirmed using microsatellite amplification of the specific chromosome. Only the samples with no molecular evidence of alteration by joint QF-PCR and HGQ-PCR were additionally studied with a SNP Array. Overall, in only 31 of our 900 cases (3%), it was not possible to reach a diagnosis because of absence or excessive degradation of fetal tissues in the placental remains received. Among the 869 diagnosed samples, 65% presented numerical or structural chromosomal aberrations. The most common anomalies were trisomies (44%), triploidy (9%) and monosomy X (5%). The most common trisomies involved the following chromosomes: 16 (24%), 21 (15%), 22 (14%) and 15 (11%). SNP array permitted the diagnoses of trisomies 6, 10, 11, 12 and 17, which had not been targeted by QF-PCR and/or HGQ-PCR. In this manner, aneuploidies were identified on all chromosomes, except 1 and 19. Additionally deletions and/or duplications and chromosomal mosaicism were detected by SNP Array in 16 cases. In conclusion, the sequential analysis of fetal tissues with QF- PCR, HGQ-PCR and SNP-ARRAY is a highly useful, rapid and cost-effective approach for the diagnosis of chromosomal alterations in spontaneous abortions.

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MOLECULAR PRENATAL DIAGNOSIS OF CHROMOSOME ALTERATIONS IN PREGNANCIES WITH ABNORMAL ULTRASOUND FINDINGS

Juliane Rocha[1]; Heloisa B. Pena[1]; Cassiana S. de Sousa[1], Betânia M.A. Pena[1]; Sérgio D.J. Pena[1*]

[1]Laboratório GENE - Núcleo de Genética Médica. Belo Horizonte, MG, Brazil

*spena@gene.com.br

Key-words: prenatal diagnosis; QF-PCR/HGQ-PCR; SNP array

The primary function of ultrasound studies in the beginning of the second trimester is the screening for malformations and evaluation of markers for aneuploidy. Abnormalities thus detected have to be quickly and efficiently assessed by genetic prenatal diagnosis in chorionic villi, amniotic fluid or fetal blood for detection of numerical and structural chromosome abnormalities. We have developed a protocol for sequential use of Quantitative Fluorescent PCR (QF-PCR), Homologous Gene Quantitative PCR (HGQ-PCR) and SNP-Arrays, which permits the examination of numerical and structural analyses of all chromosomes rapidly and powerfully. We wish to describe our results with 225 samples referred to our laboratory for prenatal diagnosis. Chorionic villi received were carefully dissected to eliminate maternal decidua using an inverted microscope. The dissected material, as well as samples of amniotic fluid or fetal blood had their DNA extracted by routine techniques. This was followed by rapid initial analysis with QF-PCR and HGQ-PCR, which together permitted the detection of numerical abnormalities of chromosomes 13, 18, 21, X and Y. Positive results were confirmed using microsatellite amplification of the target chromosome. Only the samples with no molecular evidence of alteration by joint QF-PCR and HGQ-PCR were further analyzed by SNP Array (CytoSNP). Overall, 51% of the 225 samples received proved to be abnormal. With the first part of the protocol (QF-PCR and HGQ-PCR), we found chromosome numerical abnormalities in 48% of the cases. The most common abnormal result was trisomy 21 (24%), followed by trisomy 18 (8%) and monosomy X (4%). An unexpected finding was triploidy, seen in 3% of samples examined. The samples that were normal were next examined by SNP Array, with which we found abnormalities in 18% of the cases (deletions, duplications and mosaicism). The high number of abnormal cases diagnosed by SNP Array demonstrates the great importance of including this detailed examination in prenatal diagnosis. In conclusion, our study demonstrates the importance of undertaking the molecular cytogenetics of all pregnancies with abnormal ultrasound findings, with a first step (QF-PCR and HGQ-PCR) for diagnosis of numerical abnormalities of a selected set of chromosomes and a second step (SNP Array) for diagnosis of structural alterations of all chromosomes.

FUNCTIONAL ANALYSIS OF THE MICRORNA-1914-5P IN LIPID METABOLISM IN HEPATIC CELLS: PATHWAYS TO UNDERSTAND NON-ALCOHOLIC FAT LIVER DISEASES

Thaís Porto Barbosa[1]; Letícia Ferreira Ramos[2]; Marina Bonfogo da Silveira[2]; Karen Cristiane Martinez de Moraes[1,2,3]

[1]Pós-Graduação em Biotecnologia, UNESP - Instituto de Química (IQ) – Campus Araraquara. [2]Pós-Graduação em Biologia Celular e Molecular, UNESP - Instituto de Biociências (IB) – Campus Rio Claro. [1 3]Departamento de Biologia, Universidade do Estado de São Paulo (UNESP) – IB - Campus Rio Claro, São Paulo - Brazil.

*kcm.moraes@nesp.br.

Key words: hepatic steatosis, mechanistic studies microRNA

Non-alcoholic fat liver diseases (NAFLD) has been considered a serious health problem worldwide. Such diseases are characterized by an excessive fat storage in the liver, which damages the organ and, in some cases, it is able to develop an even worse physiopathologic symptoms such as cirrhosis and hepatocarcinome. Despite several different etiologic agents, the molecular aspects that supports NAFLD progression to its worsen clinical aspects have to be described as better treatments have to be developed. Recently, our research group described the microRNA-1914-5p as an effective element that modulates the lipid metabolism in hepatic stellate cells (HSCs) LX-2, which has directed our current interests in evaluate the mechanisms by which the miR-1914-5p acts in hepatic steatosis. MicroRNAs are small molecules that modulate gene expression by targeting a complementary RNA sequence and avoiding its translation. To perform the analyses, pro-steatotic cellular model was set up using the hepatocytes HEPG-2 (ATCC® HB8065™) cell line and a mixture of fatty acids (oleic and palmitic, Sigma- Aldrich) at 400 µM final concentration, which was added to the cultures. Those cells were previously transfected or not with 40 nM of the miRNA 1914-5p mimic or inhibitor (mirVana™, Thermo Fisher Scientific). To address the effect of the investigated molecules in lipid metabolism, gene expression of relevant molecules of this molecular pathway such as FASN, ACACA, MLYCD, CPT1, CPT2, among others, were evaluated by quantitative gene expression analyses (qPCR). More prominent changes in gene expression were verified in cells transfected with the miRNA inhibitor. Moreover, the cellular effect of the investigated microRNA in lipid metabolism were addressed by Fourier Transformed - Infra Red (FT-IR) spectroscopy, which also demonstrated relevant effect of the miR1914-5p inhibitor in the controlling of lipid level in cells cultivated under pro-steatotic conditions. Currently, we are addressing the effect of the investigated molecules on κ-oxidation of the fatty acid in the fat cells, aiming to find mechanistic details that may contribute to the development of an innovative therapeutic strategy.

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Radiation-induced DNA damage cooperates with heterozygosity of TP53 and PTEN to generate high grade gliomas

Bipasha Mukherjee[1,2], Pavlina Todorova[1], Eliot Fletcher-Sananikone[1], and Sandeep Burma[1,2]

[1]Division of Molecular Radiation Biology, Department of Radiation Oncology, University of Texas Southwestern Medical Center, Dallas, TX, USA;
[2]Department of Neurosurgery, University of Texas Health, San Antonio, TX, USA

Glioblastomas (GBM) are lethal brain tumors which are treated with conventional radiation (X- rays and gamma rays) or particle radiation (protons and carbon ions). Paradoxically, radiation is also a risk factor for GBM development, raising the possibility that radiotherapy of brain tumors could promote tumor recurrence or trigger secondary gliomas. In this study, we determined whether tumor suppressor losses commonly displayed by GBM patients confer susceptibility to radiation-induced glioma. Mice with Nestin-Cre-driven deletions of Trp53 and Pten alleles were intracranially irradiated with X-rays or charged particles of increasing atomic number and linear energy transfer (LET). Mice with loss of one allele each of Trp53 and Pten did not develop spontaneous gliomas but were highly-susceptible to radiation-induced gliomagenesis. Tumor development frequency after exposure to high-LET particle radiation was significantly higher compared to X-rays, in accordance with the irreparability of DNA double-strand breaks (DSB) induced by high-LET radiation. All resultant gliomas, regardless of radiation quality, presented histopathological features of grade IV lesions and harbored populations of cancer stem-like cells with tumor-propagating properties. Furthermore, all tumors displayed concomitant loss of heterozygosity of Trp53 and Pten along with frequent amplification of the Met receptor tyrosine kinase, which conferred a stem cell phenotype to tumor cells. Our results demonstrate that radiation-induced DSB cooperate with pre-existing tumor suppressor losses to generate high grade gliomas. Moreover, our mouse model can be used for studies on radiation-induced development of GBM and therapeutic strategies.

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CYTOGENETIC ANALYSIS OF *LITHOBATES PALMIPES* (ANURA, RANIDAE) WITH CLASSIC AND MOLECULAR APPROACH THROUGH TELOMERIC SEQUENCES

Murilo D. Santos[1*]; Marcelo J. Silva[1]; Thiago Gazoni[1]; Célio F.B Haddad[2]; Luiza R. Cholak[1]; Patricia P.P Maltempi[1]

1Departament of Biology, Biosciences Institute, UNESP, Rio Claro, São Paulo, Brazil., 2Departament of Zoology, Biosciences Institute, UNESP, Rio Claro, São Paulo, Brazil.

* murilodurigon@uol.com.br

Keywords: Cytogenetics; Fluorescence *in situ* hybridization; AgNOR.

There are currently, 1.093 species of anurans described in Brazil. *Lithobates* (Anura, Ranidae) comprises 51 species, inhabiting diverse environments, mainly in the Brazilian Amazon. Taxonomic problems are often seen in Anura, and in *Lithobates* there are evidences that populations under the specific name *L. palmipes* comprise a complex of species, mainly due to their wide distribution. To obtain data to help elucidate this question, inter-population cytogenetic studies, allied to other taxonomic and molecular approaches, become extremely necessary. However, there is still a certain shortage of such studies, especially in relation to the constitution and karyotype evolution in anurans, which could provide support for different approaches. We performed conventional and molecular cytogenetic analyses in *L. palmipes* cells, in a comparative interpopulation manner (Paranaíta, MT and São Gabriel da Cachoeira, AM), searching for cytological markers that could characterize the species, as well as help to verify of the taxonomic status of *L. palmipes*. For this, mitotic chromosome analysis classical analyses with Giemsa and impregnation by silver nitrate was performed to identify the number and location of the nucleolus organizer regions (NOR), as well as telomeric sequence mapping (TTAGGG)n using fluorescense *in situ* hybridization. From the analyzes performed, the karyotype of *L. palmipes* was observed, with a maintenance of the diploid number of 2n=26 in both analyzed populations, with approximately eight submetacentric chromosome pairs and five subtelocentric chromosome pairs. It was also observed that in males and females, from both populations, the nucleolus organizing region (NORs) are in the proximal region of the long arm of par 10, suggesting the absence of structural rearrangements in the chromosomes of these populations in relation to the position and to the number of NORs. FISH with telomeric probes showed, that in both populations of *L. palmipes* only have terminal signals of all chromosomes, a pattern expected for this type of sequence, with no evidence of interstitial markings being seen for these populations. Our results provide updated data on chromosomal characteristics of *Lithobates*, contributing to the understanding of the chromosome structure and karyotype evolution in this genus, especially in relation to the telomeric sequence, which leads us to believe that there were probably no significant chromosome structural rearrangements between the two studied populations.

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DEVELOPMENT OF NOVEL MODEL FOR BACTERIAL PROMOTER PREDICTION

Murilo Henrique Anzolini Cassiano[1*]; Ananda Sanches-Medeiros[1]; Rafael Silva-Rocha[1];

[1]Systems and Synthetic Biology Lab, Cell and Molecular Biology Department, FMRP- USP.

murilo.anzolini.cassiano@gmail.com

Keys: Bacterial promoter prediction; Transcription regulation; Bioinformatics

Not all the genes are expressed constantly, they need to be regulated and a part of the control is the transcription start. The actors in this process are RNA polymerase, transcription factors (which activate or inhibit transcription), sequences in the DNA itself, so-called promoters, which are related to the frequency of transcriptional activity, and sigma factors, which recognize these promoter sequences in DNA. Finding new promoters, for example, in genomic DNA sequences, can support transcription and regulon studies as well as facilitate finding promoters with different levels of expression, which generate a fine tuning of expression levels. This would be useful for the metabolic engineering of industrial organisms in research studies on genetic control, and in several applications where accurate biological circuits are needed. In the first part of this project, we developed a position-weight matrix to find motifs of sigma 70 binding sites in *Escherichia coli* and found a cut-off value for the model, based on a work that made available promoters discovered by SELEX genome. We have seen *in silico* that the model is robust and discriminates well between false promoters and possible promoters even when the randomly generated false promoters have AT contents like those in sequences of natural promoters. Despite these results, in the genome, the model encounters a very large number of occurrences, indicating many potential false positives. As a first approach to check the model's accuracy *in vivo,* we selected putative promoters taken from the genome, predicted with high scores, and inserted then into reporter vectors, regulating the expression of fluorescent protein. We could identify 5 previously unidentified promoter regions in the genome of *E. coli* using this approach. Next, we compared our model with the available tools for promoter prediction, using accurate promoter sequences present in RegulonDB and we discuss about features and methods to predict bacterial promoter sequences. Taken together, this work shows the potential and limitations for available prediction tools for the identification of promoter regions in bacterial genomes.

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AUTOIMMUNE REGULATOR (AIRE) GENE AND ADHESION WITH THYMOCYTES MODULATE THE mRNA ISOFORM DIVERSITY IN MEDULLARY THYMIC EPITHELIAL CELLS

Natane de Araujo Miglioli[1]; Amanda Freire-Assis[1,2]; Romário Mascarenhas[1]; Leticia Agrelli de Brito[1]; Geraldo Aleixo Passos[1,3]

[1] Grupo de Imunogenética Molecular, Departamento de Genética, Faculdade de Medicina de Ribeirão Preto, USP [2] Universidade Estadual de Minas Gerais (Passos, MG). [3] Departamento de Biologia Básica e Oral, Faculdade de Odontologia de Ribeirão Preto, USP

natanearaujomiglioli@gmail.com, passos@usp.br

Keywords: Aire; mRNA isoforms; RNA-seq

The autoimmune regulator gene (AIRE) is a transcriptional controller in medullary thymic epithelial cells (mTECs), which plays a role on the expression of tissue related autoantigens (TRAs) that are presented by mTECs to developing thymocytes. This is a crucial process for the occurrence of negative selection of autoreactive thymocytes. A failure in this process is associated to onset of autoimmune diseases. The diversity of TRAs guarantees self-representation in the thymus and induction of immune tolerance towards peripheral organs and tissues. Our hypothesis is that the AIRE gene may play a role in the generation of TRA diversity throughout alternative processing of their respective mRNAs isoforms. To test this, we compare the mRNA transcriptome profiling of murine Aire wild type (WT) vs Aire mutant (Aire exon 3 KO induced by Crispr-Cas9 system, loss-of-function -LOF- mutation) mTEC cells. The mRNA transcriptome was done through RNA- sequencing (Illumina RNA-Seq) from paired-end cDNA libraries (50 million reads coverage) of Aire WT vs Aire KO mTECs, before and after adhesion with thymocytes. A bioinformatics pipeline was used to analyze the transcriptome sequencing data of these cells and to select those differentially expressed TRA mRNA isoforms through RSEM and EdgeR softwares. Our results show 238 down-regulated and 29 up-regulated isoforms when comparing together these groups of cells. Although Aire influenced isoform diversity, the most evident effect on the modulation of TRA mRNA isoform was adhesion mTEC-thymocyte regardless Aire LOF mutation.

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GENES ENCODING PHLOEM PROTEINS IN CITRUS ARE UPREGULATED IN RESPONSE TO *CANDIDATUS* LIBERIBACTER ASIATICUS

Nathália Da Roz D' Alessandre[1]; Laís M. Granato[2]; Marcos A. Machado[2]

[1]Departamento de Ciências da Natureza, Matemática e Educação, Universidade Federal de São Carlos - Faculdade de Ciências Agrárias, Araras, São Paulo. [2]Instituto Agronômico de Campinas - Centro de Citricultura Sylvio Moreira, Cordeirópolis, SP.

nathy.dalessandre@hotmail.com

Keywords: HLB; *Ca*Las; PP2.

Huanglongbing (HLB), also known as 'Greening', is one of the major phytosanitary problems in the current citrus industry. HLB is caused by the bacterium *Candidatus* Liberibacter asiaticus (*Ca*Las) and the symptoms have been related to callose deposition and phloem protein (PP2) accumulation in the phloem sieve tubes, causing clogging of the sieve elements and inhibiting the transport of assimilates along the plant. PP2 are phloem lectins widely conserved in plants, and although they are phloem structural proteins, they are abundant in the phloem sap and can be translocated together with the assimilates for long distances. In *Arabidopsis thaliana* (*At*) genome are found 30 genes, which are divided into two groups (*At*PP2-A and *At*PP2-B). However, in the sweet orange (*Citrus sinensis*) genome we verified 14 genes. Transcriptome analysis of *Ca*Las- infected citrus plants had showed overexpression of the PP2B10 and PP2B15 genes in the phloem. Therefore, the aim of this study was to evaluate the expression of genes encoding phloem proteins (PP2) in *C. sinensis* (highly sensitive) and *Poncirus trifoliata* (tolerant), infected with *Ca*Las. The plants used in this study were propagated using buds that were grafted onto rootstocks of a 6-month-old Rangpur lime (*C. limonia* Osb.) and maintained under controlled conditions at the greenhouse of the Sylvio Moreira Citrus Center, Cordeirópolis/SP. Leaves of similar age, position and at the same developmental stage were collected from four sides of the plant and petioles from five leaves were pooled for RNA extraction. For the RT-qPCR experiments, five biological replicates for mock-inoculated (healthy budwood) and ten biological replicates for *Ca*Las inoculated (infected budwood) were evaluated for *C. sinensis* and *P. trifoliata*. For relative quantification, the $2^{-\Delta\Delta CT}$ method was applied using GAPDH and EF1K as reference genes. It was observed a significant up-regulation of the evaluated genes in the HLB positive plants (HLB+) compared with healthy plants (healthy) to *C. sinensis*. To *P. trifoliata* only PP2B10 and PP2B15 genes were upregulated. It was also observed a significant up-regulate expression of all genes evaluated in *C. sinensis* when compared to *P. trifoliata*. The PP2B1 gene was the most upregulated gene in *C. sinensis*. Besides that, we also proved that *C. sinensis* plants infected with *Ca*Las had higher callose deposition in the phloem sieve tubes and higher starch accumulation in the leaves than *P. trifoliata*.

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COMPARISON OF THE MUTAGENICITY PROFILES CAUSED BY UVA AND UVB LIGHT IN XP-C DEFICIENT CELLS.

Nathalia Quintero Ruiz[1]; Natalia Cestari Moreno[1]; Camila Corradi[1]; Tiago Antonio Souza[1]; Carlos Frederico Martins Menck[1].

[1] DNA repair Laboratory, Department of Microbiology, Institute of Biomedical Sciences, São Paulo University - USP, São Paulo, SP, Brazil.

cfmmenck@usp.br , nathaliaquinteroruiz@usp.br

Key-words: ultraviolet radiation; mutagenesis; exome.

Sunlight radiation is essential for life on earth, nevertheless it can also be harmful to DNA due to its ultraviolet (UV) component that causes DNA structural damage, such as pyrimidine dimers (i.e. CPD, 6,4-PP) and oxidized bases. These type of lesions are mainly removed by nucleotide excision repair (NER) pathway, and when unrepaired may generate mutations, which causes of cancer. To date most studies are carried out using UVC as model, but only UVA and UVB are considered biologically relevant, as they are part of sunlight spectrum that reach the Earth's surface. In this work, we performed a novel strategy to detect mutations in human cells by next generation sequencing (NGS) to compare mutagenesis by UVA and UVB light irradiation. As a model, we used a XPC mutated cell line (NER deficient) and its complemented isogenic (NER proficient) control (COMP- complemented by transduction with a lentivirus vector expressing XPC protein). XP-C cells are from patients with genetic syndrome that result in a clinical phenotype, that includes an extremely high incidence of skin cancer in exposed areas to sunlight, denominated Xeroderma Pigmentosum (XP). Mutations were detected after exome sequencing. Results indicate that UVA and UVB increased cell death of XP-C cells compared to COMP control cells. The exome data point out a significant mutagenesis increase in XP-C cells irradiated with UVA and UVB, generating a significant increased of C>T, CC>TT and C>A base substitutions. Remarkably, on both types of UV-light the C>T transition in a pyrimidine dimer context, considered the mutational signature of UVC/UVB irradiation, is the main type of point mutation observed, with preference to mutate the second base of the dimer. Additionally, T>N mutation in pyrimidine dimer context is significantly increased by UVB, also with preference for the second base of the TT dimers. Finally, the mutagenic spectra generated by UVA and UVB were very similar and the irradiated XP-C cells display a mutational signature related to cutaneous skin cancer, the signature 7 of the COSMIC catalogue. These results indicate that in cells without repair, a unique dose of UVA or UVB contribute to generate the typical spectrum of mutation found in skin cancer tumors. Eventually, the data may be used for comparison with the mutational profiles of skin tumors obtained from XP patients, as well as they may help to understand this mutational processes in non-affected individuals. Lastly, our results highlight the importance of photoprotection against sunlight radiation and other artificial sources of UVA, since UVA is not innocuous.

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DEVELOPMENT OF SEX CHROMOSOME LINKED MARKERS OF RHINELLA MARINA GROUP SPECIES (AMPHIBIA)

Nayara Furtado[1]*; Carla Bessa[1]; Yrlan Sousa[1]; Rodrigo Petry[1]; Iracilda Sampaio[2], Fernando Sequeira[3] & Marcelo Vallinoto[1]

[1]Laboratório de Evolução, Universidade Federal do Pará, Campus Bragança; [2]Laboratório de Genética Molecular, Universidade Federal do Pará, Campus Bragança. [3]CIBIO/INBIO, Universidade do Porto, Portugal.

furtadonayara28@gmail.com

Keywords: Cane toad; sex-linked markers; EPIC.

Understanding the causes underlying biological diversity and factors that limit species' distribution is critical for predicting species' range shifts driven by climate and habitat changes. The morphologically cryptic Neotropical toads from the *Rhinella marina* species complex (*R. marina*, *R. schneideri* and *R. jimi*) constitutes a compelling case to study natural hybridization dynamics and adaptation. First, hybridization in contact zones seems to be a general trend in this complex, and hence it constitutes an excellent opportunity to see speciation in action. Second, documented hybrid zones between these toads largely coincide with transitional biomes (ecotones) or within interdigitate ecotonal boundaries between rain forest (Amazon), seasonal dry forests (Cerrado), and semi-arid areas (Caatinga). Third, these species share many affinities in biological terms, including reproductive mode, making comparisons between contact zones straightforward. The main goal of this study is to develop markers from the sexual chromosomes of cane toad. This will provide new markers to investigate hybrid zone dynamics between species that are permeable to genetic exchange even after a long time of divergence, and the factors responsible for the maintenance of species cohesion. To accomplish the main goal, we have designed primers from the recent published draft genome of cane toad. We used genes previously linked to sex chromosome from *Xenopus tropicalis*, but also studied in *Hyla*, *Scinax* and *Bufo* species. Primers were designed using an EPIC strategy (Exon-Primed Intron-Crossing). We have isolated ten pair of primers from eight distinct genes. All the markers were amplified in the *R. marina, R. schneideri* and *R. jimi* and may represent an important tool for future works in hybrid zones. Also, for one of those markers (DMRT1 exon 1), which it was previously reported as a sex linked in all three groups (*Hyla*, *Scinax* and *Bufo*), we have tested in males and females of *R. marina* group species. To accomplish that, we have amplified 10 *R. marina* males and 10 females, however, our data does not support the hypothesis of exon 1 of DMRT1 as a sex-linked marker for *R. marina*.

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IL1-β GENE POLYMORPHISM (rs16944) IS ASSOCIATED TO DYSLIPIDEMIA AND HYPERTRIGLYCERIDEMIA IN ELDERLY

Jamille Silva Oliveira[1*]; Ivna Vidal Freire[1]; Ícaro José Santos Ribeiro[1]; Cezar Augusto Casotti[1]; Ana Angélica Leal Barbosa[1]; Rafael Pereira[1]

[1]Universidade Estadual do Sudoeste da Bahia, Jequié, Bahia, Brasil.

oliveira.j.s@hotmail.com

Key words: dyslipidemia, interleukin 1β, Single Nucleotide Polymorphism

The Interleukin 1-beta (IL1-β) is a pro-inflammatory cytokine and some polymorphisms of the gene encoding it is associated with a propensity to a systemic pro-inflammatory status, a condition that has been gained scientific prominence, owing to impair the cell metabolism, and contribute to the development of dyslipidemias and progression of the atherosclerosis process. Notwithstanding, the elderly population is at increased risk for the atherosclerotic processes development. In this sense, the present study aimed to analyze the association of IL1-β gene polymorphism (rs16944) with dyslipidemia and hypertriglyceridemia in the elderly. The investigation included two hundred and twenty-six non-institutionalized elders (≥ 60 years), resident at Aiquara, Bahia. Peripheral venous blood was collected and separated into two types of vacuum tubes: 1) without EDTA, for biochemical dosages (total cholesterol and fractions, triglycerides); 2) with EDTA, for the isolation of DNA. The the Polymerase Chain Reaction with enzyme restriction (PCR-RFLP) was used to identify the genotypes of single nucleotide polymorphisms (SNPs). The PCR amplicons were digested using *AvaI* enzyme for 16 h at 37 °C and the digestion products (192bp and 113bp) were separated on 2% agarose gel using GelRed. The studied elders were stratified according to CC genotype (CC *vs* CT + TT). They were also stratified according to the diagnosis of dyslipidemia (total cholesterol, LDL and HDL) and hypertriglyceridemia, following the recommendations of the Brazilian Society of Cardiology. Then, the association analysis was performed using the chi-square test, with the significance level was set at $p \leq 0.05$. The results indicated that CC homozygosity was associated with abnormally low HDL cholesterol levels (odds ratio [OR] = 3.16, 95% CI = 1.48 - 6.73, p = 0.002). Likewise, the CC genotype was also associated with hypertriglyceridemia (OR = 2.45, 95% CI = 1.2 - 4.98, p = 0.012). However, no significant association was observed between the rs16944 polymorphism and LDL and total cholesterol ($p > 0.05$). These results indicate that elders carrying the CC homozygosis of the of the IL1-β gene polymorphism (rs16944) are significantly associated to dyslipidemia and hypertriglyceridemia in elders, indicating a greater cardiovascular risk. Further studies should investigate the exact role of IL-1β gene polymorphism (rs16944) in the pathogenesis of dyslipidemia and cardiovascular risk in the elderly, as well as their interaction with environmental and behavioral factors, such as the nutritional and anthropometric profile, and level of physical activity.

Challenging adult school (EJA) students to have ideas for producing transgenic organims as a learning strategy.

Joana D'arc Marçal Caxeado Oliveira[1] e Mônica Bucciarelli Rodriguez[2]

[1] Escola Estadual Pedro Alcântara Nogueira, Ribeirão das Neves, MG, Brazil and [2] Depto. Genética, Ecologia e Evolução/UFMG, Belo Horizonte, MG, Brazil.

monica-bucciarelli@hotmail.com

Key words: Genetics teaching; adult education; transgenic organisms

Teaching adult school (EJA) students usually brings more challenges than regular classes. They come to the school tired from an entire day of work and their motivation is frequently just to get the degree to get better jobs. Quite often they also have several gaps on their educational background. In order to increase the attention for improving the molecular biology concepts learning and turning evident its importance for the society several classes were used to discuss the construction of transgenic organism and the students were divided in groups to produce mini videos regarding the production of a GMO they thought was interesting and useful for the society. The tutorial used for teaching the basics of video production was downloaded from https://docs.google.com/document/d/1dQ2EWXSzF7QRTWYJsO2zIdRz_xgOd-RXPZIsn54RGaA/edit At first the students didn't feel capable of facing this challenge, but most of them presented good results. The most impressive idea was to develop a probiotic GMO with anti-depressive properties, expressing rosemary's genes. Overall the students have learnt a lot more than previous students of the same teacher and the experience had a good impact in their self-esteem.

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EVOLUTION OF SPIDER DEVELOPMENTAL GENES

Pedro Mariano-Martins[1*]; Tatiana Teixeira Torres[1]

[1]Departamento de Genética e Biologia Evolutiva, Instituto de Biociências, Universidade de São Paulo, São Paulo, Brazil.

* pedro.mariano.martins@usp.br

Key words: evo-devo; spiders; morphological novelty.

Spiders owe much of their success in colonizing terrestrial environments to their ability of producing and manipulating silk threads. The spinning apparatus is a morphological novelty exclusive of the order Araneae. It consists of silk producing glands, which lead to abdominal appendages -spinnerets- that extrude the silk. Yet, in spite of their ecological and taxonomical importance, the evolution of such structures has never been deeply studied, and neither has their development. The information on such matters is quite sparse. Given that scenario, we gathered some information on genes that have been shown to participate in spinneret development in spider embryos and selected six of them: *Abdominal-A*, *Activator Protein 2*, *Delta*, *Notch*, *Distal-less* and *Ultrabithorax*. Our aim was to search those sequences looking for molecular evidences of modification of the regimen guiding their evolution in the order Araneae compared to non-spinneret bearing arachnids. To do so, we gathered 235 publicly available arachnid transcriptomes (180 of them belonging to spider species) and made a *de novo* assembly. The BLAST tool was used to search for ortholog sequences of each gene in the transcriptomes using publicly available transcripts from *Parasteatoda tepidariorum* as a reference. The orthologs were aligned and then analyzed using CODEML to estimate the non-synonymous and synonymous substitution rates and their ratio (respectively, d_N, d_S and κ) in the sequences using an evolutionary tree as reference. Such analysis allowed us to infer whether those genes are being selected (either positively or negatively) or evolving neutrally. Our results show that sequences of all chosen genes are evolving under purifying selection, which is expected for developmental genes. Also, there is no strong evidence of differences between the evolutionary regimen guiding molecular evolution in spiders compared to the outgroups. With such results we propose that, when those genes were coopted for spinneret development, the evolution of this morphological novelty was due to other factors (*e.g.* their expression patterns), which could not be tested by our methods rather than differences in their molecular evolution regimen.

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EPHA2 AND PBX1 MEDIATE FGFR2 SIGNALLING AND MODULATE TAMOXIFEN RESPONSIVENESS IN LUMINAL A AND B BREAST CANCERS

Kelin G. de Oliveira[1]; Rafaela Nasser Veiga[2]; Jaqueline Carvalho de Oliveira[2]; Daniela Fiori Gradia[2]; Mauro A. A. Castro[3*]

[1]Department of Clinical Sciences Lund, Oncology and Pathology, Lund university, Lund, Sweden. [2]Departamento de Genética, Universidade Federal do Paraná, Curitiba-PR, Brasil. [3]Departamento de Bioinformática, Universidade Federal do Paraná, Curitiba-PR, Brasil.

* mauro.a.castro@gmail.com

Key-words: Breast cancer, EphA2, PBX1

Cancer is a group of genetic diseases that leads to a lack of control in cell proliferation. In Brazil, breast cancer is the most common type of cancer in women (excluding non- melanoma skin cancer) and with the highest mortality rate. Considering breast carcinomas subtypes, those that express the estrogen receptor (luminal A and B) are the most frequent, accounting for at least two thirds of all cases. Although there are target-specific treatments for these tumors, a portion of the patients develop resistance to therapy. Fibroblast growth factor receptor 2 (FGFR2) has often been associated with breast cancer, especially estrogen-responsive tumors, but for a better understanding of this regulatory pathway, many transducers still need to be elucidated. The present study investigated, through *in silico* analysis, which other proteins are involved in the interaction network between the genes encoding FGFR2 (*FGFR2*) and estrogen receptor (*ESR1*), and which are able to affect the therapeutic response. The purpose of these analysis is to guide the selection of transducers, which will be experimentally investigated through functional analysis in luminal A and B breast cancer cell lines in the search for potential therapeutic targets. From more than 700 FGFR2-interacting proteins obtained from databases, 434 were mapped to mammary tissues. The gene expression of these 434 proteins was evaluated against a panel of experimental data in breast cancer cell lines submitted to FGFR2 stimulation experiments. Of those proteins, 52 genes showed response to the stimuli. Of these, eight genes consistently responded to FGFR2 silencing, and all of them presented modulatory activity on transcription factors associated with breast cancer risk. In addition, analysis on database of patient-derived xenografts have identified, among the eight candidate genes, *EPHA2* and *PBX1* as possible modulators in the treatment of luminal breast cancers based on hormone therapy. Thus, EphA2 and PBX1 emerge as potential candidates for future combined therapies, and their functional evaluation is the subject of future studies, fundamental to validate the results obtained *in silico*.

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Functional polymorphisms associated with increased fetal hemoglobin (HbF) levels affect clinical outcomes in children with sickle cell anemia

Rahyssa Rodrigues Sales[1], André Rolim Belisário[3]; Marcos Borato Viana[4]; Fabíola Mendes[3]; Gabriela Faria[3]; Marcelo Rizzatti Luizon[1,2]

[1] Department of Genetic, Ecology and Evolution, Institute of Biological Sciences, Federal University of Minas Gerais (UFMG), Av. Antônio Carlos, 6627, Belo Horizonte-MG, CEP 31270-901. [2] The UFMG Graduate Program in Genetics, Institute of Biological Sciences, Av. Antônio Carlos, 6627, Belo Horizonte-MG, CEP 31270-901. [3] Fundação Hemominas, Alameda Ezequiel Dias, 321 - Santa Efigênia, Belo Horizonte-MG CEP 30130- 110. [4] Department of Pediatrics, Medical School, Federal University of Minas Gerais (UFMG, Av. Prof. Alfredo Balena, 190 - Santa Efigênia Belo Horizonte-MG CEP 30130-100

rahyssar.sales@gmail.com

Keywords: Sickle Cell Anemia, Fetal Hemoglobin, Polymorphisms, Functional Annotation

Sickle cell anemia (SCA) is a monogenic disease determined by β^s allele homozysozity, and SCA morbidities derive from the polymerization of deoxy HbS. Notably, fetal hemoglobin (HbF) inhibits this polymerization, thereby reducing the severity of SCA. Recently, we conducted a genetic association study involving 250 children with SCA and Single Nucleotide Polymorphisms (SNPs) within *BCL11A gene* and *HBS1L-MYB* (*HMIP-2*) intergenic region. They were found to be significantly associated with increased HbF levels. In this study, we have examined the effect of these SNPs in clinical outcomes and laboratory parameters of SCA. We further searched for functional data regarding these SNPs to improve the interpretation of our disease association findings. The unpaired t- test (ANOVA for more than 2 groups) and Mann–Whitney U (or Kruskal-Wallis) test were used to compare different groups for continuous variables that were normally and not- normally distributed in children with SCA, respectively. Multiple linear regression was used to identify the SNPs independently associated with HbF levels. Incidence rate ratio (IRR) was calculated for risk characterization of clinical episodes comparing genotype groups for each SNP. To correlate genotype and tissue-specific gene expression, we searched for SNPs within *BCL11A* and *HBS1L-MYB* that might influence gene expression as quantitative trait locus (eQTLs) using the Genotype-Tissue Expression (GTEx) and RegulomeDB databases. Additional functional annotations were retrieved from the University of California, Santa Cruz (UCSC) Genome Browser. Three SNPs were independently associated with higher levels of HbF in our cohort: rs4671393 (β- coefficient=0.28) in *BCL11A*, rs9399137 (β-coefficient=0.16) in *HMIP-2A* and rs4895441 (β-coefficient=0.15) in *HMIP-2B.* All the SNPs associated with increased HbF level were associated with higher total hemoglobin count ($p<0.01$). Subjects carrying the A allele of rs4671393 showed lower incidence risk of transfusion (IRR=1.62; $p<0.0000001$) and infection (IRR=1.16 $p=0.01$). Subjects carrying HbF-increasing alleles of rs93979137 and rs4895441 (C and G, respectively) showed reduced count of reticulocytes($p<0.01$), a key biomarker of SCA. The risk of acute chest syndrome was lower in carriers of the C allele of rs9399137. We found no eQTL for SNPs in *BCL11A.* However, rs4671393 overlaps with DNase I hypersensitive site and shows strong linkage disequilibrium with SNPs overlapping GATA1 and RNA polymerase II binding sites (rs7599488 and rs1427407). Regarding *HBS1L-MYB*, the SNPs annotated, including rs9399137, are eQTLs for *HBS1L* expression in several tissues. According to its score 1f at RegulomeDB and the Encyclopedia of DNA Elements (ENCODE) data related to gene regulation, the rs4895441 SNP likely affects transcription factor binding and is linked to expression of genes which encode γ-globin chain of HbF (*HBG1/HBG2*). Our findings support a reduced rate of clinical complications and milder hematological profile in children with SCA carrying HbF-increasing genotypes. Additionally, functional annotations highlighted the role of some associated SNPs on the HbF regulation pathway.

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HIGH-THROUGHPUT SEQUENCING AND IDENTIFICATION OF SATELLITE DNA IN AMPHIBIA

Raquel F. Destro[1*]; Marcelo J. Silva[1]; Thiago Gazoni[1]; Célio F. B. Haddad[2]; Patrícia P. P. Maltempi[1]

1Departament of Biology, Bioscience Institute, UNESP, Rio Claro, São Paulo, Brazil. 2Departament of Zoology, Bioscience Institute, UNESP, Rio Claro, São Paulo, Brazil.

* rfdestro@gmail.com

Key-Words: Cytogenetics; Centromere; Anura.

Satellite DNAs (satDNAs) constitute an abundant fraction of repetitive DNA in eukaryotic genomes, comprising non-coding sequences repeated in tandem and related to the presence of constitutive heterochromatin, which plays an important role in the organization and evolution of these genomes. The species *Proceratophrys boiei* (2n=22, ZZ: ZW) is considered an interesting model of genetic and cytogenetic study for repetitive sequences, because it presents a karyotype with large blocks of heterochromatin in the chromosomes and a chromosomal heteromorphism linked to sexual differentiation. Thus, in this study, we characterized a satDNA from the genome of the *P. boiei* frog, providing a physical mapping of this sequence by fluorescence *in situ* hybridization, after high-throughput sequencing Illumina® and analysis in bioinformatics programs, to know its genomic organization and chromosomal location. The analyzes were carried out through the integration of a cytogenetic, molecular and genomic approach. Sequencing data were used to search for repetitive sequences in the RepeatExplorer software, in which several satDNAs were revealed in the *P. boiei* genome. For the satDNA named PboSat57-97 an analysis of the reads was performed in which it was possible to obtain a consensus of monomer of 97 bp, with 53.6% of A+T and a genomic abundance of 5.2%, besides a grouping graph of relatively dense and characteristic satellite DNA sequences. After FISH experiments, hybridization signals were observed in the centromeric and pericentromeric region of all *P. boiei* chromosomes, in areas corresponding to constitutive heterochromatin. Satellite DNAs are major components of heterochromatin, located primarily in centromere and telomeres. Our results reinforce the tendency of satDNA matrices to occupy a more restricted chromosomal location (constitutive heterochromatin), and the presence of PboSat57-97 in *P. boiei* centromeres may result from its function in the formation of heterochromatin and centromeric function, which is playing key roles for the maintenance of the centromere.

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GENETIC IDENTIFICATION OF SHARK'S FINS SEIZED IN NATAL - RN

Ana Paula Moreira Cotrim[1]; Fernanda Dotti do Prado[1]; Carlos Egberto Rodrigues Jr[2]; Fábio Porto-Foresti[1].

[1]Universidade Estadual Paulista "Júlio de Mesquita Filho" – UNESP – Câmpus Bauru. [2]Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis (IBAMA)

anaa_cotrim@hotmail.com

Palavras-chave: DNA Barcode; Conservation; Chondrichthyes.

Nowadays the fishing industry is the main problem related to shark's population decrease, comparing environmental destruction, pollution and climate changes. This situation is getting worse due to high consumption of shark's fin, by the Asian market. So, the majority apprehensions captured by IBAMA (Brazilian Institute for the Environment and Renewable Natural Resources) are isolated fins, and it is difficult to recognize the species morphologically. Therefore, genetic-molecular methods - more specifically, the Barcode DNA method - helps to identify the species of shark, independent of fin's morphology. The main objective of this study is identifying the species of sharks apprehend by IBAMA in Natal, capital of the state of Rio Grande do Norte. Twenty-seven samples (fin muscle) were selected. The DNA was extracted from the biological material, increasing the water bath time (60 ° C) in 12 hours compared to standard protocol (2h) for fish extraction. Subsequently, the COI gene (Cytochrome oxidase I) was amplified by PCR (polymerase chain reaction) using the FishF1 and FishR2 primers and visualized on 2% agarose gel. The most successful samples were sequenced and analyzed on the BOLD platforms (www.boldsystemes.org) and BLAST (www.blast.ncbi.gov). Up to now, it was observed that five samples had 100% of similarity with the specie *Isurus paucus*, popularly known as "Anequim-preto". According to IUCN (International Union of Conservation of Nature) the state of conservation of this specie is classified as "Endangered". This study shows the necessity of genetics methods to identify, correctly, the species of sharks captured by fishing industry. And, also helps, to provide important information for projects of preserving and conserving shark populations along the Brazilian coast.

ELIMINATION OF B CHROMOSOMES IN ASTYANAX HYBRIDS

Caio Augusto Gomes Goes[1]; Duílio Mazzoni Zerbinato de Andrade Silva[2]; Ricardo Utsunomia[1]; Fausto Foresti[2]; Fábio Porto-Foresti[1*]

[1]Universidade Paulista Júlio de Mesquita Filho - UNESP. Campus de Bauru. [2] Universidade Paulista Júlio de Mesquita Filho - UNESP. Campus de Botucatu

caioaggoes@gmail.com

Keywords: B chromosome; *Astyanax*; Neotropical fishes; Reproduction.

The genus *Astyanax* (Characiformes, Characidae) is one of the most abundant species among Neotropical fish. In this group, B chromosomes have already been observed in at least ten species, among which the variant present in *Astyanax paranae* stands out for being an isochromosome with several types of repetitive DNA and single copy genes, characterizing itself as an important study model. Although many DNA sequences have already been characterized in this element, data on the inheritance patterns of these chromosomes have never been collected and analyzed. In this sense, the present work had as objective to evaluate the inheritance of B chromosomes in *Astyanax paranae* through directed crosses between females bearing B chromosomes and *Astyanax lacustris* males. The crosses were performed through the semi-natural reproduction method and were directed as follows: a control crossover (male and female of *A. paranae* non-B chromosome carriers); a cross with female carrier and non-carrier male (*A. lacustris*) (crossing 1);. The larvae resulting from these crosses were fixed in alcohol and the identification of supernumerary individuals was performed through the relative quantification of the sbno gene (known to be present on the B chromosome of this species) through the qPCR method, all of them being analyzed in triplicates independently. With these data, it was possible to calculate the rate of transmission of chromosomes B (kb) from the average number of B chromosomes of the progeny, divided by the total number of B of the parents. Twenty larvae were analyzed from the control crossing, with no supernumerary carriers. Already at crossing 1, 23 larvae were analyzed, being only 3 carriers and 20 non-carriers (Kb = 0.130). the ratios obtained have been close to extinction, suggesting a mechanism of elimination of the B chromosome by *A. paranae* females, probably related to elimination of the polar corpuscle in female gametogenesis, through preferential migration of chromosome B for this portion. In this way, it is possible to say that, in the assay performed, the transmission of the B chromosomes is in a state of elimination, being the sex of the parental carrier a factor that can impact on its heritability.

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RELATIONSHIP BETWEEN HYPOXIA-INDUCIBLE FACTOR-1 ALPHA P582S POLYMORPHISM AND DIABETIC RETINOPATHY

Bruna Letícia da Silva Pereira[1]; Daisy Crispim[2], Evelise Regina Polina[1], Renan Cesar Sbruzzi[1], Luís Henrique Canani[2]; Kátia Gonçalves dos Santos[1].

[1]Laboratório de Genética Molecular Humana, Programa de Pós-Graduação em Biologia Celular e Molecular Aplicada à Saúde, Universidade Luterana do Brasil (ULBRA), Canoas, Rio Grande do Sul, Brasil. [2]Serviço de Endocrinologia, Hospital de Clínicas de Porto Alegre (HCPA), Porto Alegre, Rio Grande do Sul, Brasil.

*brunaleticiap@gmail.com

Key-words: HIF1A; rs11549465; diabetes complications.

Chronic hyperglycemia caused by decompensated diabetes mellitus promotes changes in the oxygen homeostasis, leading to cell damage by hypoxia. The hypoxic environment in the retina contributes to the development of diabetic retinopathy (DR), which is the main cause of non-traumatic vision impairment in adults. Hypoxia-inducible factor-1 is a heterodimeric transcriptional factor, composed by oxygen-sensitive subunit HIF1-alpha, responsible for regulating several genes related to cellular response against hypoxia. The T allele of the P582S polymorphism (C1772T, rs11549465) in HIF1A gene has been related to enhanced transcriptional activity of HIF1-alpha when compared to the wild- type C allele. To elucidate whether the P582S polymorphism in the HIF1A gene is associated with DR, we conducted a case-control study with 927 South Brazilian outpatients with type 2 diabetes (T2DM). The case group included 480 patients with DR while the control group was composed by 447 subjects without DR. Additionally, 110 health blood donors were included to verify the polymorphism frequency in the general population. Genotyping was performed by real-time polymerase chain reaction using hydrolysis probes. Inferential statistics analyses were done by Pearson's chi-square and multiple logistic regression. There were no differences in the allele frequencies between blood donors and patients with T2DM (C=87.3% and T=12.7% versus C=90.1% and T=9.9%, respectively; p=0.227). However, those groups differed in their genotypic frequencies (CC=78.2%, CT=18.2% and TT=3.6% versus CC=80.9%, CT=18.5% and TT=0.6%, respectively; p=0.010). By the observation of the adjusted residuals, the data indicated that the subjects without T2DM have a higher frequency of the TT genotype. The observed genotype distribution in T2DM and blood donors were in accordance with Hardy-Weinberg equilibrium. In relation to DR, allelic frequencies were similar between cases and controls (C=90.6% and T=9.4% versus C=89.6% and T=10.4%, respectively; p=0.507). Genotypic frequencies in controls were different in comparison to cases (CC=80.6%, CT=18.1% and TT=1.3% versus CC=81.2%, CT=18.8% and TT=0.0%, respectively; p=0.037), however, after adjustment for sex, diabetes duration, bodymass index and use of insulin, the association between the polymorphism and DR was not significant (OR=0.819, 95% CI 0.577-1.162). After the covariates adjustment, the data provides no evidences that the T allele of P582S polymorphism in HIF1A gene displays a protector role against DR. Therefore, it is necessary to increase the number of individuals to detect a proper significance due to the low frequency of this polymorphism.

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Species delimitation in tonguefishes (Pleuronectiformes; Cynoglossidae; *Symphurus*) from Northeastern Brazil using DNA barcoding

Hannah Magalhães Muniz Teixeira[1*]; Leandro Araújo Argôlo[2]; Jamille de Araújo Bitencourt [1]; Paulo Roberto Antunes de Mello Affonso[1]

[1]Universidade Estadual do Sudoeste da Bahia – UESB, Jequié, Bahia, Brazil. [2]Universidade Federal da Bahia – UFBA, Salvador, Bahia, Brazil.

*hannah_teixeira_@hotmail.com

Keywords: COI; flatfish; systematics.

Estimates about the diversity in Pleuronectiformes are hindered by the controversial identificaition of species in this group of flatfishes. This is the case of *Symphurus*, the only genus of Cynoglossidae found in Western Atlantic, being characterized by several taxonomic uncertainties and evidence of species complexes. Therefore, molecular studies, such as those based on DNA barcoding, represent a potentially informative database to resolve the taxonomic status of *Symphurus* representatives. In fact, the mitochondrial cytochrome oxidase I (COI) gene has been sucessfully used to identify species in distinct animal groups since it encompasses variable regions able to discriminate closely related taxa. Thus, the goal of this study was to evaluate the taxonomic status of *Symphurus* specimens (formerly identified as *Symphurus* cf. *tessellatus* and *Symphurus* aff. *plagusia*) from Northeastern Brazil based on analyses of COI sequences. For comparative analyses, additional COI sequences available for other *Symphurus* in the BOLD platform were also included in the present study. The dendrogram based on COI data using Neighbor-Joining (NJ), Maximum Likelihood (ML) and Bayesian inference (BI) methods reliably separated the samples herein obtained of *Symphurus* cf. *tessellatus* from the single public COI sequence associated to *S. tessellatus* in BOLD. On the other hand, the samples of *Symphurus* aff. *plagusia* clustered together with *S. ginsburgi*. However, since the latter is restricted to southern and southeastern coast of Brazil, it is likely to represent a case of misidentification in the public database. Moreover, putative cryptic species lacking a formal description were also indicated in the analyzed samples. These data indicate either errors in the taxon identification in the BOLD platform or cryptic speciation in *Symphurus* that should be revisited under an integrative approach.

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RUNX2 tandem repeat associated to mandibular shape in sigmodontine rodents (Rodentia, Cricetidae)

Rafael de A. Carvalho[1]; Thales R. O. de Fretias[1]

[1]Departamento de Genética, Universidade Federal do Rio grande do Sul.

carvalhora89@gmail.com

Key-words: Evolutionary Developmental Biology, Morphological Evolution, Skeleton

The recent field of evolutionary developmental biology has revealed several manners in which genetic modification can alter developmental paths and hence produce phenotypic novelty without being deleterious. In this context, a few studies in the latest fifteen years showed that a particular transcription factor, namely *RUNX2*, is tightly related to the craniofacial diversity in relatively high diverse mammalian taxa, such as domestic dogs and others carnivorans mammals, as well as bats, primates and sigmodontine rodents. More specifically, this protein has a poli-glutamine (poli-Q) followed by a poli-alanine (poli-A) homopeptides whose stretching and shortening can affect time of bone ossification and, so, as they vary across taxa, may strongly impact skeletal evolution. Our study is a first attempt to investigate the relation between *RUNX2* and the diversity of shape in the mandible of a mammalian highly diverse – although morphologically conserved – taxon, namely the sigmodontine rodents (Neotropical mice and rats). In order to do so, we extract DNA from soft tissues and amplified the QA repeat stretches, and its flanking regions, of 19 individuals belonging to different sigmodontine species. After the sequencing, the number of Q and A, and the ration (Q/A ratio) between them, were calculated. Also, we digitalized 13 landmarks in the lateral face (right) of the mandible of each one of the 19 individuals. The landmark configurations were submitted to a Procrustes superimposition and then to a principal component analysis (PCA). To test the correlation between the Q/A ratios and mandibular shape, we performed Spearman's correlation and a phylogenetic generalized least squares (PGLS), using a phylogenetic framework obtained from the literature. The species evaluated here showed a low variability in the Poli-Q and poli-A stretches. However, we found an association of the Q/A ratio and the interspecific variation in the main axis of variation (PC1), which is tightly related to size variation (Spearman's: $r = -0.70$, $p < 0.05$; PGLS: $r^2 = 0.28$, $\lambda = 0.932$, Intercept = - 0.186, F-statistic = 6.45, $p < 0.05$). Higher values of Q/A are associated to a more robust mandibular shape, including larger processes, a more "adult-like" condition. On the other hand, lower Q/A values were found in species with a "juvenile-like" mandibular shape, that is, possessing narrower processes and narrowe horizontal ramus. Thus, our finding suggests that, besides the skull, the variability in RUNX2 tandem repeats may also impact mandibular evolution, possibly in the directions of the lines of least evolutionary resistance (i.e., size). Future studies exploring ecological specialization in the sigmodontine mandible could reveal if *RUNX2* underwent natural selection while modeling mandibular shape in sigmodontines.

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IN SILICO ANALYSES OF *daf-2* GENE IN ANHYDROBIOTIC AND NON-ANHYDROBIOTIC SPECIES

Yasmin de Araújo Ribeiro[1,2]; Danyel Fernandes Contiliani[1,2]; Vitor Nolasco de Moraes[1,2];Tiago Campos Pereira[1,2]

[1]Departamento de Biologia, FFCLRP, USP, Brasil. [2]Programa de Pós-graduação em Genética, FMRP, USP, Brasil.

yasminar@usp.br

Keywords: anhydrobiosis; *daf-2*; tyrosine kinase.

Kinases compose the largest family of proteins in eukaryotes and play key roles in intracellular communication, protein regulation and signal transduction. In *Caenorhabditis elegans*, the protein 'tyrosine kinase receptor' encoded by the *daf-2* gene plays an important role in endocrine signaling. It controls whether the animal enters into the reproductive life cycle or interrupts its development by entering into *dauer,* an alternative and desiccation tolerant (i.e., anhydrobiotic) stage. The aim of the present work is to evaluate the conservation of *daf-2* protein in anhydrobiotic and non- anhydrobiotic (desiccation sensitive) species. Search for *daf-2* homologues was conducted via tBLASTn, in ten anhydrobiotic organisms and ten non-anhydrobiotic organisms, whose identities were reconfirmed via BLASTx. These sequences were aligned in Clustal Omega, primary structures were visualized in BioEdit v 7.0.5 and Jalview (anhydrobiotic, non-anhydrobiotic and both simultaneously) and the structures in PyMOL (tertiary structures of 'kinase' and 'fibronectin type III' domains of *C. elegans* and *H. sapiens*). Our results indicate conservation of the 'fibronectin type III' and 'kinase' domains in 30% of the evaluated non-anhydrobiotic organisms, all belonging to the animal kingdom. The remaining organisms of this group presented conservation only of 'kinase' domain. Among anhydrobionts, all species display conserved kinase domain, and *C. elegans* alone has preserved the 'fibronectin type III' domain (e-value = 6.11e- 07). Alignment of the tertiary structures of the 'fibronectin type III' and 'kinase' domains of *C. elegans* and *H. sapiens* presented considerable structural similarity (RMSD = 2,938; RMSD = 0.711, respectively). We conclude that fibronectin type III is not crucial for anhydrobiosis and that both 'fibronectin type III' and 'kinase' domains of the investigated anhydrobiotic and non-anhydrobiotic are functionally similar.

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IDENTIFICATION OF MUTATION IN *MYO7A* GENE IN A FAMILY WITH AUTOSOMAL DOMINANT HEARING LOSS

André Silva Bueno[1]; Ronaldo Serafim Abreu-Silva[1]; Regina Célia Mingroni Netto[1]

Departamento de Genética e Biologia Evolutiva - Instituto de Biociências -Universidade de São Paulo[1], Brasil

andre.bueno@usp.br

Keywords: Mutation; Autosomal dominant; Hearing loss

Hearing loss is one of the most frequent sensorial disorders in humans. When inherited, it can be autosomal dominant or recessive, X-linked or mitochondrial. Sixty- seven *loci* havealready been mapped and 45 genes have been identified as associated with autosomal dominant hearing loss, responsible for about 20-30% of the hereditary cases.The development of massive parallel sequencinghas contributed to diagnosis and genetic counseling indiseases with genetic heterogeneity, allowing the sequencing of thousands of genes in a single test. Our aim was to identify the gene and the mutation that explain neurosensorial, progressive and nonsyndromichearing loss, with autosomal dominant transmission, in a family ascertained in the Laboratory of Human Genetics – (LGH) IBUSP. The age of onset of hearing impairment ranged from six to 34 years old, with a mean age of 12 years. Massive parallel sequencing of the exome was performed in three samples from affected individuals in the equipment Hiseq 2500 (Illumina INC, San Diego, California) after capture with *Sure Select QXT Target Enrichment*Kit (Agilent Technologies, Santa Clara, California). Variant filtering was performed from the Annotated VCF computational file. Computational packages to evaluate pathogenicity (*Mutation Taster*, SIFT and *Polyphen*) were used for selection of candidate variants to explain hearing loss. Sanger sequencing of the candidate mutation was used to investigate its transmission in the family. We identified the candidate variant c.689C>T p.Ala230Val in *MYO7A* gene, predicted to be pathogenic according to *MutationTaster*, SIFT, *Polyphen.* In addition, it was not identified in the main genomic databases (*6500 Exomes*, *1000 genomes*, *ExAC* and *AbrAOM*). Sanger sequencing revealed thealteration is present in all six affected individuals and absent in the two unaffected individuals from this family. The c.689C>T p.Ala230Val mutation in *MYO7A* gene had already been identified in an Italian family with autosomal dominant hearing loss.The protein Myosin VIIA localizes to actin protrusions in photoreceptor cells in retina and in cochlear hair cells. Myosin VIIA slides on actin filaments and maintains structure and function of these protrusions in auditory systems. The aminoacid substitution is located to the N-terminal motor domain of the protein, and a dominant negative effect on the wild typeprotein was speculated in the literature.In conclusion, the variant c.689C>T p.Ala230Val in the *MYO7A* gene is responsible for autosomal dominant hearing loss in this family.

Funding Agencies: CAPES and CEPID- FAPESP (2013/08028-1).

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IDENTIFICATION OF MUTATION IN *MYO7A* GENE IN A FAMILY WITH AUTOSOMAL DOMINANT HEARING LOSS

André Silva Bueno[1]; Ronaldo Serafim Abreu-Silva[1]; Regina Célia Mingroni Netto[1]

Departamento de Genética e Biologia Evolutiva - Instituto de Biociências -Universidade de São Paulo[1], Brasil andre.

bueno@usp.br

Keywords: Mutation; Autosomal dominant; Hearing loss

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Funding Agencies: CAPES and CEPID- FAPESP (2013/08028-1).

STUDY OF SOS INDUCTION BY CIPROFLOXACIN IN *PSEUDOMONAS AERUGINOSA*

Lima-Noronha, M.A.[1], Fonseca, M.R.B.[1], Valencia, E.Y.[1] and Galhardo, R.S.[1].

[1] Department of Microbiology, Institute of Biomedical Science - University of São Paulo, São Paulo, SP, Brazil.

marco.noronha@usp.br

Keywords: *Pseudomonas aeruginosa*; SOS response; Ciprofloxacin; Antibiotic resistance.

Pseudomonas aeruginosa is a gamma-proteobacterial that can cause disease in plants and animals. It is an opportunistic pathogen considered as a serious public health problem due to the high incidence of isolates that shows multi-resistance. In addition to the natural resistance to several classes of antimicrobials, many factors can contribute to the emergence of resistance in this species, one of these mechanisms being the SOS response. The SOS response is a system that controls genes directly involved in mutagenesis and may have an increased activity in the presence of antimicrobials, which contributes to the emergence of mutations that may be selected for the emergence of resistance to new types of drugs. Furthermore, this response controls several mechanisms of the bacterial physiology, such as DNA repair, homologous recombination, control of cell division and formation of biofilms. The objective of this work is to identify genes that can modify the SOS response dynamics of Pseudomonas aeruginosa and characterize them functionally. The study will be performed by the construction of a transposon library in which we will analyze the behavior of mutant strains in the presence of ciprofloxacin, an antimicrobial capable of activating the SOS response efficiently. So far, six potential genes that affect the SOS response dynamics in *Pseudomonas aeruginosa* were found.

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RESEARCH ON HEMATOLOGIC DISEASES IN QUILOMBOLA COMMUNITIES IN THE STATE OF PARÁ

Wandrey Roberto dos Santos Brito[1]; Julyanne Elyne Castilho Ribeiro[1]; Milene Raiol de Moraes[1]; Ândrea Ribeiro dos Santos[1]; Greice de Lemos Cardoso Costa[1]

[1]Laboratório de Genética Humana e Médica, Instituto de Ciências Biológicas, Universidade Federal do Pará, Belém, Pará, Brasil.

wandrey.ben1@gmail.com

Keywords: quilombola communities, hemoglobins, sickle cell anemia.

Adult hemoglobins (HbA) are proteins composed of two pairs of polypeptide chains, one with two alfa chains and the other with two beta chains, responsible for the correct transport of O_2 in the tissues. Any genetic modification that changes the structure of the molecule can be called hemoglobinopathy. In beta gene, the replacement of glutamic acid by a valine at the sixth codon in the beta chain forms hemoglobin S (HbS), which in homozygosis leads to sickle cell anemia. This hemoglobinopathy has been associated to African ancestry and it is the most prevalent hematologic disease in Brazil. During the colonization process of this country, Africans were brought on commercial routes to be enslaved and, in search of freedom, fled to regions of forests, took refuge and formed communities called quilombos. In this sense, these communities (quilombolas) have their own historical trajectory and many persist until today in Brazil. The present study investigated the type of hemoglobins present in individuals living in the Quilombola communities of Moju and Irituia (located in Pará state), estimating allelic frequency of HbA and variant HbS (HBB*S allele). This study included 100 individuals from quilombola communities Caeté (N=81) and São José do Açaiteua (N=19). From each individual, 5 mL of peripheral blood was collected in a vacuum collection tube containing EDTA as anticoagulant. Hemoglobin electrophoresis was performed to identify hemoglobin type in each individual. Chi-squared test was used in the comparison analysis. No variant hemoglobin was observed in the studied samples. However, previous studies in Pará state have shown an HBB*S allele frequency of 0.9% in Saracura community (in Santarém city) and 3.6% in Pacoval community (in Alenquer city). Thus, the absence of HBB*S allele in our findings may be due to (i) the formation of these communities being done by individuals that did not carry this allele; (ii) admixture processes with non- Quilombola groups; (iii) sample number. Nevertheless, this study contributed to a better knowledge of hemoglobinopathies in quilombolas, as well as the formation of these communities in Pará state. Therefore, this work is informative and relevant to public health. It also highlights the importance of conducting studies regarding hemoglobinopathies in quilombolas and other isolated communities, in order to assist in education and health in these communities.

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INFLUENCE OF GENETIC VARIABILITY IN THE TOMATO (*Solanum* section *Lycopersicon*) CLADE ON REGULATION OF AMMONIUM TRANSPORT ACTIVITY

Gabriel de Oliveira Ragazzo[1]; André Luiz Tagliaferro[1]; Lázaro Eustáquio Pereira Peres[2], Antonio Vargas de Oliveira Figueira[1]; Joni Esrom Lima[3].

[1]Center for Nuclear Energy in Agriculture, University of São Paulo, Piracicaba, São Paulo, Brazil. [2]Luiz de Queiroz College of Agriculture, University of São Paulo, Piracicaba, São Paulo, Brazil. [3]Biological Science Institute, Federal University of Minas Gerais, Belo Horizonte, Minas Gerais, Brazil.

* jonilimaufmg@gmail.com

Keywords: Nitrogen; *Solanum* genetic diversity; Comparative sequence analysis.

Ammonium requires less energy for its assimilation than nitrate and is preferentially taken up by *Solanum lycopersicum* roots, supporting the highest growth rates. To prevent excessive ammonium uptake, ammonium transport activity mediated by AMMONIUM TRANSPORTERs (AMTs) proteins are post-translationally modified by phosphorylation of a Thr residue in the cytosolic C-terminus of the *Arabidopsis thaliana* AMT1;1 orthologues leads to trans-inactivation of the whole quaternary AMT protein complex, that represses ammonium uptake in roots. Although *Solanum* is the largest genus in the family, and includes tomato (*Solanum lycopersicum*), tomato breeding over recent decades has focused on higher productivity but adaption to different cultivation systems including higher nitrogen (N) inputs remains elusive. Considering the overwhelming inter- species genetic variability, tomato wild species represent a gene pool with viable possibilities to address improvement of the efficiency in the use of nitrogen (NUE). Here, we have studied this genetic variation by accessing the genome sequencing of representative tomato accessions for protein-coding *SlAMT1;1* gene. In 84 wild species, the number of single-nucleotide polymorphisms (SNPs) exceeds 7% in *SlAMT1;1* coding sequence but only 3,4% of the polymorphisms are non-synonymous. Maximum- likelihood analysis for the target gene revealed SNPs found to be distinctive for the *Lycopersicon*, *Arcanum*, *Eriopersicon* and *Neolycopersicon* sections. In particular, *Eriopersicon* group has many species-specific polymorphisms in the C-terminus in spite of a highly conserved Thr residue found in all groups. To verify whether diversity in specific SNPs in the C-terminus are related to phenotypic variation in ammonium transport activity, influx studies using ^{15}N-labeled ammonium were performed in one accession for *Eriopersicon* group, one accession in the *Lycopersicon* group and one *S. lycopersicum* cultivar. Inhibition of ammonium transport capacities in the roots of *Lycopersicon* accession and *S. lycopersicum* cultivar were 75% whereas in *Eriopersicon* accession was reduced by 28% relative to control conditions. By contrast, *SlAMT1;1* expression in roots increased in all accessions upon ammonium supply, indicating that the reduced uptake levels are not related to transcriptional variation. These results suggest that SNPs in C terminus are associated with allosteric inhibition to distinctly repress ammonium transport activity in *Eriopersicon* accession, which may enhance the versatility for ammonium nutrition in response to environmental triggers.

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Timing the drugs: Implications of the circadian clock on resistance to chemotherapy

Matheus Molina Silva[1*]; Clarissa Ribeiro Reily Rocha[2]; Carlos Frederico Martins Menck[1];

[1]Department of Microbiology, Institute of Biomedical Sciences, University of São Paulo, São Paulo, Brazil. [2]Department of Clinical and Experimental Oncology, Federal University of São Paulo, São Paulo, Brazil.

matheusmolina@usp.br

Key-words: cancer; chronotherapy; DNA repair.

Currently, cancer is one of the main causes of morbidity and mortality worldwide, with approximately 8.8 million cancer related deaths in 2015, in which 1.69 million were from lung cancer. The most common lung cancer treatment is chemotherapy with cisplatin, based on the cytotoxicity caused by DNA damage induced by the drug, however, changes in the protection systems of cells may arise, causing resistance. Among these alterations, the increased expression of the DNA repair proteins XPF and ERCC1, and of the transcription factor NRF2, the master regulator of antioxidant response, stand out. Interestingly, there are evidences that these processes are under control of the circadian clock, an internal timing mechanism, orchestrating variations in behavior, physiology and metabolism, according to daily variations in our environment, such as temperature and light/dark cycles. Therefore, our main objective is to understand how the expression of these resistance factors is being controlled by the circadian clock, determining its peaks and depths, and the impact of this phenomenon on the efficacy of cisplatin treatment. To achieve this, we are using cell lines with the luciferase gene under control of a circadian clock gene promoter to follow their clock phase after synchronization with different drugs. We also perform cell viability experiments, measure protein levels by Western Blot and mRNA levels by qPCR, in different time points after synchronization. Our results show that there is a significant oscillation in the expression of NRF2 and its target genes, and also of DNA repair proteins, although in different phases. Furthermore sensitivity to cisplatin and other chemotherapeutic drugs that uses the same resistance mechanisms oscillates during the day with the same peak. This data can provide basis to the development of chronotherapy protocols that improves patients well-being.

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CHROMOSOME MAPPING OF REPETITIVE DNA IN *Symphysodon discus* (PERCIFORMES: CICHLIDAE): INSIGHTS ON CHROMOSOMAL ORGANIZATION

Ana Beatriz Moreira Ferreira; Allan Luiz Galvão Dickson; Manoella Gemaque Cavalcante; Luan Felipe da Silva Frade; Cleusa Yoshiko Nagamachi; Julio Cesar Pieczarka; Renata Coelho Rodrigues Noronha*

Universidade Federal do Pará, Instituto de Ciências Biológicas, Centro de Estudos Avançados de Biodiversidade, Laboratório de Citogenética Animal, Rua Augusto Corrêa, 1, 66075-110, Belém, PA, Brasil.

renatarcrn@gmail.com

Key-words: Karyotypic Reorganization; Histone H1; Cichlidae.

Fish of the genus *Symphysodon* representes one of the most distinct groups among South American Cichlids, possessing the largest diploid number among cichlids.It was proposed that diferent classes of repetitive DNAs may play important roles in the karyotype Evolution of these fish. In this sense, the aim of the presente study is to investigate the in situ organization of telomeric sequences, 45S rDNA and Histone H1 genes in *Symphysodon discus* karyotypes. The samples were collected in Rio Negro, Amazonas, Brazil. The repetitive sequences were amplified by polymerase chain reaction, labeled by nick translation, and used for the Fluorescence In Situ Hybridization technique (FISH). The results shows *Symphysodon discus* with 2n=60 (50m-sm + 10st- a). Telomeric sequences were observed in the distal region of all chromossome pairs. 45S rDNA sites were detected on the short arm of only one subtelocentric chromossome pair. Histone H1 genes were flagged in the subterranean regions of several chromossome pairs. The diploid number obtained for *S. discus* corroborates previous studies, reinforcing a karyotypic condition derived for the speicies, with an increase of chromossomal pairs. Structure rearrangements involving pericentric inversions, translocations and/or fissions were proposed, which led to the increase of 2n and genomic reorganization of *S. discus.* The telomeric and 45S rDNA data also corroborate previous studies for S. discus; however, it differs in localization and number of locus of the 45S rDNA relative to other species of the genus *Symphysodon*. The various subterminal signals of H1 genes open perspectives for assessments in these accumulations occur in remnants of chromossomal rearrangements, since it is a highly reorganized karyotype. It is worth to mention that histonic sequences have characteristcs common to regions of chormossome break, such as tandem repeats, periocentric and/or subtelocentric localization, and high rates of intrachromossomal and interchromossomal recombination. In this context, these sequences may promote genomic instability and representes a fragile substrate, propitious to break. In conclusion, we povide insight into the physical organization of repetitive sequences in the genus of S. discus and open perspectives fot studies on the location of the rearrangements occuring in the karyotype of the species as well as possible accumulations of repetitive DNAs in those regions.

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GUT MICROBIOME CHARACTERIZATION OF THE AMAZONIAN SURUÍ-AIKEWARA ETHNIC GROUP

Ana Paula Schaan[1*]; Giovanna C. Cavalcante[1]; Leandro Magalhães[1]; João Farias Guerreiro[1], Ândrea Ribeiro dos Santos[1,2.]

[1]Laboratório de Genética Humana e Médica – Universidade Federal do Pará, Belém/PA, Brazil. [2]Núcleo de Pesquisa em Oncologia (NPO) – Hospital Universitário João de Barros Barreto, Belém/PA, Brazil.

apschaan@gmail.com

Palavras-chave: Gut microbiome; Native Americans; Amazonia.

Humans have co-evolved for thousands of years with symbiotic communities of microbes that are most abundant and diverse in the gastrointestinal tract, the gut microbiome. In recent decades, the scientific community has gained increased awareness of the fundamental roles the gut microbiome plays in sustaining human health. However, the majority of studies focus on westernized and industrialized human groups, and do not reflect the full diversity of the human microbiome. Despite its enormous cultural and ethnic diversity, no study has ever studied the gut microbiome of Brazilian Native American communities. In order to address this issue, we characterized the gut microbiome of an Amazonian native group through V3/V4-16S rRNA metagenomic sequencing. We included 37 participants from the Suruí-Aikewara Amazonian ethnic group with ages ranging from 5 to 73 years old. The gut microbiomes of these individuals were represented by stool samples, and we additionally performed dietary questionnaires and collected medical records. Results show that the dietary habits of the Suruí-Aikewara consist largely of complex carbohydrates such as manioc and sweet potato, and small game animals such as agouti, deer, wild pig, and armadillos, although the consumption of industrialized foods is increasing. The prevalence of intestinal parasites was 51%. Metagenomic results reveal *Firmicutes*, *Bacteroidetes*, *Proteobacteria*, *Actinobacteria*, and *Spirochaetes* as the most abundant phyla in the gut microbiota of the Suruí-Aikewara. Further, samples show high abundances of taxa negatively associated to industrialized populations, such as *Prevotellaceae*, *Succinivibrionaceae*, and *Spirochaetaceae*, which is in accordance with other worldwide rural populations. Moreover, we detected the presence of several taxa belonging to the Treponema family, which has previously been found in wild hominids, ancient, and traditional rural populations. Also, there was small contribution of *Bacteroides* and *Akkermansia* genus, known as frequent among urbanized groups. Our results demonstrate that the gut microbiome of the Suruí-Aikewara resembles that of other traditional populations, and that their subsistence patterns are likely responsible for the acquisition of typically traditional microbial taxa. This may, however, transition to a westernized microbiome due to recent changes in lifestyle habits.

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Impaired late stage muscle regeneration in different murine models for muscular dystrophies

Antonio F. Ribeiro Junior[1], Lucas S. e Souza[1], Camila F. Almeida[1], Renata Ishiba[1], Stephanie A. Fernandes[1], Danielle A. Guerrieri[1], André L. F. Santos[1], Paula C. G. Onofre-Oliveira[1] and Mariz Vainzof[1]*

[1]Human Genome and Stem-cell Research Center, Biosciences Institute, University of São Paulo, São Paulo, 05508-090, Brazil.

antoniof.ribeiro@usp.br

Key-words: Satellite cells; muscular dystrophies; muscle regeneration

Satellite cells (SCs) are the main muscle stem cells responsible for its regenerative capacity. In muscular dystrophies, however, a failure of the regenerative process results in muscle degeneration and weakness. To analyze the effect of different degrees of muscle degeneration in SCs behavior, we studied adult muscle of the dystrophic strains: *DMD^{mdx}*, *Large^{myd}*, *DMD^{mdx}/Large^{myd}* with variable histopathological alterations. The expression of PAX7, an important transcription factor for SCs self-renewal, was analyzed. SCs proliferative capacity was measured by PAX7-positive/Ki67-positive cells quantification, since Ki67 is an important marker for cell-cycle. Similar results were observed in the dystrophic models as compared to wild type, with the maintenance of normal levels of PAX7 expression, retention of the PAX7-positive SCs pool, and their proliferation capacity. Moreover, an elevated expression of MYOG, an important myogenic factor, was also observed in dystrophic strains. The ability to form new fibers was proved through the presence of dMyHC positive regenerating fibers. However, those fibers had incomplete maturation characteristics, such as small and homogenous fiber caliber, which could contribute to their dysfunction. We concluded that dystrophic muscles, independently of their degeneration degree, retain their SCs pool with proliferating and regenerative capacities. Nonetheless, the maturation of these new fibers is incomplete and do not prevent muscle degeneration. Taken together, these results suggest that improvement of late muscle regeneration should better contribute to therapeutic approaches.

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MicroRNA Differential Expression in Tuberculosis

Arthur Ribeiro dos Santos[1*]; Cleonardo Silva Augusto[1,2]; Wanderson Gonçalves e Golçalves[1]; Rafael Pompeu Pantoja[1]; Pablo Pinto[1,2]; Amanda Ferreira Vidal[1]; Tatiana Vinasco-Sandoval[1]; André M. Ribeiro-dos-Santos[1,2]; Ândrea Ribeiro-dos-Santos[1,2]; Sidney Santos[1,2]; Gilderlanio Santana de Araújo[1]

[1]Laboratório de Genética Humana e Médica (LGHM) – Universidade Federal do Pará. [2]Programa de Pós-Graduação em Oncologia e Ciências Médicas – Núcleo de Pesquisas em Oncologia (NPO).

*arthurrdsantos@outlook.com

Keywords: MiRNA, Tuberculosis, Biomarkers

Molecular studies regarding regulatory elements such as small non-coding RNAs and their mechanisms are poorly understood in infectious diseases. Tuberculosis is one of the oldest infectious diseases of humanity, and it forces us to question it on a day-to-day basis. The control of the infection as well as its diagnosis are still complex, and the treatments used are linked to several side effects. Thus, the objective of this project is to investigate the miRNA's expression profile in order to identify possible biomarkers of this disease. We applied next generation sequencing to investigate the global expression profile of miRNAs from blood samples of 8 patients infected with tuberculosis, their respective hospital controls (6 samples), and 7 external controls. The samples were pre- processed by applying trimmomatic tools, STAR alignment and HTSEQ count, and finally submitted to edgeR methods for differential analysis. Altered miRNAs were observed in all the analysis. While comparing patients and hospital control, three differentially expressed miRNAs (*miR-let-7g, hsa-miR-4863p* and *hsa-miR-4732*) were found and are suggested to participate in important granuloma regulation and formation pathways involved in the three phenotypic types of granuloma immune physiopathology. These results suggested that miRNAs may be directly involved in immune modulation through the formation and maturation of granulomas, regulating the repertoire of genes expressed in cells of the immune system and encourage the application of miRNAs as potential biomarkers.

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CHARACTERIZATION OF REGULATORY REGION OF OPERON VisP-YgiV THAT CODING A REGULATOR OF THE AraC FAMILY IN *Salmonella enterica* serovar Typhimurium

Vânia Santos Braz[1]; Luana de Sales Leite[1]; Cristiano Gallina Moreira[1]

[1] Faculdade de Ciências Farmacêuticas - Campus de Araraquara, UNESP (Rodovia Araraquara Jaú, Km 01 - S/N - Campos Ville - Araraquara/Sp - Cep 14800-903).

vabraz@usp.br

Keywords: Transcription factor; regulation; gene expression.

Salmonella species are the most common cause of foodborne diseases in the world and one of the leading causes of self-limited human gastroenteritis, but some more invasive strains can cause typhoid fever. This extraordinary capacity for pathogenicity is intimately related to its virulence factors. These factors undergo a complex temporal regulation that responds to the signaling found in the microenvironments in the host. Thus, several mechanisms are involved in the regulation of these virulence factors as transcription factors (activators and repressors), regulatory RNAs, two-component systems, among other mechanisms. The periplasmic protein VisP, Virulence Stress-related Periplasmic Protein, participates in the basic cellular functions, maintenance of the cell membrane and including in the survival and virulence of *S.* Typhimurium. Besides, *visP* is co- transcribed with *ygiV* in *S.* Typhimurium that encodes a transcription regulator of the AraC family. Some studies have shown the importance of VisP in the periplasm by integrating care with membrane homeostasis to the processes of bacterial virulence and response to stress. Members of the AraC family are involved in regulating various processes such as carbon metabolism, stress response, and virulence. Therefore, the aim of this work is characterized by the expression and regulatory region of the VisP- YgiV operon besides to investigate if promoter expression pattern alters in different sugar conditions. Between *visP* and *ygiV* genes, there are 71 pb noncoding, indicating a potential promoter of the *ygiV*. Four different fragments corresponding to the regulatory region of *ygiV-visP* and *ygiV* were PCR amplified and cloned in front of the *lacZ* reporter gene. The β-Galactosidase activity assays were realized in different media. The *ygiV* promoter is not induced in different conditions, indicating the absence of a promoter in this region. The expression pattern of *ygiV-visP* showed an increase during growth in the rich medium when compared with growth minimal medium. In addition, in LB medium the P4 construction presents higher expression in relation to the other constructions. Additionally, the assays were performed in minimal medium adding glucose or maltose. In the presence of glucose and maltose the constructs P1, P2 and P3 showedinduction of the expression in relation to the minimal medium and only P4 showed no alteration of expression in the presence of maltose. All results indicated that different expression pattern.

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ASSESSMENT OF THE GENOTOXIC POTENTIAL OF PESTICIDES USING THE MICRONUCLEI TEST IN TRADESCANTIA PALLIDA

Carlos Fernando Campos[1]; Ana Maria Bonetti[1]; Cássio Resende de Morais[1]; Edimar Olegário de Campos Junior[1]; Henrique Nazareth Souto[1]; Matheus Campos Cunha[1]; Sandra Morelli[1]; Vanessa Santana Vieira Santos[1]; Boscolli Barbosa Pereira[1]

[1]Universidade Federal de Uberlândia. Instituto de Biotecnologia (IBTEC). Avenida Pará 1720, Umuarama, Uberlândia – MG, 38405-320.

carllosfernando20@hotmail.com

Key-words: Pesticides; Tradescantia; Genotoxicity.

Pesticides are defined as a large group of heterogeneous chemicals widely used to repel and combat pests in different fields of agriculture. The most common examples of these compounds include insecticides, herbicides, fungicides, rodenticides and plant growth regulators. Remarkably, pesticides can be used in public health protection programs against vector organisms implicated in the transmission of infectious diseases, as malaria, dengue and schistosomiasis. Initially, they were used due to some advantages, contributing to the increase of agricultural production, control of pests and eradication of illness. However, their residues can remain in the environment, consequently exposing several organisms to undesirable concentrations and to toxicity risk during all the steps related to the production and use of pesticides. In this sense, it is important to consider methodological tools to assess the genotoxic capacity of different pesticides. The aim of this study was to evaluate the genotoxic potential of two commercial pesticides, Roundup Original DI® (Glyphosate) and Benevia® (Ciantraniliprole), through the Micronucleus test in *Tradescantia pallida*. Firstly, the rods were excised and placed in distilled water for acclimatization. Both pesticides were evaluated in the following concentrations: 200%; 100%; 50%; 25%; 12.5%; 6.25% and 3.125%. For each treatment, 15 plants were exposed for 12 hours, including the positive control (As_2O_3) and negative control (Hoagland solution). Subsequently, the plants were placed in distilled water for 24 hours to recover. Then, the inflorescences were fixed in Carnoy solution for 24 hours at 4 °C. The inflorescences were stored in 70% ethanol at 4 °C until analysis. Five slides for each treatment were prepared. Cells were stained with 2% acetic carmine. The slides were examined under an optical microscope at 400x. 300 tetrads were analyzed per slide. Effective concentrations for genotoxicity were evaluated using logarithmic function. The genotoxic changes were expressed by the number of micronuclei per 100 tetrads after statistical analysis (ANOVA, Tukey $p < 0.05$). In all concentrations evaluated, Roundup exhibited genotoxic effect when compared to the negative control, while Benevia showed genotoxic effect in five concentrations (200%; 100%; 50%; 25% e 12.5%) in comparison to the negative control. Data obtained by this study reveal the genotoxic potential of two pesticides widely used in agriculture for pest control. These findings predict the possibility of chromosomal damage that may affect the genetic instability of humans and other organisms. In conclusion, the micronucleus test in *Tradescantia pallida* showed to be a versatile methodology in the assessment of the genotoxic potential of both pesticides, even at low concentrations. Moreover, it is important to highlight that they can be hazardous to the public health and safety.

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USE OF MITOCHONDRIAL DNA TO ANALYSE THE POPULATION STRUCTURE AND GENETIC DIVERSITY OF LONGFIN MAKO (*ISURUS PAUCUS*) IN THE ATLANTIC OCEAN.

Carolina de Oliveira Magalhães[1]; Rui Coelho[2], Claudio Oliveira[1], Fausto Foresti[1] Bruno Lopes da Silva Ferrette[4], Fernando Fernandes Mendonça[3].

[1] Instituto de Biociências de Botucatu, Universidade Estadual Paulista – UNESP, campus Botucatu, São Paulo, Brasil. [2] Instituto Português do Mar e da Atmosfera (IPMA), IP, Olhão, Portugal. [3] Instituto do Mar, Universidade Federal Paulista – UNIFESP, Campus Baixada Santista, Santos, São Paulo, Brasil. [4] Laboratório de Genética e Conservação, Universidade Santa Cecília (UNISANTA), Santos, São Paulo, Brasil.

carol17magalhaes@gmail.com

Palavras-chave: Oceanic tropical shark; conservation genetics; molecular marker.

Isurus paucus, is an oceanic tropical shark widely distributed, but only occasionally found. It is mainly fished as by-catch in pelagic longline fishing, and its catches are inadequately monitored and possibly underestimated due to errors in identification among species of the genus Isurus. This species is currently listed as "Endangered" on the International Union for Conservation of Nature and Natural Resources (IUCN) Red List because of its apparent rarity and biological characteristics. This work aimed to generate data about the population structure and genetic diversity of the *Isurus paucus* shark in the Atlantic Ocean, using mitochondrial DNA sequences. Tissue samples were collected in oceanic regions of the Atlantic equatorial region. The genetic material was extracted, amplified the region of interest in the mitochondrial DNA and performed the nucleotide sequencing. For the genetic-population analyzes, the composition and nucleotide diversity, the number of polymorphic and haplotype sites and, also, the haplotypic diversity were calculated. Molecular Variance Analysis (AMOVA) was calculated and, to verify the existence of deviation in the null hypothesis of neutrality in the sequences of the control region, FS neutrality tests of Fu and R2 were performed. The Mismatch distribution test was used and the haplotype networks were elaborated using the Median Joining method. Sequencing of the mitochondrial DNA control region of 113 individuals of the *I. paucus* species resulted in 823 nucleotides with six polymorphic sites, evidencing 11 haplotypes. The haplotypic (h) and total nucleotide (π) divergences were h = 0.761 ± 0.023 and π = 0.00247 ± 0.00016, respectively. The AMOVA analysis revealed the absence of genetic structuring (ΦST = -0.00823), with significant deviations from the null hypothesis of neutrality, suggesting the occurrence of population expansion. The R2 values were not significant at any site, while the Fu Fs test showed negative values at all sites. The unimodal patterns observed in the graphs resulting from the Mismatch analysis, suggest a hypothesis of recent population expansion. *Isurus paucus* is a highly migratory species and it can be inferred that the results of the indexes of genetic diversity, nucleotide and haplotype diversity were similar to those observed for other pelagic shark species and that the individuals analyze characterize a single panmitic population in the Atlantic, covering at least the tropical range of its distribution. Although the *Isurus paucus* populations described in the present study have a high degree of connectivity and genetic diversity indexes of a panmitic population, the maintenance of genetic variability should not be ignored. Our data highlight the importance of more effective strategies for management and conservation, which require international collaboration, in order to enable the maintenance of the genetic variability of the species.

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GENE EXPRESSION PROFILE OF MAYOR ROYAL JELLY PROTEIN (MRJP) AND YELLOW IN STINGLESS BEE *MELIPONA QUADRIFASCIATA*

Patrick Douglas de Souza dos Santos[1]*, Zilá Luz Paulino Simões[1,2]

[1]Departamento de Biologia, Laboratório de Biologia do Desenvolvimento de Abelhas, Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto, Universidade de São Paulo, Av. Bandeirantes, 3.900 - Monte Alegre, Ribeirão Preto – SP. [2]Departamento de Genética, Laboratório de Biologia do Desenvolvimento de Abelhas, Faculdade de Medicina de Ribeirão Preto, Universidade de São Paulo, Av. Bandeirantes, 3.900 - Monte Alegre, Ribeirão Preto – SP

Keywords: Age polyethism; Hypopharyngeal gland; mrjp; Hymenoptera; Caste differentiation

The stingless bees have a high level of social organization through caste differentiation, overlapping generations in the same colony, reproductive division of labour and parental care, features related to eusociality. The factors responsible for the caste differentiation in Meliponini can be quantitative-alimentary or genetic-alimentary, in other words, the food is a determining element in this differentiation. In bees of the genus Melipona, caste differentiation is genetic-alimentary and there is an influence of epigenetic factors to make larvae a queen or worker. The compounds present in larval food are rich in amino acids, lipids and proteins, being essential for the development of the larvae. The hypopharyngeal glands synthesize the proteins present in larval food, these structures play a role in the production of the royal jelly in *Apis mellifera*. Genes expressing such proteins are called Mayor Royal Jelly Proteins (MRJP) and are located in a cluster that is flanked by genes from the yellow family. However, some of these genes present in *Apis mellifera* have orthologs in *Melipona quadrifasciata* even though they do not produce royal jelly. In addition, a high amino acid similarity of MRJP suggests a common evolutionary origin between these two groups of genes. The work aims to analyse the expression of genes in the hypopharyngeal glands of bees Melipona quadrifasciata. The information of genes was collected from NCBI and Hymenoptera Mine. Orthologous genes were identified by aligning *Apis mellifera* protein sequences against the sequences of *Melipona quadrifasciata*. The genes were annotated in Artemis software and the primers were designed specifically for each gene in Primer3 software. The bees were collected in ± 22d old (foragers). The hypopharyngeal glands were dissected in saline solution and collected for RNA extraction using Trizol reagent. A pool of glands (n=5) was used with 3 replicates. The cDNA synthesis was standardized according to the amount of total RNA. From the cDNA, PCR was performed with 3 genes of interest (mrjp9-like, yellow-e3 and yellow-h). PCR analysis showed amplification of only one gene (mrjp9-like), whereas yellow-e3 and yellow-h weren't amplified perhaps due to the primers. The next steps will evaluate the expression of these genes in different ages (0, 10 and 22 days) because the activity of the hypopharyngeal glands is influenced by the age of individuals, where a young bee (nurse) has greater glandular activity than an older bee (forager).

A STUDY ON THE EVOLUTION OF HOX MULTIGENE FAMILY USING GENE PRESENCE-ABSENCE PROFILING

Janaina Lima de Oliveira[1*]; Victor Montenegro Marcelino[1]; Rafaela Vieira Bruno[2]; Sávio Torres de Farias[1]

[1]Laboratório Paulo Leminski, Universidade Federal da Paraíba. [2]Laboratório de Biologia Molecular de Insetos, Instituto Oswaldo Cruz (IOC/Fiocruz).

Palavras-chave: Birth-and-death model; Metazoan archetypes; Evo-Devo.

Understanding the dynamics of multigene family evolution is a fundamental question to both molecular and morphological evolution. Expansions in gene copy numbers may have important consequences to the evolutionary process because they can (i) change the dosage of expressed gene products and (ii) allow a relaxation of selective constraints on duplicated gene copies. In both cases, duplication events may precede the emergence of evolutionary innovations by creating genetic variation to be shaped by selection. The *Hox* multigene family is of particular interest to understand the evolution and diversification of animal form because members of this family are involved in morphological patterning along body axes (anteroposterior and proximal-distal). In fact, changes in *Hox* gene sequence and sites of expression are correlated to metazoan body form diversification. Since this family evolves under a birth-and-death dynamics, we hypothesized that the fast turnover of *Hox* genes/clusters in animal genomes could contribute to the diversification of metazoan archetypes. To test this hypothesis, we explored genome data from several animal groups available in the Ensembl database to first identify all *Hox* clusters and their gene contents in each species. This information was transformed in a large binary matrix indicating gene presence/absence across species, which was lately subjected to phylogenetic reconstruction and inference of ancestral states using the R package phangorn. Our results pinpoint events of *Hox* genes gain/loss and the inferred ancestral states of *Hox* clusters' content in relevant nodes, which helps to understand how the evolution of the *Hox* multigene family might have contributed to the diversification of metazoan body plans.

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EVALUATING THE OCCURRENCE OF ALLELE-SPECIFIC EXPRESSION IN THE POLYPLOID SUGARCANE GENOME

Guilherme Bovi Ambrosano[1*]; Fernando Henrique Correr[1]; Guilherme Kenichi Hosaka[1]; Monalisa Sampaio Carneiro[2]; Gabriel Rodrigues Alves Margarido[1]

[1]Departamento de Genética, Escola Superior de Agricultura "Luiz de Queiroz"-ESALQ, Universidade de São Paulo-USP. [2]Centro de Ciências Agrárias, Universidade Federal de São Carlos-UFSCar.

* guilherme.ambrosano@usp.br

Palavras-chave: Differential gene expression; Allele dosage; Polyploidy

Little is known about the control of gene expression in sugarcane, despite its economic importance to Brazil and to São Paulo. The genome of sugarcane is polyploid and aneuploid, with evidence of both autopolyploidy and allopolyploidy. Its complex genomic structure hinders the use of techniques developed for other crops, frequently in a diploid mindset. Differential expression analysis, used in functional genomics research, is one of those techniques. This analysis aims to find differences in the levels of gene expression between samples from contrasting experimental conditions. It can be applied not only to diploids, but also polyploid species, albeit with some particularities. In these organisms, heterozygous loci present several possible allele dosages. In other words, alleles do not necessarily show a 1:1 ratio, as they would in diploid organisms. In addition to activating and repressing specific genes, differential expression may promote the expression of a particular allele over the others (preferential allele expression or allele-specific expression). Therefore, the allelic dosage in a given polymorphic site should be considered in the differential expression analysis. The objective of this work was to investigate allele-specific expression in leaves of sugarcane infected by *Puccinia kuehnii*, the causal agent of orange rust. In addition, we aimed to analyze the association between the levels of allele expression and the dosage of SNP alleles. To that end, we used RNA-Seq data from samples of the susceptible sugarcane genotype SP89-1115, collected at 0, 12, 24, 48 hours, 5 and 12 days after inoculation with *P. kuehnii*. Allele dosage data were obtained through genotyping-by-sequencing of this genotype. We observed strong allele-specific expression over time, mainly in genes related to stress response or photosynthesis. We found many polymorphic genomic sites for which only one of the alleles was expressed, according to RNA-seq data. Also, we found evidence of increasing allele expression with higher allele dosage, although not in a 1:1 fashion. Systematic error cannot be ruled out, because this deviation occurred in a similar manner at all sampled time points, and the influence of gene dosage over gene expression should be further investigated. Our study provides indication of the prevalence of allele-specific expression in the highly complex sugarcane genome, thus highlighting the importance of taking this process into account in future differential gene expression studies.

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ANALYSIS OF THE *WUSCHEL-RELATED HOMEOBOX* GENE FAMILY IN *Passiflora* spp.

Mariana Bombardi da Silva[1]; Helena Augusto Gioppato[1]; Tatiane Yamaguchi Quijada[1]; Bruna Rafaella Zanardi Palermo[1], Marcelo Carnier Dornelas[1].

[1] Departamento de Biologia Vegetal, Universidade Estadual de Campinas - Instituto de Biologia, Campinas, São Paulo, Brazil.

mbombardis@gmail.com

Key Words: Homeotic Genes; Plant Development.

Homeotic genes encode transcription factors characterized by a homeobox sequence, composed by 60-66 amino acids, and they are intimately related to organism development. Fifteen homeotic genes, named *WUSCHEL-related homeobox* (*WOX*), were identified in *Arabidopsis thaliana* and have a wide variety of functions related to plant development, some genes being expressed at very early developmental stages. *WOX* genes can be classified into three different clades: Ancient, Intermediate and WUS clades. The Ancient clade is formed by *WOX10*, *WOX13* and *WOX14*, and is also expressed in green algae. The intermediate clade is formed by *WOX8*, *WOX9*, *WOX11* and *WOX12* and the WUS clade is formed by *WOX1* to *WOX7* and *WUSCHEL* (*WUS*). The *WUS* gene is a member of the *WOX* family and is expressed in the cells of the organizing-center of the shoot apical meristem (SAM) and of the axillary meristems and regulates and guarantees stem cell maintenance. The genus *Passiflora*, the largest within the Passifloraceae family, composed by approximately 600 species, shows great diversity in flower morphology. *Passiflora organensis* is the first species of this genus to have its genome entirely sequenced by our research group, which allowed us to identify and analyze key genes in plant development, such as the *WOX* genes. *P. organensis* develops both tendril and flower simultaneously from the axillary meristems, which does not occur in *A. thaliana.* Thus, a better understanding of *WOX* gene family functions, especially *WUS*, in *P. organensis,* allows the analysis of their expression patterns during the development of both flower and tendril. This present work aims the identification and characterization of the *WOX* genes, using RT-qPCR to analyze gene expression. The *P. organensis* genomic sequences, used as a source for the identification of putative orthologs of the *WOX* genes, were compared to published *A. thaliana* sequences. We identified 17 *WOX* genes in *P. organensis*, distributed in the same three clades mentioned above. Of the 17 genes, two are putative *AtWUS* orthologs, therefore named *PoWUSa* and *PoWUSb*. Analyzes of gene expression showed expression of both paralogs during three different developmental stages: juvenile, adult with tendril and adult with both tendril and flower.

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ASSESSMENT OF THE GENOTOXIC POTENTIAL OF AKWATON® IN SWISS MICE

Lucas Henrique Domingos da Silva, Natália Helen Ferreira, Francielle Aparecida de Sousa, Tábata Rodrigues Esperandim, Lucas Teixeira Souza de Oliveira, Francisco Rinaldi-Neto, Renato Luis Tame Parreira, Denise Crispim Tavares.

Universidade de Franca, Franca, SP.

* lucas.hdsilva94@gmail.com

Key-words: Akwaton®; Genotoxicity; Cytotoxicity.

Akwaton® of Fosfaton-Akwaton International Ltd. (FAI) is a polymerized derivative of guanidine, which has high solubility in water. Studies have shown that Akwaton® has broad spectrum activity against Gram-positive and Gram-negative bacteria, fungi, yeast and viruses. Formulations containing Akwaton® as an active component have been evaluated against the microorganisms that cause canine otitis. However, it is necessary to evaluate the genotoxicity of this product for the safety of the animals submitted to the treatment. Therefore, the present study aimed to evaluate the genotoxic potential of the 5% Akwaton® aqueous solution in Swiss mice through the micronucleus test. The assay was performed in Swiss mice bone marrow, being 5 animals per treatment group and their respective control groups. Therefore, the animals received via *gavage* different doses of 5% Akwaton® solution (375, 750 and 1,500 mg/kg body weight [b.w.]). In addition, negative (water) and positive (urethane, 400 mg/kg b.w., intraperitoneal injection) control groups were included. For the evaluation of genotoxicity, micronucleated polychromatic erythrocytes were analyzed in the 4,000 polychromatic erythrocytes per animal (20,000 cells per treatment group and control groups). For the evaluation of the cytotoxicity of the treatments, the polychromatic erythrocyte/total erythrocytes ratio was calculated by analyzing 500 erythrocytes per animal (2,500 cells per treatment group and control groups). The results showed that the frequencies of micronucleated polychromatic erythrocytes in the animals treated with the different doses of 5% Akwaton® aqueous solution did not differ significantly from the negative control. No significant difference was observed in the ratio of polychromatic erythrocyte/total erythrocytes the different treatment groups. Therefore, the aqueous solution of 5% Akwaton® showed no genotoxic or cytotoxic effect, under the experimental conditions used. The results found in the present study contribute to a better understanding of the biological action of this polymerized derivative of guanidine, Akwaton®.

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STUDY OF THE DEVELOPMENT OF THE CORONA IN *Passiflora* spp.

Tatiane Yamaguchi Quijada[1]; Mariana Bombardi da Silva[1]; Helena Augusto Gioppato[1]; Bruna Rafaella Zanardi Palermo[1]; Marcelo Carnier Dornelas[1].

[1]Departamento de Biologia Vegetal, Universidade Estadual de Campinas – Instituto de Biologia, Campinas, São Paulo, Brazil.

tatianeyquijada@gmail.com

Key-words: corona filaments; floral development; MADS-box.

The genus *Passiflora*, the most expressive in several species of the Passifloraceae, which includes about 600 species of plants, presents a remarkable floral diversity and complexity, and also particular structures typical of the genus, such as the presence of the corona filaments, which is an elaborate floral structure, located between petals and stamens of *Passiflora* flowers. The establishment of the origin of the corona filaments and its homology with other floral organs, however, has not yet been resolved. The present work aims to investigate the two main hypotheses concerning the ontogeny of the corona filaments: (1) if they are a *sui generis* floral organ, probably derived from the activation of an axillary meristem of the petals, from the premise that, since the floral organs are modified leaves, they have an axillary meristem, even if cryptic; (2) if the corona filaments are modified petals or modified stamens, as a result of duplications of floral organ identity genes. For this, we identified and analyzed the gene expression of *Passiflora organensis*, using RT-qPCR, of putative genes related to the meristematic activity and corona filaments formation, as *WUSCHEL* (*WUS*), a member of the *WOX* gene family, which is expressed in the cells of the organizing center of the shoot apical meristem (SAM) and the axillary meristems, whose function is the maintenance of the stem cells. In addition, we also analyze the expression of some members of the MADS- box gene family, focusing on B-class genes, such as *APETALA3 (AP3)* and *PISTILLATA (PI)*, once they have a key role in petals and stamens identity during flower development. Our results demonstrate that, differently from *Arabidopsis thaliana*, which has one *WUS* gene, one *AP3* gene, and one *PI* gene, *P. organensis* has a duplication with two putative *WUS* orthologs, two *PI* orthologs, and three *AP3* orthologs. It is possible to suppose that *PoWUS* genes and the expansion of the B- class genes in their genome may indicate a meristematic activity of the corona filaments, directly related to the morphological complexity of the corona filaments.

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COMPOUND HETEROZYGOUS MUTATION (K26R/I268T) OF MVK GENE IS A CAUSE OF MEVALONIC ACIDURIA IN AN ADOLESCENT WITH AUTOINFLAMMATORY AND NEUROLOGIC SYMPTOMS

Beatriz Pavarino Bertelli[1,2]; Leandro Pedro Goloni Bertollo[1,3] Glaucia Maria de Mendonça Fernandes[1], Regina Célia Ajeje Pires de Albuquerque[1,4]; Érika Cristina Pavarino[1], Eny Maria Goloni-Bertollo[1].

[1]Genetics and Molecular Biology Research United (UPGEM), São José do Rio Preto Medical School (FAMERP) São José do Rio Preto – SP, Brazil; [2]União das Faculdades dos Grandes Lagos (UNILAGO), São José do Rio Preto -SP, Brazil; [3]Universidade de São Paulo (USP), São Paulo - SP, Brazil; [4]Department of Neurology and Head and Neck Surgery, Neuropediatrics, São José do Rio Preto Medical School (FAMERP) São José do Rio Preto – SP, Brazil.

eny.goloni@famerp.br

Key-words: autoinflammatory diseases, mevalonate kinase deficiency, mutation

Autoinflammatory diseases, a heterogeneous group of disorders, are characterized by recurrent episodes of apparently unprovoked inflammation. Clinical manifestations include sudden fever attacks, inflammation of joints and serosal surfaces, skin rashes, lymphadenopathy, abdominal pain, rheumatic manifestations as well as the musculoskeletal and neurological involvement. The differential diagnosis among these disorders can be a difficult challenge due to clinical overlap. A definitive diagnosis can be confirmed by genetic analysis of different genes. In this study, we aimed to establish the diagnosis in an adolescent with symptoms suggestive of autoinflammatory and neurologic symptoms by genetic testing. Genomic DNA from blood sample was isolated according to the manufacture's protocols. The exons and splicing joint of MEFV, NOD2, PSTPIP1, MVK, NLRP3 AND TNFRSF1 genes were amplified by Polymerase Chain Reaction (PCR). The PCR products were eletrophoresed in agarose gel, purified and then submitted to next-generation sequencing (NGS) using platforms Illumina MiSeq (Illumina, USA). Sanger sequencing with the ABI 3500 Genetic Analyzer (Life Technologies, USA) was performed to validate results from NGS. Assessment of variant pathogenicity was carried out in public databases (ClinVar, HGMD and InFevers). The patient was 15 years old when was referred to the clinical genetic evaluation. The onset of symptoms occurred in the first months of life. Up to 12 years old he manifested intermittent fever, abdominal pain, diarrhea, vomiting, hypotonia, developmental delay, phychomotor retardation, cerebellar ataxia and atrophy, headache, skin rash, visual impairment, arthritis, fatigue airway recurrent infection, hepatomegaly and cervical lymphadenopathy. Laboratory findings showed elevated serum immunoglobulin D and A, neutrophilia and leukopenia. Molecular analysis revealed a compound heterozygous mutation (c.77A>G:pK26R/c.803T>C: p.I268T) in mevalonate kinase (MVK) gene. The p.I268T variant is classified as pathogenic in the ClinVar, HGMD and InFevers databases and associated with melavonic aciduria, a rare autosomic recessive autoinflammatory condition. Since the patient also carries the p.K26R variant in the heterozygous state was not describle in these databases, we associated the phenotype with double heterozygosis. Thus, with the dignostic of melavonic aciduria, the patient was referred to appropriate treatment. Since then, he showed an improvement. In conclusion, the genetic analysis can allow the identification of autoinflammatory diseases, therefore, early genetic screening is required to definitive diagnosis and improvement of patient´s quality of life. In this study, que have reported for the fisrt time a new mutation (pK26R) in heterozygosity in the MVK gene in an adolescent with melavonic aciduria.

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TRANSCRIPTOME ANALYSIS OF *PASPALUM NOTATUM* AND *PASPALUM VAGINATUM* UNDER WATER DEFICIT CONDITION

Joyce Etsuko Arakaki[1*]; Wilson Malagó Junior[2]; Mauricio de Alvarenga Mudadu[3]; Patricia Menezes Santos[2]; Alessandra Pereira Fávero[2]; Ricardo Caneiro Borra[1]; Bianca Baccili Zanotto Vigna[2].

[1]Programa de Pós-Graduação em Genética Evolutiva e Biologia Molecular, Universidade Federal de São Carlos, São Carlos, São Paulo, Brazil. [2]Embrapa Pecuária Sudeste, São Carlos, São Paulo, Brazil. [3]Embrapa Informática Agropecuária, Campinas, São Paulo, Brazil.

*joyce.e.arakaki@gmail.com

Key-words: drought; transcriptome; *Paspalum*; gene expression.

Drought is one of the abiotic stresses that most affect plant growth and productivity. Grasses of the genus *Paspalum* are successfully used as turf and forage in Australia, Argentina, Brazil and United States. *Paspalum notatum* has good forage quality, and *P. vaginatum*, high tolerance to salinity. In addition, their potential to tolerate drought has been described previously, making them interesting for transcriptome studies under water deficit. The objective of this work was to analyze the gene expression profiles of both species in response to drought. The accessions *P. vaginatum* BGP 114 and *P. notatum* BGP 216 were chosen according to their characteristics, importance and ability to tolerate drought. The drought experiment was carried out in a greenhouse at Embrapa Pecuária Sudeste (Nov/2016) and cultivated in triplicates. Leaf samples of each biological replicate were collected under two conditions, with no water deficit (28% of relative soil moisture) and under water deficit (4% of relative soil moisture), in which *P. vaginatum* achieved this percentage after eight days with no irrigation and *P. notatum*, after five days. Their RNAs were extracted and sent for sequencing using Next Generation Sequencing (NGS) technology on Illumina HiSeq 2500 equipment. The paired-end (2x100 bp) cDNA libraries (~80M reads/library) of leaves from each genotype, three from the control group and three from the water deficit, passed through FastQC quality control and Trinity software performed the *de novo* assemblies of the transcriptomes. The assemblies were evaluated using contig N50 and ExN50 values. Also, Bowtie2 software estimated the percentage of mapped reads and BUSCO3 evaluated the completeness of transcriptomes against embryophita dataset. RSEM software estimated the abundance of transcripts using Transcripts per Million Transcripts - TPM. EdgeR package considered differentially expressed genes (DEGs) those with FDR <0.05 and $\log_2$Fold-Change ≥ 2. Trinotate software suite was used for functional annotation of transcriptomes followed by functional enrichment analysis with Goseq package. The GO terms were summarized and clustered according to their p- values using REVIGO platform. Both libraries and assemblies achieved satisfactory quality results. RSEM estimated 31.835 genes for *P. vaginatum* and 38.002 for *P. notatum*. For *P. vaginatum*, 3280 DEGs (10.3%) and 658 enriched GO terms were identified while for *P. notatum*, 1978 DEGs (5.2%) and 720 enriched GO terms. Results from both species showed genes related in responses to water deficit such as LEAs, aquaporins, auxin related proteins, accumulation of carbohydrates, heat shock proteins and responses to ROS.

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CHARACTERIZATION OF CELL PROLIFERATION GENES OF CANCER STEM CELL IN PHARYNGEAL CANCER

Ludimila Leite Marzochi[1]; Glaucia Maria de Mendonça Fernandes[2]; Vilson Serafim Junior[2]; Maria Antônia dos Santos Bezerra[2], Ana Livia Silva Galbiatti Dias[2], Lennon Pereira Caires[2], Rosa Sayoko Oyama-Kawasaki[2], José Victor Maniglia[3], Érika Cristina Pavarino[2], Eny Maria Goloni-Bertollo[2].

[1]UNESP – São Paulo State University. [2]Genetics and Molecular Biology Research Unite – UPGEM, FAMERP – São José do Rio Preto Medical School, Brazil.. [3]Departament of Otorrinolaringology and Head and Neck Surgery, FAMERP – São José do Rio Preto Medical School, Brazil.

ludimila.marzochi@gmail.com, eny.goloni@famerp.br

Key-words: cancer stem cells; pharyngeal cancer; cell proliferation.

Pharyngeal cancer (PHC) corresponds around 5-10% of the malignant neoplasms present in the upper airways. Cetuximab, an epidermal growth factor receptor (EGFR) monoclonal antibody, alone or in combination with standard first-line chemotherapy can be used in the treatment of the recurrent and/or metastatic PHC. Cetuximab acts on the extracellular binding domain of the EGFR, preventing its activation by endogenous ligands, and activation of downstream intracellular signal transduction pathways, including the KRAS. The high incidence of the PHC can be related to the presence of cancer stem cell (CSC), that have capabilities of self-renewal, and resistance to conventional chemo-radiotherapy. In view of this, the identification and characterization of biomarkers can help in the prognosis of cancer and in the elaboration of therapeutic strategies. The present project aims to identify CSC, quantify EGFR and KRAS genes expression and to evaluate the effects of the treatment with Cetuximab. The cell line of pharyngeal cancer–FADU, was sorted in ALDH1+ as CSC and ALDH1- as non-CSC, by fluorescent-activated cell sorting technique (FACSAria, Becton Dickinson). EGFR and KRAS genes expression were evaluated in CSCs and non-CSCs (relative control) by quantitative real-time PCR using the TaqMan® (Thermo Fischer). Both subpopulations were treated with 0.05mg/ml Cetuximab for 24, 48 and 72 hours. Cell viability was evaluated by MTS-assay (Promega). Cells without treatment were considered a control group. Statistical analysis was performed by ANOVA Test using GraphPad 6.0 software, and $p>0.05$ value was considered statistically significant. The cell sorting showed 57% CSC and 8% non-CSC. The CSCs formed more colonies (57) than non-CSC (40), but the difference was not statistically significant ($P=0.581$). The EGFR and KRAS genes showed down expression ($RQ=0.842$ and $RQ=0.557$, respectively). The treatment of the CSC with cetuximab for 48h did not show statistically significant results when compared to the control group ($P=0.6347$), but after 24h and 72h, the results were statistically significant ($P=0.001$). The ALDH as a single marker appears not to be good to separate CSCs as was evidenced by colony formation results in that was observed more colonies in CSCs, but the difference was not statistically significant. In relation to gene expression, EGFR and KRAS genes were under-expressed, unlike the literature. The overexpression of these genes is directly related to proliferative capacity and tumor survival. In our study, the proliferative potential is probably activated by other pathways. The ineffectiveness of the cetuximab can be due to EGFR downexpression. The high amount of CSCs in cell sorting with the only ALDH1 is not efficient to differentiate CSCs and non-CSC in the FADU cell line, evidenced by results not significant of colony formation. More studies about CSC and biomarkers are important to improve the treatment of pharyngeal cancer.

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FOLLOW- UP STUDY ON GROWTH AND DEVELOPMENT IN CRI DU CHAT SYNDROME ACCORDING TO PARENTAL PERSPECTIVE

Renata Melo dos Santos[1, 2] ;Layla Damasceno Espírito Santo [2]; Mariluce Riegel[3]; Lília Maria de Azevedo Moreira[1,2].

[1]Programa Genética&Sociedade Instituto de Biologia - Universidade Federal da Bahia, Salvador, Bahia. [2]Laboratório de Genética Humana e Mutagênese- Instituto de Biologia - Universidade Federal da Bahia, Salvador, Bahia. [3]Laboratório de Citogenética do Serviço de Genética Médica do Hospital de Clínicas de Porto Alegre (HCPA), Porto Alegre, Rio Grande do Sul / RedeBRIM.

renatam.santos@outlook.com

Key words: Follow up study; Cri du Chat syndrome; Parents perspective

Human growth is a dynamic and continual process which occurs from the beginning to the end of life and is mostly regulated by genetic and environmental factors. In parallel, development is associated with the acquisition of abilities and is also subjected to continue genetic influences that affect humans at different stages of life. Children with genetic syndromes may present problems in growth and development, restricting volunteer movements, adaptive abilities of cognition, language, and behaviour. The aim of the present study was to evaluate development and growth of a 20 year-old male with Cri du Chat syndrome and Autistic Spectrum Disorder, from the parents' perspective. For the diagnosis, clinical characteristics and cyto-molecular analyzes (GTG banding; FISH; aCGH) were observed. The proband was followed up at a community genetics service, with periodic returns over 15 years. The parents were asked to answer a semi-structured questionnaire with 30 open-ended questions after informed consent, according to the bioethical norms advocated by Resolution 466/12 of the National Health Council. The reported case had a cytogenetically detectable 5p deletion, no translocation or mosaicism detected by FISH analysis and the array-CGH profiles revealed overlapping deletions of 5p15.33-p13. Nowadays he presents a unbalanced walk because of hypertonia, hyperactivity, repetitive movements, irritability, attachment to objects, communication through gestures, microcephaly, kyphoscoliosis and short stature. According to the parents the main signals which led to the suspect of CdC were the small cephalic perimeter and atypical face, characteristics that have remained during his whole development, except for the initially round face that became thin and elongated. The early diagnostic was followed by a multiprofessional assistance, allowing him to improve his psychomotor control, being able to hold objects and even to develop late march movements, but until now, he has not been able to swallow solid food. Since early childhood he has shown connection with his parents and later to his younger siblings, although without any control of emotions and expressing his interests strongly. Interactions with other people are unpredictable, sometimes with positive or indifferent reactions. His puberty started early at the age of 11, with fast development of body hair and genital organs, but without expression of sexual desires and episodes of priapism since childhood. He is not expected to continue to grow, but from a developmental perspective he has shown new interests and improvement at logical reasoning. For his future, the parents restricted to express their perspectives, just to seize daily experiences and to see him happy. As expected, changes and improvements have been observed during the probands development. The authors believe that studies in this area may exemplify models of development of syndromic children with chronic symptoms which can be softened by specific therapeutic assistance.

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ESTIMATION OF MATING SYSTEM PARAMETERS IN OPEN- POLLINATED PROGENIES OF PIPTADENIA GONOACANTHA (FABACEAE-MIMOSOIDEAE), AN IMPORTANT TREE USED IN BRAZILIAN FOREST RESTORATION

Carolina Grando[1]*; Patricia Sanae Sujii[2]; Ellida de Aguiar Silvestre[1]; Evandro Vagner Tambarussi[3]; Jaqueline Bueno de Campos[1]; Marcos Vinícius Bohrer Monteiro Siqueira[4]; Miklos Maximiliano Bajay[5]; Fabiano Lucas de Araujo[1]; José Baldin Pinheiro[6]; Pedro Henrique Santin Brancalion[7]; Maria Imaculada Zucchi[8]

[1]Instituto de Biologia, Universidade Estadual de Campinas, Campinas, São Paulo, Brasil. [2] UDF Centro Universitário, Brasília, Distrito Federal, Brasil. [3]Setor de Ciências Agrárias e Ambientais, Universidade Estadual do Centro-Oeste, Irati, Paraná, Brasil. [4]Faculdade de Ensino Superior e Formação Integral, Garça, São Paulo, Brasil. [5]Departamento de Engenharia de Pesca e Ciências Biológicas, Universidade do Estado de Santa Catarina, Laguna, Santa Catarina, Brasil. [6]Departamento de Genética, Escola Superior de Agricultura "Luiz de Queiroz", Piracicaba, São Paulo, Brasil. [7]Departamento de Ciências Florestais, Escola Superior de Agricultura "Luiz de Queiroz", Piracicaba, São Paulo, Brasil. [8]Agência Paulista de Tecnologia do Agronegócio, Pólo Centro-Sul, Piracicaba, São Paulo, Brasil.

* carolinagrando@gmail.com

Palavras-chave: allogamy; biparental inbreeding; microsatellites

The knowledge about the mating system of tree species is important for determining how genetic combinations are distributed within and between populations, supporting seed sampling in forest restoration projects in order to obtain new populations with higher effective population sizes, and lower negative effects of inbreeding and genetic drift. However, studies estimating the mating system are usually not performed in advance for most tree species used in forest restoration projects. Thus, our aim was to estimate the mating system parameters of progenies and seed trees for *Piptadenia gonoacantha*, a tree species widely used for restoring Atlantic Forest degraded lands. In a fragmented, 241 ha remnant in the State of São Paulo, Brazil, we collected seeds from 16 seed trees, obtaining 197 open-pollinated progenies that were genotyped with seven microsatellite markers. Our estimates of mating system, notably by the outcrossing rate equal to one, and the multilocus correlation of selfing equal to zero, indicated that *P.gonoacantha* is an allogamous tree species. We also observed that biparental inbreeding and correlated matings occurred, probably favored by the limited seed dispersion, the aggregated spatial distribution, and the formation of seedling banks in forests. The high average effective number of pollen donors we observed was probably indicating that pollination was responsible for maintaining the high outcrossing levels, and consequently for reducing the chance of mating between nearest plants. These findings will support further estimation of the number of seed trees required for seed collection, leading to the establishment of long-term viable populations of *P.gonoacantha* in forest restorations.

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GLUCOCORTICOID RECEPTOR VARIANTS PRESENT DIFFERENTIAL EFFECTS ON BRAIN VOLUMES ACCORDING TO ADHD STATUS

Cibele Edom Bandeira[1,2]; Eugenio Horacio Grevet[2,3]; Renata Basso Cupertino[1,2]; Bruna Santos da Silva[1,2]; Eduardo Schneider Vitola[2,3]; Felipe Almeida Picon[2,3]; Nina Roth Mota[4]; Maria Eduarda de Araujo Tavares[1,2]; Diego Luiz Rovaris[2,3]; Claiton Henrique Dotto Bau[1,2,3]

[1]Department of Genetics, Institute of Biosciences, UFRGS, Porto Alegre, RS, Brazil. [2]ADHD Outpatient Program – Adult Division, Hospital de Clínicas de Porto Alegre, Porto Alegre, RS, Brazil. [3]Department of Psychiatry, School of Medicine, UFRGS, Porto Alegre, RS, Brazil. [4]Department of Human Genetics and Psychiatry, Donders Institute for Brain, Cognition and Behaviour, Radbound University Medical Center, Nijmegen, The Netherlands.

cibele.edom@gmail.com

Keywords: ADHD; *NR3C1*; subcortical brain volumes.

Attention-Deficit/Hyperactivity Disorder (ADHD) has been associated with neuroanatomic alterations, such as reduced brain volume in certain regions and with dysregulation of the Hypothalamic–Pituitary–Adrenal (HPA) axis, involved in stress- response. The glucocorticoid receptor, encoded by the *NR3C1* gene, plays a pivotal role in the stress response and has effects on neuronal excitability. Considering the evidence linking HPA axis genetic variation in psychiatric disorders and brain volumes, we hypothesize that *NR3C1* variation could moderate the reported association between ADHD and brain subcortical volume. For this purpose, we evaluated the accumbens, amygdala, caudate, hippocampus, putamen and intracranial volumes (associated with ADHD in the largest meta-analysis published so far) in 100 adults with ADHD and 60 controls. The diagnosis of ADHD followed DSM-5 criteria and the images acquisition were conducted in a Siemens Magnetom Spectra 3T scanner. Genotyping was performed on the Infinium PsychArray-24 BeadChip platform, followed by application of genomic filters and pruning, which resulted in 47 independent variants in *NR3C1* analyzed. The hippocampus and intracranial volumes were decreased in cases when compared to controls. Several of the *NR3C1* polymorphisms evaluated presented opposite direction of effects according to ADHD status, mostly on accumbens and amygdala. The functional and well-studied rs10052957 and rs41423247 SNPs were among the significant ones. In silico analyses revealed that the significant SNPs were in strong Linkage Disequilibrium with rs6198, another variant known for its functional effect. Our findings indicate that volume differences of specific brain regions in subjects with ADHD and controls might be influenced by *NR3C1* variants.

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INVESTIGATION OF 27VNTR GENE *eNOS* VARIANT IN ASSOCIATION WITH SYSTEMIC ARTERIAL HYPERTENSION IN AN AMAZON POPULATION

Cintia Silva[1,2]; Lucas Santos[1,2]; Milene Raiol-Moraes[1,2]; Cristina M.D. Valente[1,2]; Antonio C. Modesto[1,2,3]; Ândrea Ribeiro-dos-Santos[1,3]; André M. Ribeiro dos Santos[1,2,3]

[1]Laboratório de Genética Humana e Médica – Programa de Pós-Graduação em Genética e Biologia Molecular, Universidade Federal do Pará, Belém, Pará, Brazil, 66075-970; [2]Programa de Pós-Graduação em Antropologia, Universidade Federal do Pará, Belém, Pará, Brazil, 66075-970; [3]Núcleo de Pesquisas em Oncologia, Universidade Federal do Pará, Belém, Pará, Brazil, 66073-005.

cintiahelena88@hotmail.com

Keywords: 27VNTR, Hypertension, eNOS Gene, Amazonian Population, Genetic Polymorphism.

The Brazilian Amazon is one of the most genetically diverse regions of the country. This is the product of admixture of three main ethnic groups (Native American, Europeans, and Africans) which contributed to the formation of the Brazillian population and their consequences are still observed. Systemic Arterial Hypertension (SAH) is a disease resulting from the interaction between environmental and genetic factors. One candidate gene to play an important role in this pathology is the eNOS gene. Therefore, this study evaluated the association of eNOS 27VNTR polymorphism in a SAH cohoort from the Amazon. The investigated samples come from the city of Belém- PA of the basic health unit of Sacramenta neighborhood and include 50 hypertensives (case) and 50 normotensives (control). The eNOS 27VNTR polymorphism was genotyped using a PCR (Polymerase chain reaction) and polyacrylamide gel. Logistic regression analysis indicates a strong association of the eNOS*a allele with SAH risk (OR 0,001). Moreover, significantly higher allelic frequency of eNOS*a was observed in West Africa (p-value <0.001) when reviewing the literature. Our results corroborate to previous work that found eNOS*a allele as risk a SAH risk factor and produces new evidence of such. In parallel, it highlights the importance of such polymorphism to the Nothern Brazil most West African genetic contribution was observed.

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DECONSTRUCTING THE CONCEPT OF RACE IN HUMANS USING ELETRONIC GAMES

Julio Wenceslau Macowski[1*]; Nina Amália Brancia Pagnan[1].

[1]Universidade Federal do Paraná

*juliomacowski@gmail.com

Key-words: human races, eletronic games, biology teaching

The term race or racial group has been employed to divide our species according to simple phenotypic characteristics such as skin color and other visual physical traits. The division into distinct groups leads to conflicts and strengthens racist and eugenic ideas. The school has an important role in promoting critical thinking and helping students to live successfully in a democracy contributing to the fight against racism and discrimination in a multicultural society. Respect and tolerance are values that should be taught in school, contributing to avoid hate speech. The presente work intends to encourage the decosntruction of the notion of the existence of human races using modern genetics concepts allied to the narratives of the following video games: League of Legends (Riot Games), Pikimin 3 (Nintendo) and Resident Evil 5 (Capcom). We considered that the games could influence the understanding of the so called human raciology by high school students. Pre and post-tests questionnaries were applied in order to collect information about the students's perception about race.The results were analyzed both quantitatively and qualitatively. Our observations indicate that the proposed teaching strategy, mixing genetics classes with electronic games can be used to discuss and question the existence of human races. Qualitative analysis showed a change in students's opinion and in the employment of the word race in the post-test answers. For instance, answers directly related to phenotypic characteristics as skin colour and hair type showed a difference, suggesting an influence of the game narrative. The statistical hypothesis testing revealed a p-value of 0,00028 (<0,05)instituição the analysis of a possible influence of the Resident Evil 5 game, supporting the ideia of an influence of this game on students's conceptions. The association between genetics classes and electronic games narratives can be used to improve the learning of complex issues that are difficult to assimilate by the students. This methodology also allows the discussion abou traces, which involves several areas of knowledge, promoting the interaction between teacher and student as well as between classmates.

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IDENTIFICATION OF GENES ENCODING CHAPERONES IN METAGENOMIC DATABASES

Ninna Hirata Silva[1]; Rafael Silva-Rocha[3]; María Eugenia Guazzaroni[2]

[1]Universidade de São Paulo - Faculdade de Medicina de Ribeirão Preto. [2]Departamento de Biologia, Universidade de São Paulo - Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto. [3]Departamento de Biologia Celular e Molecular e Bioagentes Patogênicos, Universidade de São Paulo - Faculdade de Medicina de Ribeirão Preto.

ninna.hirata@usp.br

Key-words: bioinformatics; metagenomics.

Metagenomic allows the biotechnological potential from bacteria, mainly from those once considered not cultivable, through mining directly DNA sequences recovered from the natural environment. In particular, metagenomics approaches take advantage of the genetic potential of microbes living in extreme environments. Deciphering the microbial diversity and metabolic activities of microorganisms under extreme conditions reveals the biochemical strategies used by them to survive in those conditions. This, in the order hand, can be used to expand the capacity of surviving in bacteria used in industrials processes. A set of families of proteins called molecular chaperones aids in several processes involving folding, unfolding, and homeostasis of cellular proteins. After denaturation of the protein caused by stress, the proteins can be unfolded, disaggregated and then refolded, or can be targeted for disposal by proteolytic systems. These molecular mechanisms are essential for the cell to overcome the harmful effects of stress, and thus increase tolerance under adverse growth conditions. Therefore, the project aims to mine genes encoding chaperones and other proteins that confer resistance to stress conditions (such as nucleic acid binding proteins) in public databases (MG-RAST and MGnify) of metagenomic sequences from extreme environments. Our dataset consists in samples extracted from different selected environments, representing diverse types of stress conditions, such as a desertic area, acid waters, acid soils, places that have high or low temperatures, among others, totalizing seven samples from different studies around the world. After that, we have downloaded complete genes from NCBI, in order to construct probabilistic's models called Hidden Markov Models, from some chaperones (ClpX, ClpA, ClpC, ClpE), genes of proteins that bind to nucleic acids (RBP, HU, DPS) and genes of proteins related to DNA replication (GyrA, RecA, DnaA). We used HMMER3.0 to create more than 40 profiles and also to compare the results tothe metagenomes mentioned above. Analyzing those, we found that some proteins appear more frequently in certain samples, others are more restrict to specific groups of countries, and others are widespread in most of the metagenomes. As next steps, we will use other bioinformatics softwares, such as PRICE or GenSeed in order to assemble the best contigs found in all the metagenomes, or in most of them, that would be the ones that constitute the core aimed to be studied in future phases.

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PROTEOMIC ANALYSIS OF CELL WALL IN MEMBERS OF THE *Paracoccidioides* COMPLEX

Ayda Luz Malaver Salamanca[1],Danielle Silva Araujo[1], Lilian Cristiane Baeza[2], Igor Godinho Portis[1], Maristela Pereira[1], Célia Maria de Almeida Soares[1].

[1]Laboratório de Biologia Molecular-Instituto de Ciências Biológicas-Universidade Federal de Goiás. [2]Faculdade de Ciências Farmacêuticas-Universidade Estadual do Oeste do Paraná.

cmasoares@gmail.com

Key words: PCM, cell wall, proteomics.

Paracoccidioidomycosis (PCM) is the most important systemic mycosis in Latin America. The fungal cell wall is an absent structure in human cells; it is the most superficial structure and is important to the infection stage. The study of the fungal cell wall is interesting for the identification of new target proteins that could potentially be used for development of new therapeutic strategies against PCM. The aim of this work is the proteomic analysis of the yeast cell wall of *Paracoccidioides* (*P. americana* and *P. brasiliensis)* species. We used a combination of method sample enrichment, proteomic analysis with liquid chromatography coupled to a mass spectrometer (NanoUPLC-MSE), and bioinformatic filters. A total of 312 proteins were identified and 154 proteins were found in both species, 104 were recovered in *P. americana* and 54 in *P. brasiliensis.* 92% of the recovered proteins were predicted as secreted by non-classical routes and 8% by classical routes. Cell wall proteins that perform functions of synthesis and remodeling of cell wall components as cell wall glucanase Crf1, a beta-1,6-glucan biosynthesis protein, cell wall glucanase Utr2 and chitinase 3 were identified. In addition, Cu/Zn superoxide dismutase involved in the response to oxidative stress, Aqualysin-1 serine protease which is an important virulence factor and proteins with adhesion function such as enolase, GAPDH, and Gp43 were also recovered. The proteomic analyses showed the presence of cytoplasmic and hypothetical proteins whose extracellular or cell wall functions are unknown. Our results guide new studies that allow establishing the function of proteins found abundantly in cell wall, and their relevance to the virulence and pathogenesis.

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IDENTIFICATION AND VALIDATION OF ENDOGENOUS GENES FOR STUDIES OF GENE EXPRESSION IN *Trichoderma harzianum* EMPLOYING RT-qPCR.

Ana Beatriz Cândido de Queiroz[1]; Maria Augusta Crivelente Horta[2]; Déborah Aires Almeida[1]; Jaire Alves Ferreira Filho[1]; Clelton Aparecido dos Santos[1]; Maria Lorenza Leal Motta[1]; Anete Pereira de Souza[1*]

[1]Centro de Biologia Molecular e Engenharia Genética, Universidade Estadual de Campinas, Campinas, São Paulo, Brasil; [2]Technische Universität München, TUM, Alemanha.

*biaqueirozc@gmail.com

Key-words: *Trichoderma harzianum*; control genes; RT-qPCR.

Trichoderma is a genus of filamentous fungi capable of colonizing several substrates under different environmental conditions. *Trichoderma* species, especially *T. reesei* and *T. harzianum*, are capable of producing numerous cellulolytic and hemicellulolytic enzymes, which guarantee advantages against the degradation of lignocellulosic substrates in the environment. This has called the attention of researchers to its biotechnological use. Currently, several studies explore the potential for the production of cellulolytic enzymes from *T. harzianum* especially for the production of biofuels. However, until now, there is no consensus as to which reference genes could be used to normalize gene expression levels in quantitative reverse transcription PCR (RT- qPCR) analyzes. This work proposes to prospect reference genes for the *T. harzianum* IOC 3844 and CBMAI 0179 strains that allow a safe analysis of different genes of interest using the RT-qPCR technique. Initially, an *in silico* analysis was performed based on the mapping of RNAseq reads in the growth conditions of the fungus in cellulose and glucose (three biological replicates for each condition), searching for genes with the lowest expression variation and, therefore, promising candidates for validation of control genes. Five genes from each lineage (IOC-3844 and CBMAI-0179) were selected for each biological condition (cellulose and glucose). After this selection, specific oligos for each gene were designed using the software Primer3Plus. From the cultures obtained in previous works, RNA extraction from the two strains of the two conditions, in triplicate, was carried out using the RNeasy Mini Kit extraction kit from Qiagen. In addition, 1% agarose gel was used to check the integrity of the extracted material. Next, cDNA synthesis was performed with Qiagen's QuantiTect Reverse Transcription kit. The RT-qPCR analysis was performed on the *CFX384 Touch Real- Time PCR Detection System* (Bio-Rad) and the final results were obtained by computing 'delta delta Ct' (ΔΔCt). Among the selected genes it was possible to validate a control gene for each line with low variation in RNA-seq and in RT-qPCR analysis. By selecting these control genes, it was possible to validate the RNAseq of differentially expressed genes of *T. harzianum* (CBMAI-0179 and IOC-3844) under cellulose and glucose conditions.

Funding Agency: CAPES; CNPq; FAPESP.

Genotoxic effects on tetrads of *Tradescantia pallida* var. *purpurea* (Commelinaceae) induced by atmospheric pollutants in the city of Joinville, Santa Catarina, Brazil

Vetorazzi, Valéria Cristina Rufo, Marques, Dalva; Pinheiro, Pedro Hartelt, Bruna Tays

Departamento de Ciências Biológicas, Universidade da Região de Joinville (Univille) Rua: Paulo Malschitzski, nº 10 – Zona Industrial CEP 89219-710 – Joinville, SC, Brasil

brunahartelt@hotmail.com

Keywords: Biomonitoring; Genotoxicity; Micronucleus; Trad-MCN.

Atmospheric biomonitoring allows the assessment of air quality in large areas using living organisms that respond to pollution by changing their functions or accumulating toxins. This study aimed to evaluate the genotoxicity of atmospheric air in the city of Joinville with the use of the Micronucleus Test on *Tradescantia* (Trad-MCN). Five flowerbeds ornamented with *Tradescantia pallida* were used as sample points: Point 1 in the Municipal Cemetery; Point 2 in a segment of São Paulo Street; Point 3 in the Public Market; Point 4 on Albano Schmidt Street; and Point 5 in a segment of Dr. João Colin Street. After the selection of the sample points, the chromosome assays were performed by means of the micronucleus count in the plant system. The analyzes were done under optical microscope (400X) and the number of MCNs in 300 tetrads was counted on each slide. No significant differences were observed between MCN frequencies at different sample points. Statistical analyzes revealed that the mean frequency of MCN was between 0.84% and 0.56%. Although Joinville is a strong industrial power in the state and a region in constant development, the data obtained show that atmospheric air pollution did not provoke an increase in the MCN frequency of the study plant.

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GENETIC DIVERSITY AND INBREEDING RATES ANALYSES OF A POPULATION OF *Astyanax altiparanae* FOR POSSIBLE USE IN RESTOCKING PROJECTS

Mariana Machado de Andrade[1]; Caio Felipe da Silva[1]; Diogo Teruo Hashimoto[2]; Fabio Porto-Foresti[1*]

[1] Universidade Estadual Paulista, Departamento de Ciências Biológicas de Bauru. [2] Universidade Estadual Paulista, Centro de Aquicultura da UNESP de Jaboticabal.

mma.mariana07@gmail.com

Key-words: Lambari, ichthyofauna, biodiversity

The Alto-Paraná river basin presents a great diversity of fish. Among all this diversity is the species *Astyanax altiparanae*, popularly known as yellowtail lambari. This species has great importance in both economy and ecology, being used in sport fishing and as food source for a wide range of vertebrates. Microsatellites are currently the molecular markers most used in genetic studies in fish, being applied mainly in kinship and paternity studies, both in culture stocks and in natural populations. The objective of this study was to analyse the genetic diversity and perform a breeding directioning using microsatellite molecular markers in *A.altiparanae* specimens from the Sapucaí-mirim river. The individuals were collected in the Sapucaí-Mirim river and taken to the Fish Genetics Laboratory in the Unesp of Bauru, where the extraction of genomic DNA from the caudal fins was performed through a commercial kit. Agarose gel 1% was them used to analyse the integrity and quality of the extractions previously done. Through the Polymerase Chain Reaction (PCR) technique, the DNA was amplified using loci previously described in the literature. For the analysis of the degree of kinship betwen the individuals and of the genetic diversity, computational programs were applied. The analysis of genetic diversity revealed that the population studied has a high degree of diversity and a low number of related individuals. The individuals presented an average of 10.4 alleles per locus. Observed (Ho) and expected (He) values of heterozygosity varied from 0.58 (Asty26) to 0.941 (Asty12) and 0.645 (Asty12) to 0.921 (Asty15) respectively. The values of the Fixation Index (Fis) ranged from -0.4798 (Asty12) to 0.3946 (Asty15). Through the calculations of the Fixation Index (FIS) it was possible to observe that among the seven loci only one presented a negative value (Asty12). The studied population in general presented high genetic diversity, indicated by the estimated indexes such as expected and observed heterozygosity, Fixation Index (FIS) and number of alleles. Through this study it became possible to see that the population is very genetically diverse, showing low levels of inbreeding. The microsatellite markers used in the present study proved to be efficient for this study, demonstrating that the individuals analysed have the potential to be incorporated into a breeder's bank for future restocking projects.

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TEACHING EVOLUTION: KNOWLEDGE AS NATURAL SELECTION IN THE CLASSROOM

Yrlan Sousa[1]; Camila Gomes[1]; Davidson Sodré[2]; Marcelo Vallinoto[1*]

[1]Laboratory of Evolution, Universidade Federal do Pará, Campus Bragança; [2]LABECA, Universidade Federal Rural da Amazonia.

*mvallino@ufpa.br.

Keywords: Evolution, teaching, natural selection.

Natural Selection is one of Science's major ideas, and is a concept fundamental to the field of Biology. However, teaching Evolution is often hampered by the growth of pseudoscientific thought, and many biology students reaching university do not necessarily accept Evolution as fact. Convincing these students of the importance of Evolution is a major challenge for today's university professors. Given this, we developed a novel approach to demonstrate natural selection using classroom learning as a model. On the first day of the Evolution course, we presented the students with 10 questions, which should be answered "I agree" or "I do not agree". The questions are very basic and do not require any profound knowledge of the course material. We then explained to the students that they, on this first day of class, represented a population, and that by the end of the course (that is, after being taught Evolution), they would represent a second, "distinct" generation, which would be presented the same 10 questions. Obviously, we explained that this second generation was only considered for teaching purposes. The answers provided by each student were transformed into a bi-allelic system, in which 9–10 correct answers represent a homozygous individual (*AA*), 6–8 correct answers, a heterozygote (*Aa*), and 0–5 correct answers represent the second homozygote (*aa*). It is important to note here that neither "allele" was considered to be dominant. Our results indicated that, while the *AA* genotype had the highest weighting, the frequencies of the two alleles were approximately equal (0.5). Following the teaching process, which involved two classes (Biology and Natural Sciences), we observed an increase in allele *A*, to over 0.80. In both cases, the results were significantly different (Chi-square). We presented the results to the students at the end of the Evolution course, and asked them to explain the findings. The response of one student is particularly representative: "Based on the results, we perceived that the change in the frequency of allele *A* represents the effectiveness of the study of evolution, which simulated natural selection, and resulted in deviations from the Hardy-Weinberg equilibrium". Despite achieving our objective, one point of concern persisted. At the end of the course, all but one of the 10 questions was answered correctly more than 90% of the time. The one exception was the question that referred to intelligent design as a theory. Overall, then, the results of the study highlighted the need to make scientific study a key point in the Brazilian school curriculum, to ensure that the students learn as soon as possible that science is the key to adequate theoretical and intellectual development.

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REPRODUCTIVE CHARACTERIZATION OF ACCESSIONS OF *Paspalum* (PLICATULA INFORMAL GROUP) USING FLOW CYTOMETRY, CYTO-EMBRIOLOGICAL ANALYSIS AND MOLECULAR MARKERS

Tiago Maretti Gonçalves[1*]; Ana Luísa Sousa Azevedo[2]; Bianca Bacilli Zanotto Vigna[3]; Alessandra Pereira Fávero[3]

[1]Universidade Federal de São Carlos. [2]Embrapa Gado de Leite, Juiz de Fora - MG. [3]Embrapa Pecuária Sudeste, São Carlos - MG.

* tiagobio1@hotmail.com

Palavras-chave: apomixis; grasses; germplasm

Belonging to the Poaceae family, the genus *Paspalum* have more than 350 species, many of them with great potential for forage or lawn purposes. The *Paspalum* Germplasm Bank (GB) of Embrapa Pecuária Sudeste has about 450 accessions from 50 species, most of them belonging to the Plicatula group. The majority of the *Paspalum* accessions is tetraploid (2n = 4x = 40) and has apomictic behavior, being rare the sexual genotypes. The identification of only apomictic materials in a collection limits the genetic breeding program because there is no possibility of intra and interspecific hybridizations. Diploid and sexual genotypes should be polyploidized and used in intra and interspecific crosses. Thus, the *Paspalum* reproductive characterization allows the identification of sexual genotypes and the choice of parents in breeding programs. The objective of the present study was to characterize reproductively 137 accessions of *Paspalum* belonging to the informal group Plicatula conserved in *Paspalum* GB of Embrapa Pecuária Sudeste based on flow cytometry technique, cytoembryological analysis and molecular markers. Based on flow cytometry data, 49 accessions were considered highly apomictic, 85 were facultative apomictic and one accession (BGP 272 - *P.rojasii*) presented behavior compatible with the sexual reproductive mode. Based on the cytoembriological analysis, three accessions (BGP 281 - *P.lenticulare*, BGP 272 - *P.rojasii* and BGP 380 - *P.compressifolium*) had a sexual behavior and two accessions (BGP 232 - *P.plicatulum*; BGP 178 - *P.compressifolium*) were characterized as highly apomictic and facultative apomictic, respectively. Based on Bulk Segregant Analysis with 78 markers in *P.compressifolium* and *P.lenticulare*, the present study found great polymorphism among samples, however no linkage was observed between the apomixis trait and the molecular markers evaluated. Thus, this study had important results that will be applied in the future at *Paspalum* genetic breeding programs.

Unveiling the black-box genome of *Deinococcus radiodurans*

Nailah Latif Ahmed[1], Ricardo Valle Ladewig Zappala[1], Wanda Maria Almeida von Krüger[1], Manuela Leal da Silva[1], Claudia de Alencar Santos Lage[1]

[1]Instituto de Biofísica Carlos Chagas Filho, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Rio de Janeiro, Brazil.

nailah.ahmed@gmail.com

Key words: astrobiology, extremophile, *Deinococcus radiodurans*

The poliextremophilic bacteria *Deinococcus radiodurans*, from the Deinococcaceae family, is resistant to extreme radiation and desiccation, and explored by the omics science since its genome sequencing. Our group identified 26 genes predicted by molecular modelling, coding possibly related proteins to those resistance functions. Among them, *dr_0491* and *dr_2073* code, respectively, a potentially heat-shock protein (acting on stress protection and protein folding control), and a putative shikimate kinase (acting on aromatic amino acids biosynthesis in bacteria, fungi and plants). The objective of this work is to investigate the contribution of *dr_0491* and *dr_2073* functions for *Deinococcus radiodurans* extreme resistance. *dr_0491::kan* and *dr_2073::kan* mutants were designed from R1 wild type *D. radiodurans*, transformed with the PGEMT-Easy vector carrying the genes interrupted by the kanR cassette, and confirmed by *dr_0491+kanR* and *dr_2073+kanR* marginal regions primer amplification. The generated phenotypes will be analyzed by survival to desiccation, radiation exposure and oxidative stress. The *dr_0491::kanR* construction is finished, and *dr_2073::kanR* mutant is being generated. This mutant is currently at *dr_2073::kanR* verification stage. We were not able to generate any *dr_0491::kanR* mutant so far. As an unprecedented information, the *dr_0491* gene, assigned as hypothetical in this species, was shown to be essential. *dr_0491* and *dr_2073* products' can take part in protein restoration and aromatic amino acids biosynthesis to regenerate damaged proteins by radiation and/or other kinds of stress.

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ANALYSIS OF *FAM49B*, *FAM84B*, *GSDMC* AND *MIR5194 GENE* EXPRESSION IN GASTRIC CARCINOGENESIS

Brunno dos Santos Pereira[1]; Fernanda Wisnieski[1]; Mariana Ferreira Leal[1], Leonardo Caires Santos[1], Camila Albuquerque Pinto[1], Renata Sanches de Almeida[1], Carolina Oliveira Gigek[2]; Ana Carolina Anauate[1]; Lucas Trevizani Rasmussen[3]; Danielle Queiroz Calcagno[4], Ricardo Artigiani[2]; Samia Demachki[4]; Paulo Pimentel Assumpção[4]; Laercio Gomes Lourenço[5]; Carlos Haruo Arasaki[5]; Elizabeth Suchi Chen[1]; Spencer Marques Payão[3], Rommel Rodríguez Burbano[4,6,7]; Marília Arruda Cardoso Smith[1]

1 Disciplina de Genética, Departamento de Morfologia e Genética, Universidade Federal de São Paulo, São Paulo, SP, Brasil. 2 Departamento de Patologia, Universidade Federal de São Paulo, São Paulo, SP, Brasil 3 Departamento de Genética e Biologia Molecular, Faculdade de Medicina de Marília, Marília, SP, Brasil. 4 Núcleo de Pesquisas em Oncologia, Hospital João de Barros Barreto, Universidade Federal do Pará, Belém, PA, Brasil. 5 Disciplina de Gastroenterologia Cirúrgica, Departamento de Cirurgia, Universidade Federal de São Paulo, São Paulo, SP, Brasil. 6 Laboratório de Citogenética Humana, Instituto de Ciências Biológicas, Universidade Federal do Pará, Belém, PA, Brasil. 7 Laboratório de Biologia Molecular, Hospital Ophir Loyola, Belém, PA, Brasil

s.brunno265@gmail.com

Key word: gastric cancer; 8q24.21; gene expression

Introduction: Gastric cancer (GC) is one of the most common tumor types in the world. The absence of specific symptoms in the early tumor stages, the frequent tumor detection at advanced stages, and the limited treatment options currently available make this disease the third leading cause of cancer-related death in the world. Thus, the continuous exploration of new strategies for early diagnosis and for identification of new therapeutic targets are of great interest in this neoplasia. Our research group has previously identified that trisomy 8 is one of the most common aneuploidies in primary gastric tumors. Moreover, we observed amplification at 8q24.21 locus, which contains important genes involved in gastric carcinogenesis, including the proto-oncogene *c- MYC*. However, little is known about the role of other genes present in this locus in GC. **Objective:** To investigate the potential impact of *FAM49B, FAM84B, GSDMC,* and *MIR5194* transcriptional alterations in GC. **Metodology:** We selected 107 pairs of neoplastic and adjacent non-neoplastic tissues from patients with primary gastric adenocarcinoma who underwent gastric resection. None of the patients had history of exposure to either chemotherapy or radiotherapy prior to surgery, or other co-occurrence of diagnosed cancers. Furthermore, we selected 40 *H. pylori* and gastritis negative gastric mucosa samples of individuals without cancer who underwent routine endoscopy. Total RNA was isolated from tissue samples and complementary DNA was synthetized. TaqMan gene expression assays were used to evaluate in triplicates gene expression. *GAPDH+B2M* were used for the normalization of target gene expression. **Results:** *FAM49B, FAM84B* and *MIR5194* expression were reduced in neoplastic samples compared with adjacent non-neoplastic samples ($p<0.01$). *GSDMC* and *MIR5194* expression were reduced in gastric cardia region tumors $p=0.02$ and $p<0.01$, respectively). On the other hand, *FAM49B*, *FAM84B*, *GSDMC,* and *MIR5194* expression were increased in neoplastic and adjacent non-neoplastic samples of patients diagnosed with GC compared with non-neoplastic gastric tissue samples of patients without cancer ($p<0.01$). **Conclusions:** *FAM49B*, *FAM84B*, *GSDMC,* and *MIR5194* are deregulated genes in GC. Our results suggest different roles for these genes, which depend on the tumor development stage. The increased expression of these genes may be an important event in preneoplastic stages and progression of the disease.

MOLECULAR PHYLOGENY OF THE EPHIPPIDAE (ACANTHURIDAE, EPHIPPIFORMES)

Greicyellem Santana[1]*; Marcelo Vallinoto[1]; Yrlan Oliveira[1]; Aurycéia Guimaráes-Costa[2]; Breno Barros[3].

Laboratório de Evolução, Instituto de Estudos Costeiros (IECOS), Universidade Federal do Pará, Campus Universitário de Bragança[1]. Laboratório de Genética e Biologia Molecular, Instituto de Estudos Costeiros (IECOS), Universidade Federal do Pará, Campus Universitário de Bragança[2]. Universidade Federal Rural da Amazônia, Campus Universitário de Capanema[3].

greicysantana1@gmail.com

Keywords: Ephippidae; biogeography; diversification.

The Family Ephippidae (Ephippiformes *sensu* Betancur *et al*, 2003) is a coastal-marine fish group, comprising eight genera and fifteen species, distributed in all tropical and subtropical coasts of the world, where the Indo-Pacific presents the greatest diversity. Evolutionary relationships within the family are scarce and poorly understood, and there are no phylogenetic analyzes based on molecular data that elucidates the relationships of these organisms. Thus, this study tested hypotheses about the relation ship of the Ephippidae off a phylogeny comprising different biogeographic distributions, through information COI and Rhodopsin markers. Database was achieved from eleven species collected from almost all genera, including sequences available in public databases to complement the analyzes. Species of family Drepanidae were included as outgroup. Our results show strong evidence that the Ephippidae family constitutes a monophyletic group, with the formation of eleven distinct clades, with three large clades separated according to biogeographical areas, with high values of support. Indo-Pacific species *Tripterodon orbis* and *Ephippus* are sister-groups, diverging at the beginning of the Miocene (~23,2 ma). The monospecific clade *Zabidius novaemaculatus* constitutes a sister-group within *Platax* species, both genera distributed in the Pacific Ocean, diverging in the mid-Miocene (~16 ma). With high probability values, the genus *Chaetodpiterus* (western Atlantic) is the sister-group of *Parasepttus panamensis* (eastern Pacific), with the estimated divergence time of 7.8 million years. Our results show that the times of divergence between clades predate important geomorphological events in the distribution of species in both regions, such as the uplift of the Andes and the Isthmus of Panama, suggesting that the distribution of the family may have accompanied vicarious events derived from the movement of tectonic plates.

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INTERACTION BETWEEN HIGH-FAT DIET AND ETHANOL INTAKE LEADS TO CHANGES ON THE FECAL MICROBIOME

Renato Elias Moreira Júnior[1*]; Luana Martins de Carvalho[1]; Ana Lúcia Brunialti Godard[1]

[1] Universidade Federal de Minas Gerais

*renatoe@ufmg.br

Key words: High-fat diet; ethanol intake; gut microbiome.

The gastrointestinal tract holds a highly diverse bacterial set that comprises a complex ecosystem in which different bacterial species cooperate and compete between them and with the host cells to proliferate and to survive. The byproducts of this interaction affect physiological processes in the host organism. A growing body of evidence suggests that consumption of high-fat diet or ethanol intake affecting directly the abundance and composition of the gut microbiome. In response to these changes, the gut microbiome could modulate cellular responses in the host organism that might be related to the onset of a broad range of phenotypes, such as obesity, metabolic syndrome and drug addiction. However, the effects of the interaction between high-fat diet, its withdrawal, and ethanol intake in gut microbiota remain unclear. In order to evaluate the effects of high-fat diet, ethanol intake and your interaction in gut microbiome, the present study use an animal model chronically fed with high-fat diet and free-choice ethanol intake. In the first experimental stage (T1) forty-four C57BL/6 mice were submitted dietary treatment for 8 weeks, in which 30 animals received a high-fat diet, the high sugar and butter diet (HSB group) and 14 animals received the American Institute of Nutrition 93-Growth standard diet (AIN93G group).In the second stage (T2) the animals for four weeks were divided in six subgroups: [1] AIN93G + H_2O (n=7), [2] AIN93G + EtOH (n=7), [3] HSB + H_2O (n=7), [4] HSB + EtOH (n=7), [5] HSB-AIN93G + H_2O (n=8), and [6] HSB-AIN93G + EtOH (n =8). Three groups (+H_2O) had only access to water, while the remaining three (+EtOH) had a free choice between water and a 10% ethanol solution. In the HSB-AIN93G groups, the HSB diet was replaced by the AIN93G diet. Throughout the experiment the body weight, diet, ethanol consumption and adiposity index were evaluated. At the end of T2, animal feces were collected for total bacteria DNA extraction, followed by sequencing through the Illumina's MiSeq platform, and posterior analysis with the aid of bioinformatics tools. The result obtained with this model, point out that the interaction between HSB diet and ethanol intake directly affected the structure, composition and abundance of bacterian groups on fecal microbiome. In addiction, the HSB consumption induced weight gain; increase in the adiposity index and *Firmicutes*/*Bacteroidetes* ratio (commonly observed in obese individual). Finally, we also pointed out that the withdrawal of the HSB diet affects the preference for alcohol and shows a structural resilience in the fecal microbiome.These findings suggests that interactions between high-fat diet, its withdrawal, and ethanol intake, trigger changes in the gut microbiome that might take an important role in the maintenance of the observed phenotypes as well as in the increase consumption ethanol.

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EVALUATION OF LEVELS OF GLOBAL DNA METILATION IN THE GRAVITY OF ACUTE CORONARY SYNDROME

Ester A. S. Amorim[1]; Romário M. Araújo[1], Roberto P. Werkhauser[1], George T. N. Diniz[1], Viviane D. C. V. Carvalho[1], Lilian C. A. Silva[1], Sérgio T. Montenegro[3], Clarice N. L. M. Fonseca[1], Danyelly B. G. Martins[2], Fábia C. S. Soares[1], Sílvia M. L. Montenegro[1]

[1]IAM-FIOCRUZ-PE. [2]Laboratório de Imunopatologia Keizo Asami (LIKA) - UFPE. [3]Real Hospital Português - Recife-PE.

esteralves6@gmail.com

Key-words: acute coronary syndrome; TIMI risk; global methylation.

Aberrant profiles of DNA methylation have already been associated with Cardiovascular Diseases. This study evaluated the influence of global DNA methylation level on the severity of acute coronary syndrome (ACS) in 221 patients with ACS and 93 patients without ACS (sSCA) of the Real Hospital Português, Recife-PE. The severity of the patients was assessed by TIMI risk and stratified into low, intermediate and high risk. The levels of methylation were determined using an Enzyme Linked Immunosorbent Assay-based (ELISA) kit. Patients with ACS were subdivided into expected and early age for the development of the disease. The male gender was the majority, besides being found an association between the factors gender, smoking, diabetes and dyslipidemia and ACS through logistic regression. The SCA and sSCA groups had different levels of methylation, and patients with ACS were hypermethylated ($p = 0.0121$). There was no difference in methylation between genders. The intermediate risk level was the majority (46.61%) and there was a difference in methylation between the severities ($p < 0.0001$), with the TIMI risk group being lower with higher methylation. The expected and early age groups showed a global methylation difference ($p = 0.0486$), and a negative correlation was also observed between methylation level and age ($p = 0.0387$; $r = -0.15$). The hypermethylated profile of the global DNA in patients with ACS may become a promising biomarker for risk assessment for acute myocardial infarction (AMI).

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IN VITRO PHENOTYPE OF iPSCs DERIVED MUSCLE CELLS FROM TWO DISCORDANT DUCHENNE MUSCULAR DYSTROPHY AFFECTED BROTHERS

Joyce Esposito de Souza[1*], Felipe de Souza Leite[1*], Igor Neves Barbosa[1], Carolini Kaid Davila[1], Danyllo Felipe de Oliveira[1], Leonardo Galleni Leão da Silva[1], Raul Hernandes Bortolin[2], Vanessa Luiza Romanelli Tavares[1], Danielle de Paula Moreira[1], Uirá Souto Melo[1], Claudia Ismania Samogy Costa[1], Mayana Zatz[1]

*These authors contributed equally to this work
[1] Human Genome and Stem Cell Center, Biosciences Institute, University of São Paulo, São Paulo, Brazil. [2] Departament of Clinical and Toxicological Analysis, School of Pharmaceutical Sciences, University of São Paulo, São Paulo, Brazil

*mayazatz@usp.br

Keywords: Duchenne Muscular Dystrophy, induced pluripotent stem cells, discordant phenotype

Duchenne Muscular Dystrophy (DMD) is a progressive muscular disease caused by absence of dystrophin due to null mutations in the dystrophin gene. Despite many efforts to find a treatment aiming an up-regulation of dystrophin expression, results are still inconclusive and suggest that maybe to restore dystrophin is not enough to recover or slow down muscle degeneration. We have described two DMD affected half-brothers with discordant phenotypes: one of them, DMD-mild (DMD-m) is now 20 years old and still is able to walk. His younger brother, DMD-severe (DMD-s), was wheelchair bounded at age 9 and has a classic DMD clinical progression. They both have no muscle dystrophin and muscle degeneration, which suggests that DMD-m has a protective mechanism independently of dystrophin expression. In order to study and elucidate these mechanisms, we have generated induced pluripotent stem cells (iPSC) from these DMD brothers and from control patients. The cells were characterized by expression of pluripotent markers and then subjected to myogenic differentiation, with a previously described protocol. Comparison between brothers' iPSCs-derived skeletal muscle progenitors and mature fibers in different time points was performed by light microscopy, immunofluorescence and qPCR for myogenic markers. Important differences between brothers' *in vitro* phenotype were observed. A greater number of skeletal muscle fibers was seen in DMD-m, while DMD-s showed impairment in differentiation, excessive extracellular matrix deposition and cell proliferation without terminal differentiation. Gene expression analysis showed a different pattern between patients, showing a muscle differentiation potential similar to normal controls in DMD-m. Our results reinforce the importance of iPSCs as tools for disease modeling to recapitulate *in vivo* phenotype and open new questions about DMD protective mechanisms, which can be applied in future DMD treatment approaches.

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ETHANOL EXPOSURE AND THE IMPAIRMENTS ON LEARNING AND MEMORY BEHAVIOR ASSOCIATED WITH DIFFERENTIAL GENE REGULATION IN ZEBRAFISH BRAIN

Bárbara Miranda Sartori[1], Isadora Marques Paiva[1], Renato Elias Moreira Junior[1], Luis David Solis Murgas[2], Ana Lucia Brunialti Godard[1]

[1]Laboratório de Genética Animal e Humana - Departamento de Genética, Ecologia e Evolução - Pós-Graduação em Genética - ICB - UFMG.
[2]Universidade Federal de Lavras, Ala de Peixes do Biotério Central da UFLA

bsartori9@gmail.com

Keywords: alcohol; learning and memory; genes

Alcohol use harm process of learning and memory in humans and other animals. This drug consumption can cause brain damage even after few exposures. In this context, animals models, like zebrafish (*Danio rerio*), has emerged as model organisms to study behavioral and molecular mechanisms of numerous diseases, including those related to the Central Nervous System, such as neuropsychiatric disorders. Thereby, this study aimed to evaluate the effects of ethanol on learning and memory consolidation and their relationship with the regulation of target genes in the brain. We performed an Object Recognition Behavioral Test (OR) in 108 adult zebrafish (*Danio renio*). In the first step of the test - Familiarization Phase (FP) -, animals were exposed for 10 minutes to two identical objects, each one placed in one side of the tank. After FP, the animals were divided into two groups: Control (C) and Treatment (T), exposed to water and ethanol (1%, v/v), respectively, for 20 minutes. In a second step - Test Phases (TP) – animals of both groups were exposed to the familiar object from the FP and a novel one 2 hours (TP2), 24 hours (TP24) and 8 days (TP8) after ethanol/water exposure, for object recognition. At the end of each TP, 16 animals of each group were euthanized to brain collection and transcriptional quantification of target genes (*lrfn2, grin1a* and *lrrk2*) by qPCR. Regarding behavioral analysis, in TP8, the C group spent more time exploring the novel object when compared to TP2. However, for the animals of the T group, we could not observe any difference when compared to Control or between TFs. For molecular results, in TP8 all genes showed a decrease in their mRNA transcription, comparing T to C. In TP24, we observed down-regulation of *grin1a*. Finally, for both groups – C and T - we found an up-regulation of *lrfn2* in TP8 in relation to TP2 and TP24. Therefore, we suggest that ethanol has an influence on gene regulation after an acute alcohol treatment, especially after a period of 8 days. In the behavioral analysis, considering data dispersion and the lack of considerable difference for animals in the T group, we observed a high variability, which suggests that individuals in this group may have different sensibilities and responses to alcohol effects. A multivariate analysis will be conducted to generate scores based on different behaviors - e.g. the results of novelty preference index, velocity and time spent in profundity. These data will be used to divide animals of group T into new subcategories in order to work around those phenotypic differences.

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NEGATIVE AND POSITIVE REGULATORY ROLES OF PHOSPHORYLATION SITES WITHIN THE ACTIVATION LOOP OF NIK1 IN RESISTANCE AGAINST BEGOMOVIRUSES

Virgilio Adriano Pereira Loriato[1], Laura Gonçalves Costa Martins[1], Ruan Maloni Teixeira[1], Gabriel A. S. Raimundo[1], Larissa G.M. Ávila[1], Caio C. A. Pádua[1], Marco Aurélio Ferreira[1], Anésia Aparecida dos Santos[1,2], Elizabeth Pacheco Batista Fontes[1,2]

[1]National Institute of Science and Technology in Plant-Pest Interactions, Bioagro, Universidade Federal de Viçosa, Viçosa, Minas Gerais, Brazil.
[2]Departament of General Biology, Universidade Federal de Viçosa, Viçosa, Minas Gerais, Brazil.

virgilio.loriato@gmail.com

Key-words: Begomovirus; viral DNA; Solanum lycopersicum.

The receptor-like kinase NIK1 (NSP-Interacting Kinase) was first identified as a virulence target of the begomovirus nuclear shuttle protein. Activation of NIK1 by oligomerization- dependent phosphorylation relays an antiviral signal to the downstream components RPL10 and LIMYB, which repress the expression of translational machinery-related genes. Therefore, the signaling module NIK1-RPL10/LIMYB protects plants against begomovirus by suppressing global translation of host and viral mRNAs. We have previously shown that expression of the constitutively activated mutant NIK1-T474D confers resistance to begomovirus in tomato. Here, we examined the potential of the double mutant NIK1-T469A/T474D to potentiate resistance against begomovirus. We first showed that the double mutant displays higher phosphorylation activity than NIK1- T474D. To assay for constitutive activation of NIK1-T474D, we measured the readouts of an activated NIK1 pathway, including down-regulation of protein ribosomal (PR) genes and resistance to begomovirus. We showed that ectopic expression of the double mutant in tomato plants represses RP gene expression and is effective against begomovirus. To potentiate further the NIK1-mediated resistance, we co-transformed tomato plants with NIK1T469A/T474D and LIMYB or NIK1-T474D and LIMYB. The extend of NIK1- mediated antiviral signaling activation in the double transformants was compared with transgenic lines expressing each transgene alone. Reconstitution of a fully NIK1 activated pathway by expressing NIK1T469A/T474D and LIMYB in transgenic lines caused a stronger repression of RB genes and an enhanced resistance against begomovirus as compared to the other combinations. Our results demonstrated thatthe NIK1-mediated antiviral signaling holds the potential to be a target for engineering begomovirus resistance in susceptible genotypes.

Funding Agency: CAPES, CNPq, FAPEMIG, INCT.

FUNCTIONAL CARACTERIZATION OF HUMAN N- ACETYLTRANSFERASE 2 CODING HAPLOTYPES IN THE BRAZILIAN POPULATION.

Victória de Moraes Silva, Eloah Aguiar Soares da Silva, Márcia Quinhones Pires Lopes, Adalberto Rezende Santos, Harrison Magdinier Gomes, Philip Noel Suffys, Raquel Lima de Figueiredo Teixeira

Laboratory of Molecular Biology Applied to Mycobacteria,Instituto Oswaldo Cruz – IOC, Rio de Janeiro, Brazil.

victoriamoraess97@gmail.com

Key-words: N-acetyltransferase2; SNPs; Gene Expression.

The human enzyme N-acetyltransferase 2 (NAT2), encoded by the *NAT2* gene, is a phase II biotransformation enzyme that catalyzes the N-acetylation of arylamines, heterocyclic amines and hydrazines and plays a significant role in the clearance and biotransformation of many drugs and carcinogens. Point mutations, known as single nucleotide polymorphisms (SNPs), within coding region of *NAT2* can alter its enzymatic activity resulting in the individual phenotypes of slow, intermediate and fast acetylation. Genetic variability in *NAT2* can contribute to individual differences in drug response or toxicity and has been attributed to therapeutic failure and adverse drug reactions (ADRs) in the treatment of different disease models. In Brazil, recent studies have demonstrated a considerable allelic diversity of *NAT2*, including the description of SNPs and new alleles with unknown functional effects. Knowledge of the functional role of variations in the *NAT2* sequence may contribute to a better understanding of the relationship between the genotype and the human N-acetyltransferase 2 phenotype and the prediction of unfavorable therapeutic outcomes for drug-treated diseases metabolized by this enzyme. Therefore, the objective of this study is to investigate the functional effect of the haplotypes *NAT2*6M* (152G> T, 282C> T, 590G> A) and **12E* (282C>T, 578C>T, 803A>G) on the acetylation phenotype and the mechanism by which this combination may result in a slow acetylation. After cloning of wild-type human *NAT2* gene in a prokaryotic expression system, site-directed mutagenesis was performed for insertion of the different mutations of interest in the *NAT2* coding region using the Gene Art® Site-Directed Mutagenesis PLUS kit. Subsequently, recombination reactions were performed followed by transformation of recombinant DNA into specific *E. coli* strains. After confirmation by sequencing, the different clones were submitted to gene expression assays with subsequent extraction of total proteins, SDS-PAGE and Western blot. Finally, to verify the effect of the combination of the mutations on the heterologous protein levels, the immunoreactive band densitometry assays was performed comparing with wild-type NAT2 expression. Preliminary results show that the haplotype *NAT2*6M* reduced the amount of *NAT2* seen in Western blot with specific antibody. These findings suggest that these polymorphisms could reduce the catalytic activity of NAT2 and therefore contribute to slow acetylation phenotype.

Funding Agency: PIBIC-CNPq; CNPq and Strategic Actions for the Development and Strengthening of Accredited Laboratories and Research Support Areas of the Oswaldo Cruz Institute (PAEF/FIOCRUZ – IOC–023-FIO-18-2-13).

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RELATIVE CONTRIBUTION OF ENVIRONMENTAL AND SPATIAL VARIABLES TO THE GENETIC DIVERSITY OF *AVICENNIA GERMINANS* AND *AVICENNIA SCHAUERIANA*

Michele Fernandes da Silva[1]; Mariana Cruz[1]; João Vidal[2]; Gustavo Mori[3]; Maria Zucchi[3]; Anete Pereira de Souza[1]

[1]Centro de Biologia Molecular e Engenharia Genética, Universidade Estadual de Campinas – Instituto de Biologia, Campinas, São Paulo, Brazil. [2]Universidade Estadual Paulista - Instituto de Biologia, São Vicente, São Paulo, Brazil. [3]Escola de Agricultura Luiz de Queiroz, Piracicaba, São Paulo, Brazil.

michele.uerj@gmail.com

Key-words: Landscape Genetics; Climate change; Black mangrove

Mangroves are a polyphyletic group of trees and shrubs that exhibit convergent adaptations, such as aerial roots, salt exclusion mechanisms and especially, the presence of propagules that float in salt water over long distances, allowing them to survive in an environment considered hostile to various other types of plants. Despite their ecological and social importance, these organisms are part of one of the ecosystems most threatened by constant climate changes due to their coastal distribution, where they act as sentinels of elevation of the sea level and increase of the frequency of extreme events such as storms, heat waves and droughts.Yet, their protection and restoration is urgent for fighting climate change, as they can store more atmospheric carbon dioxide per hectare than any other tropical forest in the world. In this scenario, the identification of the abiotic factors that determine the distribution of genetic diversity in mangrove tree species is crucial for the definition of conservation plans and for predicting the consequences of future changes to these species. Therefore, we aim to identify the contribution of environmental and spatial variables to the structure of genetic diversity of two widespread mangrove species of the genus *Avicennia* (L.) along the Atlantic coast of South America. Seventy-seven individuals of *Avicennia schaueriana* and forty-eight individuals of *Avicennia germinans* were genotyped for a total of 6170 and 2297 biallelic SNP loci, respectively. Pairwise FST between sampling sites was calculated and interpreted as genetic distances. Similarly, we determined pairwise geographic distances between localities. Twenty-nine oceanographic and climatic variables from global public databases were used for extracting environmental values for all occurrence points of the species, downloaded from GBIF. Then, this matrix was transformed through a principal components analysis (PCA), which was used to obtain pairwise euclidean distances between sampling points, which were interpreted as environmental distances. In a Landscape Genetic approach, we identified that the geographic distance contributes 81% and 61% and the environmental distance with 56% and 42% of the genetic divergence for *A. schaueriana* and *A. germinans*, respectively. For *A. schaueriana*, the presence of the oceanographic barrier was the most important factor in the genetic differentiation of these populations. Our results elucidate that even with a long distance dispersion, the floating propagules of these species are geographically restricted and those that reach different places may undergo divergent selective pressures leading to the evolution of locally adapted populations. With this information we intend to help predict the future of these species in the face of climatic changes and in the definition of strategies for the management, conservation and recovery of degraded mangroves on the Atlantic coast of South America.

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IDENTIFICATION AND CHARACTERIZATION OF *MEGALEPORINUS ELONGATUS* (ANOSTOMIDAE) DNA SATELLITE WITH THE BIOINFORMATIC TOOLS

Évelin M. Gonçalves[1]; Carolina Crepaldi[1]; Patricia P. Parise-Maltempi[1]

[1]Departament of Biology, Biosciences Institute, UNESP, Rio Claro, São Paulo, Brazil.

* evelin.mariani@hotmail.com

Keywords: DNA satellite; genomics; Neotropical fishes

Much of the eukaryotic genome is composed of non-coding repetitive sequences, with the satellite DNAs being included in this group. For being highly dynamic and variable, it is believed that satDNAs are related to the processes of heterochromatin formation, such as in the emergence, differentiation and evolution of sex chromosomes. By means of bioinformatic analyses from genome sequencing, it is possible to identify and characterize satDNAs present in the genomes as a whole. The present work is a complement of a previous analysis of the fish species *Megaleporinus elongatus* female genomic satDNA content, carried out previously in the laboratory of animal cytogenetics of UNESP Rio Claro, which obtained interesting results with several sequences isolated and mapped in the species' chromosomes. In the present analysis, a second iteration of the RepeatExplorer, together with the TAREAN tool, was performed in the search for novel satDNAs that may not have been isolated in the first analysis. As a result, 265 repetitives were found by the software, and 77 were characterized as satDNAs after screening the characteristic graphs of tandem repeat sequences. In a second step, we performed an individual and manual inspection of the 20 most abundant, analysing their contigs and aligning them to recover the consensus sequences of each one. Homology analysis using alignments through Muscle tool demonstrated that they are new sequences, not showing significant similarities with the sequences isolated previously and are in fact individual satDNAs belonging to different families. Searches through BLAST and GiriRepbase did not show relevant results of similarities with sequences already deposited in them, nor homologies with possible transposable elements. The isolated sequences have a mean of 53 bp, genome representation of 0.228%, and A+T of 60.6%. For this study, divergent primers were constructed for the 8 most abundant satDNAs, of which 4 were recovered through PCR using DNA extracted from the species. Bioinformatics resources linked to genome sequencing enable a more in-depth study of the molecular structure of its components, as well as in the present work. It was possible to verify a large number of repetitive DNAs in the sequenced genome, and with new repetitive sequences being isolated from the species. The identification of these satDNAs provide support for further studies related to the genetic mapping of the identified sequences and the possible relationship of the satellite DNAs with the heteromorphic sex chromosomes present in the *M. elongatus* species.

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CHARACTERIZING THE RIVERINE MICROBIOME EXPOSED TO EXTREME ENVIRONMENTAL STRESSFUL CONDITIONS CAUSED BY A MINING SLUDGE TSUNAMI

Maria Luíza S. Suhadolnik[1]; Mariana Reis[1]; Marcela F. Dias[1]; Marcelo P. Ávila[1]; Amanda M. Motta[1]; Francisco A. R. Barbosa[1]; Andréa M. A. Nascimento[1] *.

[1]Departamento de Genética, Ecologia e Evolução, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais.

* amaral@ufmg.br

Keywords: mining disaster; riverine microbiome; 16S rRNA gene.

In the present work we report the effect of the worst global environmental disaster of the mining industry over the ecology of the riverine bacterioplankton and sediment microbiomes. The collapse of Fundão Dam into the Doce River basin in Brazil, on November 2017, generated a mud tsunami of 50 million cubic meters of iron ore tailings, inevitably damaging the biodiversity. Water and sediment samples were collected 7, 30 and 150 days after the disaster from two impacted (do Carmo and Casca) rivers and a reference one (Água Fria) from the Doce River basin. Characterization of their microbiomes was done through 16S rRNA genes deep sequencing and metals concentrations were quantified. Diversity indexes showed that do Carmo River (the closest to the dam disruption site) riverine bacterioplankton and sediment communities contained the lowest richness and diversity on day 7 after the disaster, and that dissimilarity between the microbial communities of impacted and reference rivers reduced over time, being this partial recovery faster in water than in sediment communities. *Sediminibacterium* (Bacteroidetes), *hgc_clade* (Actinobacteria) and *Methylophilaceae* (Betaproteobacteria) were the clades that predominated in the waters of impacted rivers rather than in Água Fria, while in the impacted sediment the taxa that reached higher abundance than in the non-impacted one were *Cloacibacterium*; *Acinetobacter*; *Sediminibacterium*; *Geothrix*; *Erysipelothrix Novosphingobium*; Comamonadaceae; Rhodocyclaceae; Desulfuromonadales; Pseudomonadaceae; Aeromonadaceae and Acidobacteria Subgroup 2. In addition, network analysis will be performed to enhance comprehension of microbiome dynamics. The results show that the microbiomes composition was shaped by spatiotemporal variables, including the sites' metal content and taxa interactions.

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DMSO TOXICITY IN DIFFERENT CONCENTRATIONS IN TONGUE CARCINOMA AND NORMAL CELLS

Myllena Mayla Santos de Oliveira[1]; Mayra Carolina da Silva Ferreira[1]; Luana Pereira Cardoso[1]; Flávia Cristina Rodrigues-Lisoni[1]

[1]São Paulo State University (UNESP), Faculty of Engineering (FEIS), Campus Ilha Solteira, Department of Biology and Animal Science.

*E-mail myllenaharuno@gmail.com

Key words: cell culture; proliferation assay; cytotoxicity

DMSO is a polar and aprotic solvent, differentiating agent with a respective anti- neoplastic activity, free radical scavenger that acts in the transport and solubility in water and has been used as an inactive ingredient in several products approved by the Food and Drug Administration (FDA). In the search of alternative treatments using active plant principles, DMSO has been widely used to dilute and optimize the penetration of herbal medicines in normal and tumorigenic cells. But it has already been noted in some studies that it modulates the tumorigenicity and other characteristics of some malignant cell lines (in vitro), mimics the inhibitory effect of thalidomide on endothelial cell proliferation choriocapillaris and may act as differentiation-inducing agent. It is considered to be cytotoxic, but in amounts not yet well specified. Thus, the present work had as objective to investigate the effect of DMSO, in different concentrations, on morphology, proliferation and cytotoxicity, observing how this compound acts and how these alterations can participate in the tumorigenic process. The tongue squamous cell carcinoma (SCC-25) and normal keratinocyte skin (HaCaT) cell lines were treated with DMSO at concentrations of 1, 5 and 10%. The morphology was evaluated by the invertoscope and no modification was observed when compared to the control. The cell proliferation index was investigated by growth curve, in which the proliferation changed at concentrations of 5 and 10%, while the concentration of 1% decreased the proliferation from 48h in both cell lines. In the cytotoxicity assay, the cell viability decreased in the SCC-25 cells at concentrations of 5 and 10% (24 and 72h), while in the HaCaT cells the viability decreased at concentrations of 5 and 10% at all times, both cell lines at 1% concentration did not suffer a decrease in cellular viability. Reports in the literature of cell growth inhibition following DMSO treatment are ancient, ranging from melanoma cells to human rectal adenocarcinoma. From the results, we observed that the two cell lines responded similarly to the concentrations and times tested, but the concentration of 1% for both SCC-25 and HaCaT would allow better solubilization of drugs and/or phytotherapics that present difficulties of dilution at lower concentrations with a time limit of 24 hours. It can be concluded that the DMSO treatment does not modify the cellular morphology of the cell lines, it alters the cell proliferation mainly in the concentrations of 5 and 10% in all studied times and decreases the cell viability of the HaCaT line in the concentrations of 5 and 10%.

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IDENTIFICATION AND CHARACTERIZATION OF SATELLITE DNA PRESENT IN THE *THOROPA* GENOME (ANURA, AMPHIBIA).

Giselle P. Pessoa[*1]; Luiza R. Cholak[1]; Célio F. B. Haddad[2], Patrícia P. P. Maltempi[1]

[1]Department of Biology, Institute of Biosciences of UNESP Rio Claro, São Paulo, Brazil [2]Department of Zoology, Institute of Biosciences of UNESP Rio Claro, São Paulo, Brazil

gispessoa@gmail.com

Palavras-chave: Populations; Repetitive; Cytogenetic.

Repetitive sequences act on important cellular processes and arrangements, as well as providing relevant information on the speciation and phylogeny of related species. The most represented category of repetitive DNA in the genome is satellite DNA, which has been widely used as cytogenetic markers in studies on evolution and biological diversity. In anurans, cytogenetic studies are scarce, especially when considering their great diversity. For *Thoropa miliaris* (Anura: Cycloramphidae) these data are minimal, and nothing is known about the organization of their genomic repetitive content. When applying the C-banding technique in the *T. miliaris* chromosomes of the populations of Santa Teresa (ES) and Paraty (RJ), a superabundance of centromeric heterochromatin was observed in the first one, possibly indicating an accumulation of satellite DNA that could lead to its genetic isolation. In this study, satDNA sequences present in the *T. miliaris* genome of the Santa Teresa (ES) population were identified and characterized for the first time. We propose to locate them in their chromosomes and make comparative analysis with the Paraty (RJ) population, generating data that may help to understand their genomic organization and for future taxonomic studies of the species. The genome of *Thoropa miliaris* from the population of Santa Teresa (ES) was sequenced and submitted to analysis in RepeatExplorer, that quantified 122 clusters grouped as repetitive sequences. From these, 24 clusters (19.67%) showed compounds by highly repetitive sequences, and were selected as candidates for satDNA. These clusters were manually analysed to confirm their tandem distribution and their consensus sequences were generated through softwares MEGA and Geneious. Two satDNAs presented considerable similarities with deposited sequences, like the COI gene in the mitochondrial genome of the same species and a repetitive DNA fragment homologous to the ribosomal DNA 5S of the slime carp. From the consensus sequences, divergent primers were manually designed. Validation through PCR is still in progress, using the genomic DNA of the individuals owning the sequenced genome and the primers synthesized. The bioinformatic tools enabled the discovery of 21 satDNA in the species genome, which number reveals its repercussion on the centromeric heterochromatin superabundance and indorses its connection with the Santa Teresa population's genetical segregation. Comparisons of the satellite distribution among the target populations of *Thoropa miliaris,* using the primers as markers for the chromosomal mapping, may lead us to understand their influence on the geographic and genetic isolation processes of the species.

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GENOMIC OF *CLOSTRIDIUM DIFFICILE* STRAINS: INVESTIGATION OF VIRULENCE-ASSOCIATED GENES

Anna Beatriz Ferreira Rocha[1,2]; Victor Hugo Giordano Dias[2]; John Chris Vitucci[3]; Allan Cézar de Azevedo Martins[2]; Turan Peter Ürmenyi[2]; Wilson J da Silva Junior[4]; Waldir Balbino[4]; Rodrigo Soares de Moura Neto[5]; Rosane Silva[2].

[1]Instituto Federal de Educação, Ciência e Tecnologia do Rio de Janeiro, Rio de Janeiro, Brazil. [2]Instituto de Biofísica Carlos Chagas Filho, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil. [3]University of North Texas Health Science Center, Texas, USA. [4]Departamento de Genética, CCB, Universidade Federal de Pernambuco, Pernambuco, Brazil. [5] Instituto de Biologia, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil.

beatriz.fr.bio@gmail.com

key-words: *Clostridium difficile*; virulence-associated genes; nosocomial infection.

Clostridium difficile is an anaerobic, Gram-positive, toxigenic and spore-forming rod that colonizes the intestinal microbiota of approximately three percent of adults and sixty six percent of children without causing any problems. The occurrence of the symptoms, as mild diarrhea and fever, depends on both the virulence of the infective strain and the immune system of the patient. The broad-spectrum antibiotic usage as treatment for others diseases can disrupt the intestinal microbiota, leading to the bacteria multiplication, the toxin release (TcdA and TcdB) and it may result in death of the patient. *C. difficile* strains are found worldwide and some isolated strains are more virulent than others. We investigate the genetic influence on the difference of virulence between *Clostridium difficile* strains, in order to confirm the responsables genes for the different levels of virulence identified between the strains in the study. Thirteen *C. difficile* strains that was considered morphologically identical were selected (008, 101-110, 196 and 210), as 107, 108 and 109 the most virulent strains and 101 and 102 the least virulent strains. The virulence of each strain was verified by our collaborating laboratory of the University of North of Texas, through experiments using infected mices. The DNA was extracted and sequenced using the Nextera XT DNA Library Prep and MiSeq Reagent kit v3 kits for Illumina MiSeq equipment. The reads obtained were analyzed by the online browser RAST - Rapid Annotation Using Subsystem Technology, and from that, a database was created with information as functional categories according to the annotation of each different strain, for analyzing and detecting prospective virulence genes. About 1.820.504 reads were obtained from the thirteen strains and ninety-seven virulence-associated genes were annotated for each strain. Thirty mutual annotations between the most and the least virulent strains were obtained; two annotations were exclusive of 102 strain and three annotations were exclusive between 101, 102, 108 and 109 strains. Virulence-associated genes related to antibiotic resistance are being investigated to have an additional source of information to compare all the thirteen strains. Besides that, we intend to construct metabolic networks to understand the virulence mechanisms better. The identification of virulence-associated genes in morphologically identical strains facilitate to understand the differences of pathogenicity levels and mechanisms of action that allows the strains characterization and to find alternative manners to intervene in major metabolic pathways.

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GENOMIC ORGANIZATION OF MICROSATELLITES SEQUENCES IN ELOPIFORMES

Carolina Pinheiro Vasconcelos[1]; Diovanna Mirella dos Santos da Silva[1]; Rodrigo Petry Correa de Sousa[1]; Glaucia Caroline Silva de Oliveira[2], Marcello B. Cioffi[3], Edivaldo Herculano Correa de Oliveira[4,5]

[1]Universidade Federal do Pará, ICB, Belém, PA, Brasil. [2]Universidade Federal do Pará, IECOS, Bragança, PA, Brasil. [3] Universidade de São Carlos, Departamento de Genética e Evolução, São Carlos, SP. [4]Universidade Federal do Pará, ICEN, Belém, PA, Brasil. [6]Instituto Evandro Chagas, SAMAM, Laboratório de Cultura de Tecidos e Citogenética, Ananindeua, PA, Brasil.

*carolpinvasco@gmail.com

Keywords: Microssatelite mapping; FISH; Chromosome structure.

The order Elopiformes includes species of fish distributed in marine and estuarine environments along the tropical and subtropical regions, grouped into two families (Megalopidae and Elopidae). Although cytogenetic studies with the application of different techniques has brought important contributions to a better understanding of the genomic organization, evolutionary history and phylogenetic relationships of different groups of organisms, including fishes, there are few karyotypical studies in Elopiform species. The data generated by these studies, based on conventional staining, have shown karyotypes varying between 2n = 48 and 2n = 52, with a predominance of acrocentric elements. Among the different techniques of chromosome structure studies, the mapping of repetitive sequence by fluorescent *in situ* hybridization (FISH) is a valuable tool for population and evolutionary studies. Microsatellites are repetitive DNA sequences formed by short repeats of 1 to 6 bp, which participate in the organization of chromatin, DNA replication, recombination and regulation of genetic activity, being widely used as genetic markers. Thus, the present study had the objective of mapping eight microsatellite sequences in two species of Elopiformes (*Elops smithi* and *Megalops atlanticus*) of the Amazon coastal zone. The species were collected in the Caeté river in Bragança, Pará, and metaphase chromosomes were obtained from tissue cultures. Probes with the following sequences were used in FISH experiments: (GAC)10, (GAG)10, (TA)15, (CA)15, (CAG)10, (C)30, (CAT)10 and (GA)15 . The results showed that *E. smithi* and *M. atlanticus* show a very similar distribution of these sequences. Probes (CA)15, (C)30, (CAT)10 and (GA)15 hybridized in the telomeric and centromeric regions, while probes (GAC)10, (GAG)10 and (CAG)10 were distributed throughout the extension of the chromosomal arms, with accumulation of the sequence (CAG)10 in the secondary constrictions of pair 20, in both species. Probe (TA)15 was the only one to present different pattern between the species, with distribution throughout all the chromosomes of *Elops smithi*, while in *Megalops atlanticus* it was restricted to the telomere regions. The distribution of repetitive DNA sequences in centromeric and telomeric regions is quite common, probably because they are associated with structural stability in these regions of the chromosomes. Differently, distributions in specific regions along the chromosome may be related to the protection of genes or remnants of rearrangements. The observed similarity of the distribution of most sequences may be a reflection of the phylogenetic proximity of these species. Overall, these results may be useful for taxonomic as well as in understanding chromosomal organization and evolution of these sequences in fish.

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QUORUM SENSING POSSIBLY CONTROLS THE *XANTHOMONAS CITRI* SUBSP. *CITRI* CRISPR-CAS SYSTEM

Julia Lopes Nalin[1*]; Laís Moreira Granato[1]; Paula Maria Moreira Martins[1]; Alessandra Alves de Souza[1]

[1]Centro de Citricultura "Sylvio Moreira" – Cordeirópolis/SP.

* julia.nalin@hotmail.com

Key-words: CRISPR-Cas, quorum sensing, *X. citri*

Bacteria commonly exist in high cell density populations, making them susceptible to virus attack. To combat these invaders, bacteria possess an arsenal of defenses, such as CRISPR-Cas systems. *Xanthomonas citri* subsp. *citri* (*X. citri*) present a Type I-C CRISPR-Cas system). *X. citri* populations coordinate their behavior as cell density increases, using quorum sensing (QS) signaling. The QS of *X. citri* consists of three major components: RpfF, RpfC, and RpfG. The *rpfF* gene encodes a putative enoyl-CoA hydratase that catalyzes the synthesis of the signal molecule (DSF). It is known that strains unable to communicate via QS were less effective at defending against invaders targeted by the CRISPR-Cas system. However, little is known about how *X. citri* CRISPR-Cas systems are regulated. The aim of this study was to evaluate the expression of the Cas operonin the *rpfF* mutant (Δ*rpfF*) comparing with the wild-type strain (wt) in starving conditions. For this purpose, Δ*rpfF* and wt were grown in 50 mL of minimum medium at 28°C for 48 hours with shaking. Four biological replicates were used for each strain. The culture samples were collected, centrifuged and cell pellets were used for RNA extraction. PCR primer efficiency of each primer pair was determined using the Real-time PCR Miner and RT-qPCR was performed using three replicates of each sample with appropriate negative controls. For relative quantification, the $2^{-\Delta\Delta CT}$ method was applied. Gene expression was showed as fold change in Δ*rpfF* samples compared to the wt, using the 16S as reference gene. It was observed that the genes *cas1*, *cas2*, *cas3*, *cas4* and *cas5* were upregulated in the *rpfF* mutant comparing to the wt. Its show that these *cas* genes are modulated by the QS system. Besides, it is possible that bacteria can use DSF communication to modulate community-level defense requirements in high cell density populations and the costs of basal CRISPR-Cas activity.

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IN SILICO AND FUNCTIONAL ANALYSES OF THE REGULATORY ACTIVITY OF *MIR-21* OVER THE *TBX2B* GENE IN ZEBRAFISH

Lucas Di Pietro[1], Arthur Casulli de Oliveira[1], Pedro Nachtigall[1], Luiz Augusto Bovolenta[2], Danillo Pinhal[1]

1 UNESP, Departamento de Genética, Instituto de Biociências, Botucatu, SP, Brasil. 2 UNESP, Departamento de Física e Biofísica, Instituto de Biociências, Botucatu, SP, Brasil.

dipietroneves@gmail.com.br

Key-words: miRNA; *zebrafish*; heart development.

The zebrafish (*Danio rerio*) is a well-established animal model. Two useful traits of this model are a fully sequenced genome and functional homology to the human genome. In vertebrate animals, the heart is one of the first organs to develop, since many subsequent processes can only happen if the cardiac muscle is functioning properly. There are many processes happening inside an animal´s heart during its development that need to be rigorously controlled, one of these is the rhythmic contraction of the heart's chambers. Broadly speaking, it is necessary that some chambers contract slightly before other ones. This creates what we call the atrium-ventricle delay (AV delay). Among all the genes that act in synergy to generate the AV delay, the *tbx2b* gene is one of high importance because of its ability to modulate the action of the *nppa* gene, which is necessary for the formation of fast-twitch muscle fibers. For the heart and the rest of the *zebrafish* embryo to develop, it is significant that these elements are controlled accordingly. In this context, the regulatory molecules called microRNAs (miRNAs) have proven to be valuable to the correct modulation of the heart´s development. With that in mind, we wished to find more about the role of miRNAs within the heart development context. Firstly, we made a series of *in silico* target predictions in our laboratory. We used three target prediction tools (TargetScan, miRanda and RNA22) to search for miRNAs/mRNAs interactions that have the potential to be biologically relevant. We united the results of the prediction tools and focused on conserved miRNAs and zebrafish genes that have human homologues. Among all the miRNA/gene interactions, we found that the *tbx2b* gene could be actively regulated by dre-miR-21-3p (miR-21). The *tbx2b* gene and *miR-21* are well conserved among species. Then, we were able to further validate the *tbx2b/miR-21* relation in zebrafish embryos, using the GFP (green fluorescent protein) qualitative validation method. Injecting a GFP-capable expression cassette attached to the *tbx2b* 3´UTR, in conjunction with inhibiting morpholino molecules, we were able to see variable levels of green fluorescence and determine the *tbx2b/miR-21* relation *in vivo*. Finally, we concluded that *miR-21* can regulate the *tbx2b* post-transcriptionally, to a certain degree in *zebrafish*. Further studies are necessary to better assess the impact of this miR-21 in heart development and its implications to both zebrafish and humans.

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Expression and Purification of Recombinant Proteins of *Erysipelothrix rhusiopathiae* for Future Evaluation of Murine Model Antigenicity

Godoy, N.L.[1,3*]; Silva, A.J.[2]; Zangirolami, T.C.[2]; Novo-Mansur, M. T.M.[1]; Anibal; F.F.[3]

[1] Federal University of São Carlos, São Carlos, SP, Brazil - Department of Genetics and Evolution. [2] Federal University of São Carlos, São Carlos, SP, Brazil - Department of Chemical Engineering.[3] Federal University of São Carlos, São Carlos, SP, Brazil - Department of Morphology and Pathology

[*] naigodoy@gmail.com

Key words: *Erysipelothrix rhusiopathiae*, Recombinant vaccines, SpaA

Erysipelothrix rhusiopathiae is a Gram-positive bacillus, which causes the swine erysipelas. This disease is characterized by the appearance of hemorrhagic, purple, diamond-shaped lesions in any part of the body, and has great economic importance, since it entails the loss and poor development of the pigs. Existing vaccines for immunization against erysipelas are formed by killed or attenuated *E. rhusiopathiae* agent, and according to reports in the literature, has failed to induce protection. Potentially antigenic proteins of *E. rhusiopathiae* were previously detected by immunoproteomics approach. This work aims the heterologous expression and purification of two recombinant proteins of *E. rhusiopathiae* (P1.1 and SpaA) to evaluate the immune response induced by these proteins in murine model. Proteins were induced by IPTG in *Escherichia coli* BL21 (DE3) containing the coding regions of the genes cloned in pET28a (+). Expression of these proteins was tested at temperatures of 37°C, 30°C and 20°C; IPTG concentrations of 0.4 mM and 0.1 mM and in different culture media, such as Luria Bertani medium (LB) and richer media such as Dynamite and Terrific Broth (TB). Protein P1.1 was found in soluble form, while SpaA in insoluble form, and after determining the best expression conditions (20°C and 0,1mM IPTG), the proteins were subjected to purification by affinity chromatography using 1 and 5 ml of immobilized nickel column, for better evaluation of the purification efficiency. In order to achieve the desired purity, elution of the proteins from column were tested in buffers containing NaCl or urea, at different pH values and imidazole concentrations. The purified proteins concentrations were 6 µg/µl (SpaA) and 3 µg/µl (P1.1). Further they will be used to immunize mice to be challenged with the bacteria for evaluation of the efficacy of the immunization in terms of protection levels and types of antibodies. It is expected that the results could give support to propose a possible new vaccine against this disease.

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Characterization of a gene involved in the modulation of the SOS response in *Pseudomonas aeruginosa*

Marina Rocha Borges da Fonseca[1]; Marco Antonio de Lima Noronha[1]; Rodrigo da Silva Galhardo[1].

[1]Departmento de Microbiologia, Instituto de Ciências Biomédicas, Universidade de São Paulo, São Paulo, SP, Brasil.

marinarbfonseca@gmail.com

Keywords: SOS response; *Pseudomonas aeruginosa*; Regulation.

Constant exposure to low concentrations of antibiotics is an important factor in the selection of antimicrobial resistant strains. In addition, some antibiotics can induce genetic variation through the generation of DNA damage, which activate an emergency response,referredtoastheSOSresponse.ThissystemisregulatedbytheLexArepressor autolysis after the binding of RecA protein to single strands of DNA during replication fork blockage. The SOS response promotes events such as increased rate of homologous recombination, expression of error-prone DNA polymerases, inhibition of cell division. In *Pseudomonas aeruginosa* the SOS response directly regulates 15 genes, which seems little when compared to the over 40 LexA-regulated genes in *Escherichia coli*. Despite this difference, the primordial functions of the SOS response are contemplated in *P. aeruginosa*,however,someofthe15genesremainwithunknownfunction. Homologues of the PA0922 gene, regulated by the LexA repressor, are noted as transcriptional regulatorsandasaDNArepairgene. FromtheconstructionofaPA0922deletionmutant in PAO1, we were able to hypothesize its function, based on the mutant phenotype. The mutantPA0922showssensitivitytogenotoxicagents,andwhenoverexpressed,thegene disrupts bacterial growth. Transcriptomic analysis showed that the PA0922 mutant does not express LexA regulated genes in the same level as the wild type PAO1, whentreated with ciprofloxacin, an antibiotic known to activate de SOS response. In general, the PA0922geneappearstointerferewithSOSresponseexpressionandmodulation. Further experiments will be performed to investigate and define the PA0922 genefunction.

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IDENTIFICATION AND CHARACTERIZATION OF HUL5 AND UBP6 PROTEINS AND ANALYSIS OF GENE EXPRESSION IN RESPONSE TO THERMAL AND OXIDATIVE STRESS IN THE FUNGUS *Aspergillus fumigatus*

Fernanda Farisco[1]; Juliana da Silva Viana[1]; Natália Silva de Trindade[1,2]; Matheus de Souza Gomes[1,2]; Enyara Rezende Morais[1,2].

[1]Universidade Federal de Uberlândia – Instituto de Biotecnologia (IBTEC/ UFU Campus Patos de Minas). [2]Programa de Pós-graduação em Biotecnologia (PPGBIOTEC).

*fernandafarisco@ufu.br

Key words: *Aspergillus fumigatus*; ubiquitin-proteasome system; gene expression.

Aspergillus fumigatus is a saprophytic and opportunistic fungus responsible for causing serious diseases in humans as pulmonary invasive aspergillosis. The UbiquitinProteasome system is the main responsible for protein turnover in eukaryotic organisms, proteins such as ubiquitin ligase Hul5 and deubiquitinase UBP6 act during this process. Eukaryotic cells are exposed to various types of stresses during their life cycle; these to survive develop various defense mechanisms. Due to the lack of specific and effective treatments for aspergillosis and the high virulence of the fungus, the objective of this work was to identify and characterize the Hul5 and Ubp6 proteins in A. fumigatus and to understand how the genes encoding these proteins are expressed during thermal and oxidative stress. Bioinformatics tools were used to predict the protein sequences. The oxidative stress evaluation was performed through the measurement of lipid oxidation and the analysis of gene expression was performed by real-time PCR for the different stress conditions. The proteins Afu1g04210 (Hul5 homologue) and Afu6g02380 (Ubp6 homolog) were identified, it was also possible to observe the presence of conserved domains and catalytic residues HECT and HECTc for Hul5 and UCH, ubiquitin and Peptdase_C19A for Ubp6 respectively, both results prove the functionality of the predicted enzymes. Analysis of the gene expression of both HUL5 and UBP6 genes showed that they were inhibited during the stress situations proposed in the study. Despite the results obtained, more studies should be done to better understand this pathway.

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MITOCHONDRIAL GENETIC PROFILE OF YORUBA AND IGBO POPULATIONS FROM NIGERIA

Masinda Nguidi[1]*; Beatriz Martínez[2]; Laura Catelli[3]; Carlos Vullo[3]; Victoria O. Okolie[4]; Samuel O. Keshinro[5]; Elizeu F. Carvalho[1]; Leonor Gusmão[1]; Filipa Simão[1]

[1]DNA Diagnostic Laboratory (LDD), State University of Rio de Janeiro (UERJ), Brazil. [2]Molecular Genetics Lab, Institute for Immunological Research, University of Cartagena, Cartagena, Colombia. [3]DNA Forensic Laboratory, Argentinean Forensic Anthropology Team (EAAF), Córdoba, Argentina. [4]Molecular Biology Research Laboratory, Lagos University Teaching Hospital (LUTH), Lagos, Nigeria. [5]FCIID Annex, Nigeria Police Force, Lagos, Nigeria.

*masi.nguidi@gmail.com

Palavras-chave: Nigeria; mitochondrial DNA; control region;

Africa is the place of origin of modern human and is the most diverse continent in the world, culturally and genetically. Therefore, more and more studies are performed on its populations, comprising 54 countries and 2000 ethnic-linguistic groups. A high amount of the African diversity can be found in Nigeria, known as the "Giant of Africa", the most populated country in the continent with at least 250 distinct ethnic-linguistic groups. The mitochondrial DNA molecule (mtDNA) has particularities that make it useful for evolutionary studies, population genetics and forensics. Namely, its maternally inheritance with no recombination, its high mutation rates and high copy number per cell. For a better understanding of the genetic composition of Nigerian tribes, the analysis of mtDNA control region was used to trace the maternal ancestry of the Yoruba and Igbo ethnic groups, to evaluate intra and inter-population genetic variation. Furthermore, the goal was also to contribute to the elaboration of a database, that is, at the moment, not representative for African populations, which will be available on EMPOP platform, a mtDNA database for forensic purposes. For this study, 155 samples were collected from two tribes of Nigeria: Yoruba (105 samples) and Igbo (50 samples). Samples were sequenced by the Traditional Methodology of Sanger. The total mtDNA control region analyze resulted in 146 unique haplotypes (94.2%). The haplotype diversities were 0.9967 ± 0.0048 for the Yoruba sample and 0.9991 ± 0.0015 for Igbo. The majority of haplotypes belong to macrohaplogroup L, which is characteristic of populations from Sub-Saharan Africa. Were also found, at very low percentages, two haplogroups from Eurasia and North Africa. F_{st} genetic distances were calculated, including previously publish data from Central-West Africa populations. No statistically significant differences were found between Yoruba and Igbo, as observed by the low F_{st} and high differentiation probability values (F_{st} = - 0.00221, p = 0.60133). These two groups showed a high proximity to the Nigerian samples of Esan and Yoruba, from 1000 Genome Database, and to the geographically close populations of Ghana, Niger and Chad. In summary, similarly to other populations belonging to West Africa region, a high heterogeneity was found among the Nigerian populations, as well as a relatively high homogeneity with neighboring populations, most probably due to continuous gene flow and ethnic affinities among them.

CHARACTHERIZATION OF A LUNG CANCER MODEL KNOCKOUT FOR STAT3 GENERATED BY CRISPR/CAS9 SYSTEM AND ITS ASSOCIATION WITH CISPLATIN CHEMORESISTANCE

Ana Paula Morelli[1], Isadora Carolina Betim Pavan[1,2], Mariana Rosolen Tavares[1], Luiz Guilherme Salvino da Silva[1], Nathalie Fortes Pestana[1], Tharcísio Citrângulo Tortelli Jr.[3], Roger Chammas[3], Fernando Moreira Simabuco*[1].

Laboratório Multidisciplinar em Alimentos e Saúde (LabMAS), Faculdade de Ciências Aplicadas, Universidade Estadual de Campinas (FCA-UNICAMP), Limeira, São Paulo, Brazil. Laboratório de Mecanismos de Sinalização, Faculdade de Ciências Farmacêuticas, Universidade Estadual de Campinas (FCF- UNICAMP), Campinas, São Paulo, Brazil. Instituto do Câncer do Estado de São Paulo (ICESP), Faculdade de Medicina da Universidade de São Paulo (FMUSP), São Paulo, Brazil.

Palavras-chave: NSCLC, cisplatin, CRISPR/Cas9.

Lung cancer is the most frequent cancer and the leading cause of cancer death in the world among men, followed by prostate and colorectal cancer (for incidence) and liver and stomach cancer (for mortality). Lung cancer is strongly associated with chemoresistance to platinum compounds, as cisplatin, which corroborates with poor prognosis and 5-years survival rate. The common strategies used to improve the sensitivity are the combination with other treatments, such as radiotherapy, antibodies, selective inhibitors or drugs already prescribed. Metformin is widely associated with a decreased cancer incidence risk and in lung cancer cells metformin decreases cell proliferation in combination with cisplatin. In a previous study we showed that STAT3 is increased in cisplatin resistant cells, which is followed by increased mTOR signaling. STAT3, a transcription factor member of JAK pathway, has been constantly found activated in many types of cancer and associated with poor prognosis and 5-years survival rate. STAT3 is largely described as an oncogene and STAT3 persistent signaling leads to tumor progression. Studies reported that inhibition of STAT3 signaling may induce apoptosis and sensitize cancer cells to DNA damage agents such as cisplatin and doxorubicin. Here we report that rapamycin, an inhibitor of the mTOR pathway, in ctrl and cisplatin-resistant cells, reduces viability, colony formation and sensitizes cells to new cisplatin exposures. We hypothesize that STAT3 sensitizes cells to cisplatin and potentiates effects of sensitizing agents, such as rapamycin and metformin. The A549 STAT3 knockout cell line has been established using CRISPR/Cas9 system and western blotting assay shows an inhibition of the mTOR pathway after metformin treatment in these cells. Viability assays, colony formation, migration and cell cycle will be performed to analyze the effects of STAT3 knockout in A549 lung cancer cells, highlighting new information for the crosstalk between STAT3 and mTOR signaling pathways and potentially new therapeutic strategies for lung cancer.

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Directed Evolution of *Pseudomonas aeruginosa* in Continuous Culture

Vinicius Sousa Flores[1], Beny Spira[1], Luiz Gustavo Almeida[1]

[1]Departamento de Microbiologia, Universidade de São Paulo – Instituto de Ciências Biomédicas.

vinicius.flores@usp.br

Key-words: Pseudomonas aeruginosa; Chemostat; Phosphate.

Pseudomonas aeruginosa is a *Gammaproteobacteria* present in several environments. Phosphorus is an important element for the constitution and metabolism of all the organisms. The bacterial genome encodes several proteins involved in the uptake and metabolism of phosphate (Pi), collectively known as PHO regulon. Low concentrations of Pi activate the PHO regulon genes, like the alkaline phosphatase (PhoA), the uptake system Pst and the two component system PhoB-R. Two important pigments of *P. aeruginosa* is the siderophore pyoverdine and the antimicrobial pigment pyocyanin, where both are controlled by *quorum sensing*. The continuous culture in Chemostat is a method based on constant renovation of the culture medium. A constant dilution of the culture and the limitation in one nutrient create a selection pressure which favor the emergence of mutations for the relief of nutritional stress. The purpose of this project is to study the evolution of *P. aeruginosa* under phosphate limitation and to isolate bacteria with increased uptake of Pi through the culture-directed evolution in Chemostat. A long-term culture in Chemostat was conducted for 14 days. A increase in the activity of alkaline phosphatase, production of pyoverdine and pyocyanin was observed. Bacteria isolated from the last day of the experiment show great variation for the activity of alkaline phosphatase and the uptake of Pi. A long-term experiment in Chemostat favors the emergence of a diversity of phenotypes for the activity of the alkaline phosphatase, uptake of Pi and production of pyoverdine and pyocyanin in *P. aeruginosa*.

Funding Agency: FAPESP.

Aire gene regulates the expression of molecules related to antigen presentation in medullary thymic epithelial cells

Letícia Agrelli de Brito [1], Max Jordan Duarte [1], Geraldo A. Passos [1,2], Eduardo A. Donadi [3]

[1]Grupo de Imunogenética Molecular, Departamento de Genética da FMRP, USP. [2]Departamento de Biologia Básica e Oral, FORP, USP. [3]Departamento de Clínica Médica, FMRP, USP.

leticia.brito@usp.br; passos@usp.br

Keywords: Aire; antigen presentation; Crispr-Cas9.

The autoimmune regulator (Aire) gene is a non-classical transcription factor implicated in the central immune tolerance that occurs within the thymus. It induces the expression of peripheral self-antigens (PTAs) in medullary thymic epithelial cells (mTECs) which in turn present these PTAs to developing thymocytes through peptide-MHC complex expressed on their membrane surface so that the thymocyte maturation process can occur in the thymus. During the negative selection process, thymocytes that recognize PTAs with high affinity die by apoptosis, whereas those that bind with low affinity survive. The antigen presentation pathways are therefore extremely important for proper discrimination between self and non-self-antigens. In this work, we ask on the influence of Aire on the modulation of downstream genes encoding antigen presentation molecules in mTECs. For this, we used data generated by microarray RNA (cDNA) hybridizations from samples of Aire wild-type mTECs (Aire $^{+/+}$ WT) or mutant Aire mTECs (mTEC Aire $^{-/-}$ KO, clone 3.10E6, generated by the Crispr-Cas9 system) that were co-cultured with thymocytes establishing mTEC-thymocyte adhesion. The bioinformatics analyzes of data in R statistical environment revealed differential expression of 22 genes involved with antigen presentation in mTECs, including alleles of Type I Major Histocompatibility Complex (MHC I and MHC II) and CCl2, Cox2, H2Q7 and CxCl5 genes. As these genes were downregulated in Aire $^{-/-}$ KO (clone mTEC 3.10E6) during its thymocyte adhesion, it suggests that Aire modulates antigen presentation process in mTEC cells.

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INFLAMMATORY MARKERS IN DOWN SYNDROME: microRNAs AND GENE EXPRESSION PATTERN

Olívia Borghi Nascimento[1]; Marlon Fraga Mattos[1]; Victor Miranda Henandes[1]; Geraldo Aleixo da Silva Passos Junior[2]; Joice Matos Biselli[3]; Eny Maria Goloni, Bertollo[1] e Érika Cristina Pavarino[1].

[1]Unidade de Pesquisa em Genética e Biologia Molecular da Faculdade de Medicina de São José do Rio Preto – FAMERP. [2]Faculdade de Medicina de Ribeirão Preto da Universidade de São Paulo – USP. [3]Universidade Estadual Paulista Júlio de Mesquita Filho- UNESP.

olivia-borghi@hotmail.com

Key-words: Down syndrome, microRNA and expression gene

The intrinsically deficient immune system in Down syndrome (DS) may contribute to the increased susceptibility to infections, one of main causes of hospitalization and death of DS individuals. Trisomy 21 can cause global changes of gene expression, including those not located on chromosome 21, which regulate immune/inflammatory processes and other physiological mechanisms. Thus, knowledge of the expression profile of genes that regulate inflammatory processes and microRNAs that possibly regulate these genes may provide information concerning the molecular basis of clinical manifestations of the syndrome. Previously, we identified six microRNAs and 37 immune-related genes differentially expressed in DS individuals. In the present study, we searched for potential target genes of microRNAs among differentially the expressed genes and evaluated the expression of these predicted target genes in a larger sample of DS patients and controls. MicroRNA and gene expression analyses were previously performed by quantitative real-time PCR (qPCR) in six SD and six non- SD healthy individuals. miRNA target prediction was performed using the online database DIANA-MicroT-CDS v.5.0 (diana.imis.athena-innovation.gr) considering as cut-off value (treshlod) the score 0.8. To confirm the differential expression of the miRNA target genes predicted by the *in silico* analysis, RNA samples were obtained from peripheral blood mononuclear cells (PBMC) of 23 healthy individuals with SD and 21 individuals without the syndrome. Expression of CCR7, IKBKB and PLA2G2D genes was analyzed by qPCR using Taqman assays in the StepOnePlus Real-Time PCR system (Applied Biosystems). Statistical analysis was performed using the one- sample Wilcoxon signed rank test in the GraphPad Prism 6 software. The group of individuals without SD was used as reference. *In silico* analysis showed association between three microRNAs and three immune-related genes previously identified as differentially expressed in DS: miR-378a-3p (↑) - CCR7 (↓); miR-942-5p (↑) - IKBKB (↓); miR-668-3p (↓) - PLA2G2D (↑). This association was consistent with the microRNA and gene expression pattern of our previous study. Validation of the immune-related genes expression in our larger sample confirmed that individuals with DS present lower expression of IKBKB and CCR7 genes (median RQ = 0.26 and 0.44, respectively, values of P <0.0001) as compared with individuals without the syndrome. However, PLA2G2D was significantly down regulated in the present DS group (mean RQ=0.045, P value= <0.0001). This low expression, unexpected, may be explicated due to larger number of individuals analyzed in the present study, which provide a higher statistical power. Our results reinforce the hypothesis that trisomy 21 leads to secondary alterations in the expression of genes located throughout the genome. In conclusion, we show that DS individuals exhibit differential expression of CCR7, IKBKB and PLA2G2D genes, which may contribute to the deficiency of immunological and inflammatory processes in DS.

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FUNCTIONAL STUDIES OF NEW SMALL ORFS DURING *TRIBOLIUM CASTANEUM* DEVELOPMENT

Alessandra da Silva de Alvarenga[1], Giovana Carvalho Candido[1], Caren Santos Martins[1], Júlia Vitorino de Souza[1], Carlos Eduardo da Silva Pereira[1], Diego Guerra de Almeida[1], Lupis Ribeiro Gomes Neto[1], Rodrigo Nunes da Fonseca[1]

[1]Laboratório Integrado de Ciências Morfofuncionais, Universidade Federal do Rio de Janeiro, Instituto de Biodiversidade e Sustentabilidade, Macaé, Rio de Janeiro, Brazil.

alvarenga.sa.bio@gmail.com

Key-words: gene; *Tribolium castaneum*; expression

Search for alternatives to chemical insecticides as control agents has been carried out over the past years. Research in the area of Evolutionary Developmental Biology (EVO-DEVO) has shown great potential in revealing proteins, molecular mechanisms and signaling pathways that can be evaluated as targets in the development of new approaches to control insect vectors of disease or agricultural pests. Several studies of EVO-DEVO have been carried out in recent years with the Diptera model *Drosophila melanogaster* and with the flour beetle *Tribolium castaneum*. Genes encoding small open Reading Frames (smORFs) belong to a new gene class possibly essential for various processes during insect development. Genes with smORFs contain less than 100 translatable codons, a typical structure of non-coding RNAs, and can encode for more than one peptide in a single messenger RNA sequence, called polycistronic messenger RNA. A gene containing polycistronic smORFs was identified in the *T. castaneum* beetle *mille-pattes (mlpt)* since its silencing generate a beetle larva with multiple legs. The goal of this work is to analyze the function of new smORFs in the development of the beetle *T. castaneum*. Eggs will be collected and total RNA isolated and complementary DNA synthesis (cDNA) for conventional RT-PCR was performed. The quantitative analysis of the gene expression in real time (qRT-PCR) will also be performed, which will aim to identify at what time of development the new smORFs are expressed. In order to identify the region of the embryo in which a given gene is expressed, the *in situ* hybridization technique will be performed on fixed eggs of different stages. Gene silencing by interfering RNA will be performed with double- stranded RNA injection in adult females of *T. castaneum.* Preliminary results showed that in *T. castaneum* the smORF smallTc35 displays maternal expression in the dorsal region of the egg; the smORF smallTc112 presented expression only in embryos of late stage of embryogenesis and smORF smallTc6 showed typical staining of the mesoderm during the gastrulation period, between 8 and 11 hours of development. Together, these results point to a possible important role of these genes encoding smORFs for development and as targets for vector control and agricultural pest control mechanisms.

Funding Agency: CAPES, CNPq, FAPERJ

EXPLORING THE PROCESS OF HOST REGULATION ON *SPODOPTERA FRUGIPERDA* FOR THE DEVELOPMENT OF ECDYSONE OXIDASE INTERFERENCE RNA

Eloísa Torrezan[1]; Fernando L. Cônsoli[1].

[1] Universidade de São Paulo - Escola Superior de Agricultura "Luiz de Queiroz", Departamento de Entomologia e Acarologia, Laboratório de Interações em Insetos, Av Pádua Dias, nº 11, Piracicaba, SP, Brasil.

eloisa.torrezan@usp.br

Key words: RNAi; Host Regulation; Pest Management.

RNAi technology has a great potential in applied pest control and can be integrated to other control tactics in sustainable pest management strategies. This project aimed to explore the mechanism of host regulation by parasitoids for the selection and testing of candidate genes based on the effects of their regulation on host metabolism, growth and development. We selected the gene encoding for the enzyme ecdysone oxidase to produce the double strand RNA to silence this gene in *Spodoptera frugiperda*. To produce it, we designed specific primers based on sequences available from host regulation studies in our group. Total RNA was extracted from randomly selected insects from a laboratory population of *S. frugiperda*. We produced the cDNA from RNA samples using a commercial reverse transcriptase kit. PCRs were carried out to amplify the gene of interest, isolate and purify this product; the dsRNA was synthetized using MEGAScript. GFP dsRNA was used as a control treatment. We tested the synthesized dsRNA against larvae of S. frugiperda using two different strategies to deliver the dsRNA, and sampled larvae at 12, 24 and 72 h post-application to investigated changes in gene expression, and allowed the treated larvae to develop up to the adult stage in order to observe phenotypic changes. Our data support the use of RNAi as an investigative tool of physiological processes of this important pest.

Funding Agency: FAPESP.

miRNOME PROFILING IN COLORECTAL CANCER: A SEARCH FOR POTENTIAL BIOMARKERS

Diego Marques[1,2], Layse R Ferreira-Costa[2], Lorenna L Ferreira-Costa[2], Ana BB de Oliveira[2], Romualdo da S Correa[3], Gloria TV Sandoval[1], Katia P Lopes[1], Ricardo V Assunção[1], Amanda F Vidal[1], Vivian N Silbiger[2], Ândrea Ribeiro-dos-Santos[1,4]

[1]Laboratório de Genética Humana e Médica, Programa de Pós-Graduação em Genética e Biologia Molecular, Universidade Federal do Pará, Belém-PA. [2]Laboratório de Bioanálise e Biotecnologia Molecular, Universidade Federal do Rio Grande do Norte, Natal-RN. [3]Departamento de Cirurgia Oncológica, Liga Norte Riograndense Contra o Câncer, Natal-RN. [4]Programa de Pós-Graduação em Oncologia e Ciências Médicas – Núcleo de Pesquisas em Oncologia (NPO).

diegomarquescs@outlook.com

Keywords: Colorectal cancer; miRNome; biomarker.

Colorectal cancer (CRC) is the third most common cancer worldwide. Several genetic and epigenetic alterations are related with its development and progression, being one of those the deregulated microRNA (miRNA) expression profile. miRNAs are small non- coding RNAs that negatively regulate the expression of thousands of genes, impacting on cell proliferation, development, differentiation and apoptosis. In this present study, we aimed to identify differentially expressed miRNAs (DEmiRNAs) in CRC patients that may be potential biomarkers of this disease. We collected 21 tissues samples (Tumor = 7; Adjacent = 7; Control = 7). Then, we performed small RNA sequencing using an Illumina Next Generation Sequencing (NGS) platform. Differential expression analysis identified 20 DEmiRNAs between the three groups of samples. Based on the DEmiRNAs, we evaluated their expression profile according to the tumor staging and found ten overexpressed and four down expressed miRNAs in advanced stage tumor (III and IV) in comparison with stages I and II. We observed that six of these miRNAs also presented an aberrant expression in adjacent tumor tissue. Considering that the functional role of the miRNAs is context and tissue-dependent, it is possible that this aberrant expression in adjacent tissue may be a response in order to combat cell proliferation (*hsa-miR-133a- 3p, hsa-miR-145-3p, hsa-miR-378a-5p*, and *hsa-miR-125b-5p*) and a reflection of the inflammatory processes *(hsa-miR-1248*) occurring in the cellular environment. Therefore, the miRNAs seen to be involved in several cancer-related processes. Our findings contribute to the understanding of the molecular pathways involved in the development of CRC, and eventually may allow the application of the miRNAs as biomarkers in clinical practice. Moreover, this study corroborated with field-effect theory, which demonstrated that histologically normal tissue adjacent to the tumor might have expression profile influenced by the tumor, reinforcing the strand of studies that do not recommend the use of this tissue as a reference for molecular studies.

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APPLICATION OF PHYLOGENOMIC TOOLS FOR NOROVIRUS ANALYSIS.

Dielle Monteiro Teixeira[1]; Luciana Damascena da Silva[1]; Hugo Reis Resque[1]; Yvone B. Gabbay[1]; Rafael Azevedo Baraúna[2]

[1]Seção de Virologia, Instituto Evandro Chagas (IEC). [2]Centro de Genômica e Biologia da Sistemas, Universidade Federal do Pará (UFPA).

Key-words: norovirus; genogroups; phylogenomic.

Introduction: Human norovirus (NoV) are the main etiological agents involved in outbreaks of non-bacterial gastroenteritis worldwide. They are genetically diverse and have evolved into genogroups, genotypes and lineages, mainly due to the accumulation of mutations generated from viral replication failures in the infected host and genetic recombination between related strains within the same genogroup. These events have generated classification conflicts, mainly during the emergence of new strains and lineages. **Objectives**: To perform phylogenomic analysis of NoV using computational biology tools, based on complete genomic sequences obtained from a public database (NCBI), and then compare them with the phylogenetic analyzes based only the sequence of a single coding region (CDS-VP1 protein) already described in the literature. **Methodology**: NoV strains sequences (GI to GVI genogroups) were obtained from the NCBI nucleotide database. The sequences in the FASTA format were obtained In the Artemis program and submitted online to the RAST platform for the gene annotation standardization obtaining sequences in the EMBL format. The FASTA sequences were used for the comparative genomic ring construction in the BRIG program and to obtain the dissimilarity matrix produced in the Gegenees program (version 2.2.1) where the file was generated in Nexus format which was visualized in the program SplitsTree (version 4.14.4) in the form of a phylogenomic tree. For SNP analysis, genome files in the EMBL format were submitted to the PanWeb platform, which performed the pan-genomic analyzes based on the PGAP pipeline. For this, only GII sequences (genotype GII.4) were selected because it is the most predominant in the world and evolves in variants every two to three years, some of which are pandemic. **Results and Discussion**: In the comparative ring analysis it was observed that the six genogroups genomes evaluated showed identity varying between 100 and 50%, with the intervals presence with 0% of identity mainly between strains inter-genogroups. In the clustering analysis performed with UPGMA algorithm, a wide distinction was observed between the GI and GVI genogroups, which were organized in well defined clusters that comprised all genotypes described for each genogroup analyzed. By the SNP analysis of the GII.4 strains using the maximum likelihood method, there were three distinct and divergent clusters of a common ancestor of 2008 (Alperdoon and Osaka variants). The great advance in the area of genomics, especially with the arrival of new generation sequencers (NGS) could contribute with the development of new investigations aiming at a more detailed study of NoV genome and its epidemiological and Public Health implications, besides elucidate a more comprehensive classification, when the emergence of new strains of this genetically diverse virus. **Conclusions**: Further studies involving analysis of the complete NoV genome are needed to describe the phylogeny of emerging strains and unusual genotypes.

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DENTIFICATION OF DEVELOPMENT BONE GENES IN TAMBAQUI (*Colossoma macropomum*)

José Dirley Mendes Alborado[1]; Alex Alberto Vela Facundo[1]; Gilvan da Costa Ramos[2]; Vanessa Tourinho da Costa[1]; José de Ribamar da Silva Nunes[1,2] *

[1]Instituto de Natureza e Cultura – Universidade Federal do Amazonas. [2]Programa de Pós-graduação em Ciência Animal – Universidade Federal do Amazonas.

*ribamarnunes@ufam.edu.br

Key-words: Alignment; skeletal disorders; GBS.

The bone formation is associated to important aspects to fish aquaculture, such as growth factors, skeletal disorders, swimming disorders and nutrition. Some bone disorders are often a complex mixture, including vertebral and spinal malformations associated to genetics and nutritional factors. In some fish hatcheries, neck deformities, and vertebral and spinal disorders are abundant. The challenge in the study of the bone development is to identify the genetic process involved in bone formation. Bone and cartilages may develop during embryonic, larval, juvenile when the expression of development bone genes is most common, or in adult stages under normal ontogeny as well as during pathological states, wound repair and bone regeneration. The knowledge genetic control of bone formation and regulation can be a key to control of skeletal diseases. The understanding of these process can give us information to select specific markers to be applied in breeding program, and to plan effective strategies for genetic improvement. In this study we carried out on Genotyping by Sequencing (GBS) libraries obtained from 424 farmed and wild tambaqui (*Colossoma macropomum*) from three different Brazilian regions to SNPs discovery. We perform a functional annotation in BLAST using 65,4980 nucleotides sequences of bone development from GenBank and RefSeq data base and Magablast Algorithm. A total of 182,416 GBS sequences was used to the alignment. Using these associated loci, we also carried out a positional annotation using Bowtie 2.0 and VEP program and zebrafish genome (*Danio rerio*) data base. The BLAST generated 103 alignments in 31 species. With positional annotation we observed that nonsense mutations are common in the tambaqui genome in relation to the zebrafish genome.

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INFLUENCE OF PHARMACOGENETIC MARKERS IN THE TUBERCULOSIS THERAPEUTIC RESPONSE: A PRELIMINARY RESULTS

Cecilia Alvim Dutra[1]; Afrânio Lineu Kritski[1]; Elisângela Costa da Silva[1]; Marize Quinhones Pires[2]; Márcia Quinhones Pires Lopes[2]; Philip Noel Suffys[2]; Raquel Lima de Figueiredo Teixeira[2]; Adalberto Rezende Santos[2]

[1]Instituto de Doenças do Tórax, Hospital Universitário Clementino Fraga Filho, Complexo Hospitalar, Faculdade de Medicina, Universidade Federal do Rio de Janeiro, Rio de Janeiro, RJ, Brasil [2]Laboratório de Biologia Molecular Aplicada a Micobactérias, Instituto Oswaldo Cruz-Fiocruz, Rio de Janeiro, RJ, Brasil

cecilialvim@hotmail.com

Key-words: tuberculosis; polymorphisms; pharmacokinetic.

Single nucleotide polymorphisms (SNPs) present in genes involved in the pharmacokinetics of first-line drugs administered during treatment of tuberculosis including *NAT2* and *CYP2E1* (isoniazid metabolism), *SLCO1B1*, *SLCO1B3* and *ABCB1* (rifampicin transport) affect the exposure of these drugs in plasma and its toxicity, influencing the therapeutic outcomes and compromising the safety and efficacy of the treatment. The primary objective of this project is to identify patients with a higher risk of failure/relapse associated to the variability of the above mentioned genes. After written consent, a cohort of 96 patients from the Health Unit of Caxias (RJ) was enrolled and genomic DNA was obtained through the extraction of whole blood, followed by PCR and sequencing. To date, three single nucleotide polymorphisms (SNPs) were identified in the coding region of the *ABCB1* gene: C3435T; T3548G and C3569T, and eight SNPs in the coding region of the *NAT2* gene: G191A, C282T, T341C, C481T, C578T, G590A, A803G and G857A. The acetylation phenotype of individuals characterized by the presence of polymorphisms in the *NAT2* gene interferes in the serum levels of isoniazid during the treatment of tuberculosis and is associated with hepatotoxicity, as well as polymorphisms in the *CYP2E1* gene, besides therapeutic failure or delay in response to treatment or reactions adverse effects. Data from the literature show that polymorphisms in the *SLCO1B1*, *SLCO1B3* and *ABCB1* genes are associated with the serum concentration of rifampicin leading to a decrease of up to 28%. In our evaluation of *ABCB1*, two of the SNPs found have no description in the literature yet and can be considered new SNPs. Although their functional role are not established yet, our preliminary results show important new data to be considered in the whole final context of association with therapeutic outcomes. The second part of our project will include the genotyping of *SLCO1B1*, *SLCO1B3* and *CYP2E1* in the cohort and the carrying out of an association study of the association between the genetic data obtained versus the socio-demographic, laboratorial and clinical data having as main outcome the culture positivity for *M. tuberculosis* 2 months after starting treatment. In view of the high failure rate in tuberculosis treatment in Brazil, early tuberculosis failure/relapse predictors are needed to optimize and improve treatment efficacy.

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THE USE OF BARCODE DNA AND CYTOGENETICS AS A TOOL FOR MOLECULAR IDENTIFICATION OF NEOTROPICAL ANURANS

Renata Furtado[1]*; Rodrigo Petry[1]; Marcelo Vallinoto[1]; Iracilda Sampaio[2]

[1]Laboratório de Evolução, Universidade Federal do Pará - Campus Bragança. [2]Laboratório de Genética e Biologia Molecular, Universidade Federal do Pará - Campus Bragança.

*renat4furtador@gmail.com

Keywords: Phylogeny; Scinaxcinae; chromosomes.

The subfamily Scinaxcinae corresponds to a newly created group to encompass the genus *Scinax*, *Julianus*, *Ololygon* and *Sphaenorhynchus*. The numbers of Scinaxcinae's species are highly underestimated, and additionally there is difficulty in identification of these species, and the lack of many distribution data of some species of this group. Considering the great diversity in Scinaxcinae, and its wide distribution, we performed a DNA barcode analysis and associated these to cytogenetic data, in order to evaluate the phylogenetic relationship of this subfamily. The cytogenetic data of Scinaxcinae were obtained by bibliographic review, in which the diploid numbers and chromosome morphologies of the species were analyzed. The database was consisted by 428 bp of 16S rDNA sequences of 51 species of Scinaxcinae. The results of the maximum likelihood analysis grouped *Scinax* and *Julianus* as a single group, sister group of *Ololygon*, while *Sphaenorhynchus* was most basal. The cytogenetic data were obtained of 46 species, in which we observed the predominance of 2n = 24, with NF = 48, except *Sphaenorhynchus dorisae* and *S. carneus* of 2n = 26 and NF = 52, all bi- armed chromosomes. In addition, we observed that the morphology of first two chromosome pairs of the species are potentially a cytotaxonomic marker for Scinaxcinae. Based on the results, the phylogeny obtained from 16S rDNA were not decisive for the taxonomic status between *Scinax* and *Julianus* because of low divergence of this gene. However, this data is important to pointed out that *Ololygon* and *Sphaenorhynchus* is a monophyletic group, corroborating with data described in other studies. Cytogenetic analysis showed that Scinaxcinae has a conserved karyotype (2n = 24), resulting from chromosomal fusions of 2n = 26 to 2n = 24. Variations on first two chromosome pairs between the genera of this subfamily is important to resolve some questions, as *Scinax* and *Julianus* present first two pairs with metacentric chromosomes and *Ololygon* submetacentric, while on *Sphaenorhynchus* both patterns were observed. We conclude that the implementation of more molecular markers may be useful in understanding the phylogeny of this subfamily; in addition, the observed cytogenetic data are potential cytotaxonomic markers at genus level, while other techniques, such as FISH and new markers, may be important to be used at species level.

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MOLECULAR EVIDENCE OF A NEW SPECIES OF Erotelis (GOBIIFORMES: ELEOTRIDAE) IN THE NORTHERN COAST OF BRAZIL

Wanny Pâmela Gomes de Lima[1*]; Tomasso Giarrizzo[2]; Fabíola Machado[2]; Iracilda Sampaio[1]; Aurycéia Guimarães-Costa[1]

[1]Instituto de Estudos Costeiros, Universidade Federal do Pará, Alameda Leandro Ribeiro, Bragança, 68600000, PA, Brasil. [2]Núcleo de Ecologia Aquática e Pesca da Amazônia, Universidade Federal do Pará, Av. Perimetral 2561, Terra Firme, Belém, 66040-170, PA, Brasil.

*wanny.gomes@hotmail.com

Key-words: Fish; Neotropical realm; COI.

In our study on fish composition of the Parque Nacional de Jericoacoara (PNJ), we captured two *Erotelis* specimens, which were submitted to molecular identification. This genus includes four valid species distributed in the Neotropical region: 1) *Erotelis smaragdus* (Western Atlantic), *Erotelis armiger* (East Pacific), *Eroletis clarki* and *Erotelis shorpshirei* (endemic in Panama). For the identification of the specimens, we generated sequences of 650 bp of the COI gene, which were compared to the sequences of other neotropical eleotrids in a database composed of 48 species previously collected, with the exception of *Erotelis clarki* and *E. shorpshirei*. The sequences were edited, and phylogenetic analysis of Bayesian Inference (IB) and nucleotide divergence (Neighbor Joining tree and distance matrix) were performed. After the analysis, it was verified that the topologies of IB and NJ recovered a clade of *Erotelis* formed by three different and delimited lineages: two of them formed by the valid species *E. smaragdus* and *E. armiger*, and a third formed by the specimen captured in the PNJ. The means of nucleotide divergences among the lineages were relatively high, varying from 14.9% to 17.5%, showing unequivocally the existence of an unknown *Erotelis* species for the region. The DNA barcoding proved to be effective in showing clear separation between recognized species and distinct lineage of *Erotelis* from northeastern Brazil. When we explore the molecular diversity of Eleotridae, it is not uncommon to recognize potential new species in the Western Atlantic, since the use of Barcoding DNA has been increasingly helping to delimit these cryptic species, as verified in the discovery of diferente lineages of *Eleotris*, *Gobiomorus dormitor* and *Dormitator maculatus*.

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EVALUATION OF THE MUTAGENIC AND RECOMBINOGENIC EFFECT OF PYRIPROXYFEN® COMPOUND IN SOMATIC CELLS OF *Drosophila melanogaster*

Michely Magalhães Araújo[1]; Rosiane Gomes Silva Oliveira[1]; Matheus Gustavo Soares Santos[1]; Matheus Fernandes da Silva[1]; Juliana Miron Vani[2]; Rodrigo Juliano Oliveira[2].

[1]Laboratório de Citogenética e Mutagênese, Centro Universitário de Patos de Minas – Faculdade de Medicina Veterinária, Patos de Minas, Minas Gerais, Brazil. [2]Universidade Federal do Mato Grosso do Sul, Campo Grande, Mato Grosso do Sul, Brazil.

*michelymagalhaes@unipam.edu.br

Keywords: Pyriproxyfen®; *Drosophila melanogaster*; SMART.

Among the various branches of veterinarian science, precaution is taken with the pathogens disseminated by vectors, thus, the use of insecticides covers the control and prophylaxis of diseases. In this sense, the compound Pyriproxyfen®, physiological insecticide regulator of growth, it presents functionality by contact and translaminar action, especially to the eggs and nymphs, presenting relevant to promote disorder in the hormones, which makes impossible the maturation of the insects and posture of infeasible eggs. Thus, although also used in the human sciences for the control of the vector *Aedes aegypti* (transmitter of Dengue, Chikungunya, Zika and Yellow Fever), the compound Pyriproxyfen® presents factors that are harmful to the biotic and abiotic beings. However, few studies were developed on the presumed effects of the compound Pyriproxyfen® on somatic cells of *Drosophila melanogaster*. This study aimed to evaluate the mutagenic and recombinogenic effect of different concentrations of Pyriproxyfen® compound by the somatic mutation and recombination test (SMART) in *Drosophila melanogaster* tested in individuals from the standard cross (ST). In order to perform the test, the standard cross (ST - Cross), which composes virgin females of the line flare^3 (flr3 / In (3LR) TM3, ri p^p sep I (3) 89Aabx^{34e}Bd^s) and mwh/mwh males were manipulated. From this cross, the descendants with the trans-heterozygous marker (H: mwh + / fl3 +) were characterized as having the phenotypically normal wings, with smooth-border, which come from mutagenic and recombinogenic events. The compound Pyriproxyfen® was supplied by researchers from the Federal University of Mato Grosso do Sul (UFMS). The experiments were carried out in the Laboratory of Cytogenetics and Mutagenesis of UNIPAM. The compound Pyriproxyfen® was diluted in reserve osmosis water to obtain the concentrations 2.5; 1.25 and 0.625 µg/mL. In the present study, doxorubicin hydrochloride (DXR) was used as a positive control, at a concentration of 0.4 mM and as a negative control the reverse osmosis water. The results indicated a significant increase in the total number of spots at the concentration of 2.5 µg / mL when compared to the negative control. The results obtained show that in the highest concentration tested, the compound Pyriproxyfen® showed mutagenic / recombinogenic effect. In addition, the heterozygous balancer (BH: mwh + / TM3, Bds) will be analyzed, which changes only come from mutagenic events to quantify the percentage of mutation and recombination.

ORF1776 coding for a xylose isomerase is related with *Xanthomonas citri* subsp. *citri* pathogenicity

Evandro Luis Prieto*[1], André Vessoni Alexandrino[1], M. Teresa M. Novo-Mansur[1]

[1]Federal University of São Carlos, SP, Laboratory Biochemistry and Applied Molecular Biology- LBBMA

*evandrolprieto@gmail.com

Key words: Citrus canker, *Xanthomonas citri*, xylose isomerase

Citrus canker is one of the most devastating diseases for citrus growing, being caused by the bacterium *Xanthomonas citri* subsp. *citri* (XAC). Proteomic analyses performed previously by our group detected several differentially abundant proteins when XAC was cultured in XAM-M medium, comparatively to *X. fuscans* subsp. *aurantifolii* (Xau-B), the milder agent of cancrosis. One of these proteins identified was xylose isomerase, a bifunctional enzyme that interconverts D-xylose in D-xylulose and D-glucose in D-fructose. The present work aimed to investigate if there is a relationship between the ORF1776 coding for xylose isomerase and the virulence of XAC, since xylose is a constituent of the cell wall of plant hosts and can be used as carbon source during bacterial infection. XAC1776 ORF was deleted by means of a methodology based on the homologous double recombination between XAC genomic DNA and a suicide vector (pNPTS138), in which two fragments of approximately 1 kb corresponding to the upstream and downstream regions to the target gene were cloned in tandem. The deletion confirmation was performed by PCR using oligonucleotides that hybridize in regions adjacent to the flanking fragments. Growth curve of XAC and deleted mutant was performed in xylose-added pathogenicity-inducing medium (XAM-X) and without the presence of this monosaccharide (XAM-M). *In vivo* infection assays were conducted to evaluate the relationship between the deletion of the target gene and the virulence of XAC. The results obtained allow us to infer that the growth of XAC under induction of pathogenicity *in vitro* was not affected by the gene deletion, independently of xylose presence. In the *in vivo* assay, the apparent greater aggressiveness of the deleted mutant strain was verified when compared to the wild strain, which allows concluding that the xylose metabolism is related to XAC virulence.

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EVALUATION OF THE ANTIPROLIFERATIVE ACTIVITY OF BETULINIC ACID TRITERPENE IN TUMOR CELL LINES

Fernanda Santos Fernandes; Nayanne Larissa Cunha; Iara Silva Squarisi; Heloiza Diniz Nicolella; Wilson Roberto Cunha; Denise Crispim Tavares

University of Franca, Franca, São Paulo, Brazil.

ferfernandesss321@gmail.com

Keywords: Betulinic Acid; Cytotoxicity; XTT assay.

Natural products can be exploited for their therapeutic potential due to their various biological activities. Betulinic acid (BA) is a triterpene widely found in the plant kingdom. However, it can be obtained in abundance from the bark of *Betula alba* species known as "vidoeiro". BA has shown several biological activities as anti-inflammatory, antiviral, antibacterial, antimalarial, hepatoprotective, and immunomodulatory. Previous studies have shown that BA exhibits potent antitumor activity in apoptosis resistant neuroblastoma cells. Considering the pharmacological potential of this compound, the present study aimed to evaluate its the cytotoxicity in non-tumor (GM07492-A) and glioblastoma (M059J, U343MG and U251MG) human cell lines using the XTT colorimetric assay. Cell cultures were treated with concentrations of BA ranging from 34.2 to 4379.2 µM. Negative (no treatment), solvent (dimethylsulfoxide, 0.5%) and positive (dimethylsulfoxide, 25%) controls were included. The results showed IC_{50} (50% inhibition of cell viability) of 233.60, 449.80, 463.30 and 467.30 µM for GM0749A, M059J, U343MG and U251MG cell lines, respectively. The observed values did not differ significantly between the lineages. BA presented cytotoxic action in glioblastoma cell lines at concentrations higher than that observed in non-tumor cell line. Therefore, BA showed antiproliferative effect on glioblastoma cell lines, but revealed absence of selectivity, under the experimental conditions used.

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Expression and function of the genes encoding knickkopf during the integument formation in Apis mellifera: Characterization of the isoforms knk1, knk2 and knk3.

Leonardo Nascimento de Paula[1]; Thiago da Silva Depintor[2]; Flávia Cristina de Paula Freitas[3]; Tiago Falcon Lopes[4]; Márcia Maria Gentile Bitondi[1].

[1]Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto (FFCLRP-USP); [2]Faculdade de Medicina de Ribeirão Preto (FMRP-USP); [3]Instituto de Ciências Biomédicas da Universidade Federal de Alfenas (ICB-UNIFAL); [4]Núcleo de Bioinformática, Hospital de Clínicas de Porto Alegre (NBHCPA).

leonardo.nascimento.paula@usp.br

Key-words: knickkopf; exoeskeleton; gene-silencing.

The periodic molts that allow the growth of arthropods culminate in the degradation of the exoskeleton (cuticle) while a larger new one is de *novo* synthesized. The cuticle layer firstly deposited during exoskeleton renewal is the impermeable envelope. It protects the developing exoskeleton against the action of chitinases, which degrade chitin, the main polysaccharide of the cuticle. Recently, this protective action has also been assigned to Knickkopf (Knk), a protein that binds chitin and prevents its degradation by chitinases. The main goal of this project is to test the hypothesis that the expression of the knk paralogues (knk1, knk2 and knk3), which encode proteins in the Knk family (Knk1, Knk2 e Knk3), is fundamental for molting success and formation of the chitinous exoskeleton in A. mellifera. The expression profiles of knk1, knk2 and knk3 in the integument (formed by cuticle and epidermis) were established during the pupal-to-adult molting using RT-qPCR. These profiles based the design of RNA interference (RNAi)-mediated gene silencing experiments for functional studies. Results: Our data show high levels of knk1 transcripts in the integument of pupae and subsequent stages of development, which are marked by apolysis and synthesis of the adult cuticle. The transcript levels then decay, as the cuticle becomes melanized and intensely sclerotized at the end of the pharate-adult period. The lower levels of knk1 transcripts were detected in adult bees that were performing foraging activities. Significantly lower densities of knk2 transcripts were found in the integument of pupae, pupae in apolysis, and adult bees than in pharate-adults and newly-emerged bees. The expression profile of knk3 showed a significant lower expression in pupae than in the other developmental phases. RNAi-mediated knk1 gene-silencing showed specific effects in the abdominal integument, the local of double-stranded RNA injection. The thoracic integument was not affected. Our data highlighted distinct expression profiles for the knk paralogue genes, suggesting that they may differ functionally. The data also suggest that knk1 is functionally related to the molting process and synthesis of the adult cuticle. Gene-silencing mediated by RNAi is also being used for investigation of knk2 and knk3 functions.

Funding Agency: CNPq.

Construction of a genetic regulatory network for signal amplification of riboregulators responses

Rafael Augusto Lopes Franco[1]; Gabriela Barbosa de Paiva[1]; Vitória Fernanda Bertolazzi Zocca[1]; Danielle Biscaro Pedrolli[1].

[1]Departamento de Bioprocessos e Biotecnologia, Universidade Estadual Paulista "Júlio de Mesquita Filho" - Faculdade de Ciências Farmacêuticas, Araraquara, São Paulo, Brazil.

rafael.agostoo@gmail.com

Key-words: RNA switch; viral diagnostic; synthetic biology.

Dengue and Zika are arboviruses, genus Flavivirus, and together represent a risk of infection for billions of people around the world. The main form of virus transmission to humans is through the bite of an infected mosquito of the genus *Aedes*, mainly *Aedes aegypti*. Nowadays, the precise diagnosis of these diseases is made by ELISA and RT- PCR techniques. These procedures need high laboratory resources and the handling of trained professionals, turning the diagnosis expensive and time-consuming. Besides, the laboratory structure required turns the diagnosis impracticable in remote regions of Brazil and other affected tropical countries. This project aims at developing a synthetic DNA circuit for *in vitro* gene expression to amplify the signal of riboregulators. Highly specific riboregulators (RNA switches) previously constructed in the SynBio AQA research group were used to detect Zika and Dengue viral RNA. Individually, the switches were cloned into the plasmid pSB1C3 between the J23119 promoter and the T7 RNA polymerase gene. A second transcription unit was added to the plasmid containing the gene reporter EPIC luciferase under the control of the T7 promoter. Thereby, transcription and translation reactions were performed *in vitro* in the presence of either the specific trigger RNA or a nonspecific trigger RNA. Preliminary tests confirmed the functionality of the network. An activation fold change of 5 times has been achieved, meaning that the activation of the reporter gene expression was 5 times higher in the presence of specific RNA trigger. More tests are planned to adjust reaction parameters such as concentrations and reaction conditions to achieve a higher specific response. Additionally, a second synthetic circuit is in construction using the integrase Bxb1. In this approach, the switches are cloned in the plasmid pSB1C3 between the T7 promoter and the gene *bxb1*, the second transcription unit will be added to the plasmid containing the reporter gene under the control of T7 promoter. Initially, the T7 promoter will be placed in the opposite strand to the reporter gene, surrounded by the attB and attP specific recombination sites for the integrase. Therefore, in the absence of the target viral RNA the gene reporter will not be expressed. However, in the presence of target viral RNA the switch will activate the expression of the *bxb1* gene, which in turn will flip the promoter and activate reporter gene expression. It is expected that the synthetic genetic circuits built can be used in a diagnostic kit that is fast, simple to use, and presents a visual response for the detection of such viral illnesses, with the potential to speed up the diagnosis allowing the appropriate treatment to be applied as soon as possible.

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IDENTIFICATION OF RAYS OF THE SPECIES *Pseudobatos horkelii* IN THE COAST OF RIO GRANDE DO SUL, BRAZIL USING DNA BARCODING

Souza Bruno[1]; Adachi Aisni[1]; Ribeiro Giovana[1]; Oliveira Cláudio[1]; Cruz Vanessa[1]; Foresti Fausto[1]

[1]Universidade Estadual Paulista "Júlio de Mesquita Filho" – UNESP/IBB, Botucatu, SP, Brasil.

brunocampos.ibb@gmail.com

Keywords: Molecular markers; Mitochondrial DNA; Conservation

The elasmobranchs, is a group of organisms that includes sharks and rays, itshave a high morphological, ecological and behavioral diversity. The ray *Pseudobatos horkelli* popularly known as in Brazil as Viola ray, which presents a wide distribution, occurring from the State of Rio de Janeiro (Brazil) to northern Argentina, had its population drastically reduced to 16% in the 1980s compared to the original condition existing in the South of Brazil. This has resulted in a prohibitionon fishing and commercialization of this species in subsequent years and is currently classified as "critically endangered," according to the IUCN (International Union for Conservation of Nature) Red List and "critically endangered," according to ICMBio (Instituto Chico Mendes de Conservação da Biodiversidade). However, although this species has prohibited fishing, it is still commercialized and found in the majority of the times in the form of fillet, which hinders the correct identification of the fish An analysis methodology developed in the last decade, using a single gene sequence, proved to be efficient to molecularly identify most of the biological species, was based on the use of the mitochondrial DNA gene COI (Cytochrome Oxidase Subunit I), and became a global bio-identification system called DNA barcoding. The present study aimed to identify specimens of the genus *Pseudobatos* sampled with the application of the molecular tool DNA barcoding. Samples of 13 specimens obtained from fish markets and product landings in the region of Torrinha, Rio Grande do Sul, Brazil, were analyzed. In order to perform this study, fragments of muscle tissue were coleted, from which the total DNA extraction, the amplification of the COI gene and its sequencing were performed, and the obtained nucleotide sequences were edited and aligned using the Geneious 4.8.5 program. To confirm the identity of the sequences, the BLAST algorithm (Basic Local Alignment Search Tool), obtained through NCBI (National Center for Biotechnology Information) was used. All analyzed samples were identified as being of *P. horkelii* species. This result, in addition to confirming the suitability of this molecular tool (DNA barcoding) and its efficiency in the identification of biological products that are usually decharacterized in the market, also identifies a tool that may be useful in future fishing management and conservation actions of the species.

Financing: Fapesp, CNPq and Capes.

Telomeric genes are overexpressed in metastatic prostate cancer cell lines.

Gabriel Arantes[1]; Ruan Pimenta[1]; Poliana Romão[1]; Juliana Camargo[1]; Vanessa Guimarães[1]; Nayara Viana[1]; Iran Silva[1]; Kátia Ramos[1]; Sabrina Reis[1]; William C Nahas[2]; Miguel Srougi[1]

[1] Laboratory of Medical Research (LIM55), Department of Urology, Faculty of Medicine (FMUSP), University of São Paulo, São Paulo, SP-BR. [2] Instituto do Câncer do Estado de São Paulo.(ICESP)- (São Paulo – SP)

arantes_gabriel@hotmail.com

KeyWord: Prostate Cancer; Telomere; Gene Expression

Prostate cancer (PC) is the most common neoplasia in men and cause of thousands of deaths worldwide when progress to the metastatic stage. The search of biological mechanisms related to PC progression is urgent, aiming to find new diagnostic and prognostic biomarkers, as well as therapeutic targets. Telomeres might be one of these targets. The telomeres are the structural ends of the eukaryote chromosomes, essential for the genome stability and control of the maximum number of cell divisions. The telomere homeostasis depends on some protein complexes, being the three main complexes: i) telomerase, ii) CST complex, and iii) shelterin complex. Telomeric dysfunction and abnormal expression of telomeric components are reported in most cancers and has been related with initiation and progression of PC, being one of the hallmarks of the disease the telomerase reactivation when a critical telomere shortening is reached. Despite the importance of these mechanisms, there are few studies about the expression of the main telomere complexes and their relationship with the PC. Thus, in this work we evaluate the gene expression of TERT (telomerase), POT1, TRF2 (shelterin) and CTC1 (CST) between the DU145 and PC3 metastatic cell lines. The gene expression was performed in triplicate using the qRT-PCR and the control group was composed of 10 tissue samples from patients with Benign Prostatic Hyperplasia (BPH). Our results show that there is no expression of TERT in BPH and presence in both cell lines. Besides that, there is a overexpression of TERT (175,46) in PC3 when compare with DU145, this is important because PC3 cells have high metastatic potential compared to DU145 cells. All other three genes are over expressed in the PC3 (POT1= 3673,08 ; TRF2= 4024,70 and CTC1= 2013,37) and DU145 (POT1= 55,13 ; TRF2= 36,11 and CTC1= 11,75) when compared with the control. These results are preliminary and further investigations are needed to confirm the data, but we can observe a trend that telomeric genes are highly overexpressed in metastatic prostate cancer cells, especially in the PC3 which has an aggressive phenotype with a high genomic instability. There are some studies with relate the TRF2 and POT1 overexpression with a genomic instability, which is in accordance with our findings. Thus, we can conclude that overexpression of telomere associated genes can be related with metastatic prostate cancer and can be used as a biomarkers in the disease.

UPREGULATION OF HOX GENES PROMOTES CELL MIGRATION AND PROLIFERATION IN HEAD AND NECK SQUAMOUS CELL CARCINOMA.

Graziela de Moura Aguiar[12*], Anelisa Ramão[2], Jessica Rodrigues Plaça[12], Sarah Capelupe Simões[1], Natália Volgarine Scaraboto[12], Cibele Cardoso[12], Josane Sousa[2] Wilson Araújo da Silva Jr.[12]

[1]Ribeirão Preto Medical School, University of São Paulo, Ribeirão Preto, SP, Brazil. [2]Center of Cell-Based Therapy (CEPID/FAPESP); National Institute of Science and Technology in Stem Cell and Cell Therapy (INCTC/CNPq), Regional Blood Center of Ribeirão Preto, Ribeirão Preto, SP, Brazil.

* graziela.aguiar@usp.br

Palavras-chave: HOX genes, Cell migration / proliferation; HNSC

Dysregulation of HOX homeobox genes expression has been observed in several cancers, including head and neck squamous cell carcinoma (HNSC). Although characterization of HOX gene roles in HNSC development has been reported, there is still a need to better understand their real contribution to tumorigenesis. HOX genes expression was analyzed in HNSC RNA-Seq data from The Cancer Genome Atlas (TCGA) and by RT-qPCR from different tumor cell lines. Thirty-one out of the 39 mammalian HOX genes were found upregulated in HNSC tissues and cell lines. siRNA- mediated knockdown of HOXC9, HOXC10 or HOXC13 in HNSC cell lines attenuated cell migration, and lead to downregulation of epithelial-mesenchymal transition (EMT) markers, which were predicted as transcriptional targets of these three HOX genes by bioinformatic analysis. Diminished colony formation and cell cycle arrest after HOXC10 or HOXC13 knockdown were also observed, corroborating the fact that there was an enrichment for genes in proliferation/cell cycle pathways among predicted targets of the selected HOX genes. In summary, we revealed roles for HOXC9, HOXC10, and HOXC13 in cell migration and proliferation/cell cycle progression in HNSC cells, and suggested that those HOX members contribute to HNSC development possibly by regulating tumor growth and metastasis.

Funding Agency: FAPESP, CNPq, CAPES.

MOLECULAR EVOLUTION OF HACNS1 GENE ENHANCER IN PRIMATES

Gabrielle Azevedo Rizzato[1]; Renan B. Lemes[1]; Maria Cátira Bortolini[2]; Vanessa Paixão-Cortês[3], Tábita Hünemeier[1]

[1]Departamento de Genética, Universidade de São Paulo. [2]Universidade Federal do Rio Grande do Sul. [3]Universidade Federal da Bahia.

gabriellerizzato@gmail.com

Palavras-chave: HACNS1; enhancer; primates; new world primates; Transcription factors; TFBS.

Molecular analyses were always an important component when studying primate evolution. *Cis*-regulatory elements (CREs) are non-coding DNA sequences containing binding sites for transcription factors or other regulatory molecules that are needed to activate and sustain transcription, with enhancers being the ones CREs that when binded to a transcription factor increase the transcription of an associated gene. *HACNS1* is a highly constrained enhancer in the genome of all terrestrial vertebrates, but it has accumulated 16 human-specific changes after the human chimpanzee split, with 13 of these 16 mutations being found within an 81-pb segment of this region and having a specific role in humans, possibly related to the development of hand dexterity. The divergence between old and new world monkeys happened about 45 to 37 million of years ago, and is possible that there were other mutations between these groups that lead to the development of other specific characteristics. Hereby, this study aims at the molecular characterization of the *HACNS1* enhancer as to the presence of predicted transcription factor binding sites (TFBS) in 22 species of new world and 20 species of old world monkeys. The *HACNS1* sequences were obtained in 18 new world monkeys through sanger sequencing, whereas the sequences from other monkeys and from *Homo sapiens* were obtained from NCBI database. Prediction of TFBS was made through the software Genomatix. Computational analysis of predicted TFBS in *HACNS1* orthologs suggested that multiple sites have been gained and lost in this enhancer during primate evolution. Predicted TFBS gains include some human-specific gains such as the ones related to the genes: *ZSCAN4*, involved in telomerase maintenance; *CAMTA1*, a calmodulin-binding transcription factor; *E2F3*, involved in cell-cycle regulator; and *GLIS2*, that plays a role in kidney development and neurogenesis. One of the main findings of our work is the gain of *MYRF* TFBS in great apes. *MYRF* is a myelin regulatory factor that participates in the regulation of the myelination of central nervous system. Since *HACNS1* is related to hand dexterity, the gain of *MYRF* TFBS can also be related to the same process. For the new world monkeys there is a gain for a binding site for *ZF02*, a transcriptional repressor that participate in lipid metabolism, in *Saimiri boiliviensis*. Regarding losses, we found the loss of one copy of *PITX1*, a transcription factor that may play a role in the development of anterior structures (particularly related to the structure of hindlimb), interestingly lost in humans and chimps. This unexpected loss remains unclear and should be further explored. In summary, some of our findings were, as far as we know, not described in the literature and we expect our work contributes to a greater understanding of the role of *HACNS1* in primates.

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CARCINOMA PAPILLARY THYROID RELATED TO THE POLYMORPHISM EcoRI OF THE GENE *APOB*

Jéssica Caroline C. Mendes[1]; Rafael Martins de Morais[2]; Izabel Cristina R. da Silva [3*]

[1]Universidade de Brasília. [2]Imagens Médicas de Brasília. [3]Universidade de Brasília.

jessicacaroline.unb@gmail.com

Key-words: Thyroid Cancer; polymorphism; *APOB*.

Apolipoprotein B is involved in the metabolism of LDL. The thyroid gland is responsible for the production of triiodothyronine and thyroxine which are related to lipid metabolism. Studies have shown that decreased serum levels of these hormones lead to increased levels of LDL. Thus, genetic alterations in the *APOB* gene and changes in the thyroid can lead to differentiated phenotypic expressions, and it to influence changes in serum cholesterol levels. It has been reported that higher Serum lipid levels are associated with approximately 2-fold higher thyroid cancer risk. Apolipoprotein B is an important protein related to the clearance of LDL in serum, it mediates cellular uptake of cholesterol and is the ligand that binds to the LDL receptor. The gene coding for apolipoprotein B (*APOB*) has been cloned and is located on the short arm of chromosome 2 (q23q24). Many variants of the *APOB* gene has been found to be directly linked with lipid levels. In our study, among these variants, we paid particular attention to the most investigated *EcoRI* (rs1042031) in exon 29 single nucleotide polymorphisms in the *APOB* gene, where there is the exchange guanine base for adenine, resulting in the exchange of glutamic acid by lysine. When the mutation occurs, the *EcoRI* enzyme loses the restriction site, giving rise to the E- allele. There have been no previous studies examining this gene in the Brazilian population with papillary thyroid carcinoma. Thirty participants, of both sexes and over 18 years old, were recruited at the Medical Institute of Brasília (IMEB), who had diagnosis of thyroid cancer and were treated with Radiopharmaceutical Sodium Iodide (131I). Genotyping was conducted by the PCR-RFLP method, to evaluate *EcoRI* polymorphisms located in exon 29 of the *APOB* gene. The data were compiled in the SPSS program version 25.0. The present work was approved in the UNICEUB ethics committee n ° 57382416.6.0000.0023. Of the total number of patients, 86.7% presented the E-E- genotype, with a mean age of 47 years and IMC of 27.5 (overweight). Since all the male participants (11) had polymorphism. All patients who had stage 3 and stage 4 tumor, stage 1 nodules and metastasis 1 also carried the mutation. The antiglobulin was at a counting value less than 20 UI/mL in 16 participants. In the mean of the other biochemical tests the TSH = 75.37 μUI/ml and Thyroglobulin = 49.53 ng/mL. In radioactive iodine treatment, 14 of the 26 participants who had the polymorphism treated at a dose equal to 150 mCi. The *EcoRI* polymorphism of the *APOB* gene is related to papillary thyroid carcinoma, especially in males and in patients who are likely to have advanced stages of the disease.

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ANALYSIS OF THE INFLUENCE OF MOTHER CYP1B1 GENE POLYMORPHISMS IN THE GESTATIONAL AGE OF BIRTH: A CLINICAL AND MOLECULAR CORRELATION

Jéssica Aflávio dos Santos[1]; Lyvia Neves Rebello Alves[1]; Eric Arrivabene Tavares[1]; Diego do Prado Ventorim[1]; Fernanda Mariano Garcia[1*]; Gisele Queiroz Carvalho[2]; Djanilson Barbosa dos Santos[3]; Raquel Spinassé Dettogni; Raquel Silva dos Reis[1]; Iúri Drumond Louro[1].

[1]Universidade Federal do Espírito Santo. [2]Universidade Federal de Juiz de Fora. [3]Universidade Federal do Recôncavo da Bahia.

* f.marianogarcia@gmail.com

Key words: Gestational age at birth; Preterm birth; CYP1B1.

Currently, both prematurity and births occurring in the late preterm and in the early term have been highlighted by their negative influence on infant mortality and morbidity. Several risk factors have been associated with decreased gestational age at birth. Environmental risk factors include cigarette smoking, excessive alcohol intake, ethnicity, weight and maternal age. Regarding genetic factors, genetic polymorphisms involved in oxidative stress and xenobiotics metabolism have been strongly associated with preterm birth. This may generate oxidative stress, considered a primary etiological factor for preterm birth because it can cause a compromised placenta. This is the case of the *CYP1B1* gene, which also plays an important role in the estrogen synthesis, one of the most important hormones in pregnancy maintenance. The action of the enzyme CYP1B1 may result in the production of reactive and carcinogenic compounds due to the preferential hydroxylation of 17β-estradiol at the 4-hydroxy position. The polymorphisms rs10012, rs1056827, rs1056836 were selected for this work because they are associated with variations in this enzyme activity. Thus, the present study aims to investigate the possible influence of these polymorphisms, as well as clinical data and maternal life habits in gestational age at birth. For such, a prospective cohort study was carried out with pregnant women from the Santo Antônio de Jesus city, in State of Bahia, Brazil. Mothers were followed during gestation, delivery and puerperium between 2009 and 2016. Maternal characteristics and habits were obtained through standardized questionnaires. Genotyping was performed from blood samples from pregnant women with TaqMan® assays using the Real Time Polymerase Chain Reaction (PCR) technique. Pregnant women who delivered in the initial full-term period had higher mean maternal age and pre-gestational Body Mass Index (BMI) than the preterm and full-term periods. Pre-gestational BMI intervals, race, smoking, and alcoholic showed no significant association with gestational age at birth. A significant correlation was detected between the SNP rs1056836 and the maternal age and the pre-gestational BMI. None of the other analyzed variables showed a significant association with any of the other polymorphisms. Non direct association was detected between any of the analyzed polymorphisms and gestational age at birth. From the results obtained by this work, a possible relationship of the *CYP1B1* gene with the gestational age at birth cannot be ruled out. Subsequent studies should analyze the possible association of these polymorphisms with gestational age at birth in other ethnic groups, as well as carry out more extensive investigations including other genes and polymorphisms, as well as prematurity environmental risk factors.

DEVELOPMENT AND APPLICATION OF A PANEL OF BACTERIAL AND ANTIBIOTIC RESISTANCE GENES IN GREEN TURTLE (CHELONIA MYDAS) AS A MARINE POLLUTION BIOINDICATOR

Fernanda Sobral Short[1]; Gisele Lôbo-Hajdu[2]; Rosane Silva[1]

[1]Laboratório de Metabolismo Macromolecular Firmino Torres de Castro, Universidade Federal do Rio de Janeiro – Instituto de Biofísica Carlos Chagas Filho, Rio de Janeiro, Brazil. [2]Departamento de Genética Marinha, Universidade do Estado do Rio de Janeiro. – Instituto de Biologia Roberto Alcântara Gomes, Rio de Janeiro, Brazil.

*shortfernanda@gmail.com

Palavras-chave: resistance genes; green turtle; marine pollution

The number of strains of antibiotic-resistant bacteria has been increasing every year due to constant consumption and, mainly, the indiscriminate use for human, agricultural and veterinary purposes, these bacteria with resistance genes are dispersed by the environment and can reach marine waters due to the dumping of waste. The green turtle (Chelonia mydas), the most abundant species in the Brazilian coast, has several characteristics that make it a bioindicator of marine pollution, for spending most of its life in coastal environments, being constantly exposed to anthropic factors. In adult life, they migrate to the high seas and return to the coastal areas in reproductive times, which contributes to the dissemination of these microorganisms. Thus, the objective of this project is to develop a panel of antibiotic resistance genes from bacteria derived from green turtles from the Itaipu beach region, Niterói - Rio de Janeiro / Brazil. Samples were collected with the aid of swabs from the cloacal cavity of green turtles, stored on ice for DNA extraction from the bacteria and later sequenced. From the resistance genes found, we will make a panel with the respective classes of antibiotics. The determination of these genes is important to understand their transmission dynamics and potential effect on human health.

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MODULATOR EFFECT OF THE AQUEOUS EXTRACT OF THE LEAVES OF *Eugenia pyriformis* (CAMBESS) UNDER THE ACTION OF DOXORUBICIN IN *Drosophila melanogaster*

Matheus Fernandes da Silva[1]; Michely Magalhães Araújo[1]; Matheus Gustavo Soares Santos[1]; Rosiane Gomes Silva Oliveira[1]; Priscila Capelari Orsolin[1].

[1]Laboratório de Citogenética e Mutagênese (LABCIM) do Centro Universitário de Patos de Minas (UNIPAM).

matheusfs@unipam.edu.br

Keys-words: Fruit Fly. Uvaia. *Warts*.

Neoplasms are among the main causes of death in the world, losing only to heart diseases. Despite the advances in medicine in recent decades, the causes of cancer have not yet been fully understood, and it is possible to assume innumerable causes, as diverse as the evolution of the disease itself. Considering that 80% of the chemotherapies used in the treatment of neoplasms are derived from natural compounds, the plants stand out as the source of antineoplastic drugs. Some species of the Myrtaceae family, such as *Eugenia pyriformis* CAMBESS (uvaia), are widely used in folk medicine, but they still have few scientifically proven phytotherapy effects. Thus, the objective of this study was to evaluate the modulating effect of the aqueous extract of the leaves of *E. pyriformis* on the doxorubicin (DXR) action in *D. melanogaster*. To assess this effect, the larvae descended from the *wts+/+mwh* cross were used. This experiment was performed in four replicates for each treatment. The larvae were treated with different concentrations of the aqueous extract of uvaia leaves (2, 5. 5 and 10 mg/mL) alone and in combination with doxorubicin (0,4 mM). They were also treated with the negative (ultrapure water) and positive (DXR) control. After collecting adult individuals, 200 flies of each concentration were analyzed using stereoscopic loupes. Based on this, the isolated concentrations showed frequencies of tumors that did not differ significantly from the negative control ($p>0,05$), which shows the absence of carcinogenicity in these three concentrations. Regarding the associated concentrations, there was a modulating effect on the action of doxorubicin, because a reduction in the frequency of tumors was observed when compared to the positive control ($p<0,05$). Substances such as phenolic compounds, tannins, among other metabolites, present in species of the same genus suggest similar antiproliferative and antioxidant effects for uvaia, which could justify the results obtained. Therefore, the test for detection of epithelial tumors clones in *D. melanogaster* demonstrated that the aqueous extract of the leaves of uvaia had no carcinogenic potential, however when associated with DXR, it showed a modulating effect.

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ABSENCE OF MUTAGENIC AND RECOMBINOGENIC EFFECTS OF THE CELECOXIB THROUGH SOMATIC MUTATION AND RECOMBINATION TEST, IN SOMATIC CELLS OF *DROSOPHILA MELANOGASTER*

Matheus Gustavo Soares Santos[1]; Matheus Fernandes da Silva[1]; Michely Magalhães Araújo[1]; Mirley Alves Vasconcelos[1]; Rosiane Gomes Silva Oliveira[1]

[1] Laboratório de Citogenética e Mutagênese (LABCIM) - Centro Universitário de Patos de Minas – UNIPAM.

soarmatheus@hotmail.com

Key-words: cancer; COX-2 inhibitor; SMART.

Cyclooxygenase-2 (COX-2), an enzyme expressed in inflamed tissues, has also been described in some neoplasms. In addition, DNA changes due to the process of mutation or recombination are also associated with cancer development. Thus, substances which have the ability to inhibit this enzymatic expression and have no side effects on the genetic material, are targets for new studies in oncology area, such as the celecoxib, a nonsteroidal anti-inflammatory drug, COX-2 inhibitor. Hence, this study aimed to assess the mutagenic and recombinogenic potential of the celecoxib through the *Drosophila* wing spot test (SMART). For this, trans heterozygous larvae descended from the *High Bioactivation Cross* (HB - ♀*ORR/ORR; flr³* x ♂*mwh*) and the *Standard Cross* (ST - ♀*flr³* x ♂*mwh*) were subjected to a 48-hour chronic treatment using three different alone concentrations of the celecoxib (0.375, 0.75 and 1.5 mg/mL). A negative (reverse osmosis water) and a positive (doxorubicin – DXR 0.4 mM) control were also included in the treatment. After undergoing metamorphosis, the wings of adult flies were detached and arranged on slides. Five pairs of male wings and five pairs of female wings were attached on each slide and six slides were analyzed for each concentration. The analysis were done under an optical microscopy and mutant hairs were classified by number, type (simple or twin), size, and position. After statistical analysis, the results showed that isolated concentrations of the celecoxib did not increase ($p > 0.05$) the frequency of mutant spots when compared to negative control. Such results may be associated by its chemopreventive actions, preventing oxidative DNA fragmentation and reducing DNA damage. In conclusion, based on the experimental conditions presented, celecoxib had no mutagenic and recombinogenic effects.

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NA-SEQ EVIDENCED THAT MEDULLARY THYMIC EPITHELIAL CELLS EXPRESS LONG NON-CODING RNAS UNDER CONTROL OF THE AIRE GENE

Romário de Sousa Mascarenhas* [1]; **Amanda Freire-Assis** [1,2]; **Natane de Araújo Miglioli**[1]; **Geraldo Aleixo Passos** [1,3]

1 Molecular Immunogenetics Group, Departament of Genetics, Ribeirão Preto Medical School, University of São Paulo, Ribeirão Preto, São Paulo, Brazil. 2 State University of Minas Gerais, Passos, Minas Gerais, Brazil. 3 Departament of Basic and Oral Biology, School of Dentistry of Ribeirão Preto, University of São Paulo, Ribeirão Preto, São Paulo, Brazil.

romariomascarenhas@usp.br

Keywords: Aire; mTECs; lncRNAs.

Central immunological tolerance concerns non-responsiveness to self-antigens and this mechanism involves maturation and selection of T lymphocytes within the thymus. During this process, those thymocytes autoreactive against self tissue-restricted antigens (TRAs) are eliminated by apoptosis (negative selection) before reaching the periphery. In homeostasis, this process avoids the development of aggressive autoimmunity. The main gene involved in the control of TRA expression is the Autoimmune regulator (Aire), responsible for the modulation of about 40% of all expressed TRAs in medullary thymic epithelial cells (mTECs). Aire is considered a pleiotropic gene because it is associated with functions other than TRA transcription. Studies have shown that Aire also acts in the control of the expression of non-coding RNAs (e.g. microRNAs) in mTECs. Our hypothesis is that the Aire also regulates the expression of long non-coding RNAs (lncRNAs) in mTECs. This study aimed to identify and characterize lncRNAs differentially expressed in mTEC cells comparing Aire wild type (WT) vs Aire KO cells out in the Aire gene by means of in silico prediction algorithms. We use an Aire KO mutant mTEC cell line previously obtained in our laboratory by means of the CRISPR-Cas9 system. The total RNA from WT or Aire KO mTECs was extracted and then sequenced through RNA-seq (Ilumina RNA-Seq). Bioinformatics analysis of RNA-Seq data, (FASTQC program, trimming, genome assemble (Mus musculus) and statistical analysis in the R platform environment) showed the differential expression of lncRNAs that were further characterized. In the absence of Aire, 136 lncRNAs were differentially expressed, being 72 up-regulated and 64 down-regulated. Among these, we highlight the lncRNAs Ifi30, Gas5, and Neat1, whose respective functions are related to the immune system. These results show mTEC cells express lncRNAs and that the Aire gene is implicated in its regulation.

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Gene expression profile of heat shock proteins from Caracu and Nelore cattle breeds in response to thermal stress

Bianca Vilela Pires[1], Sérgio Pereira Lima[2], Nedenia Bonvino Stafuzza[2], Claudia Cristina Paro de Paz[1,2]

[1]Departamento de Genética, Universidade de São Paulo – Faculdade de Medicina de Ribeirão Preto, São Paulo, Brasil. [2]Centro Apta em Bovinos de Corte - Instituto de Zootecnia, Sertãozinho, São Paulo, Brasil.

biancapires@usp.br

Key words: adaptation; beef cattle; heat stress.

Heat stress cause the elevation of cortisol levels and rectal temperature that triggers the action of heat shock proteins (HSPs) in the beef cattle production. The currently study aimed detect the hsp60, hsp70 and hsp90 gene expression pattern, rectal temperature and cortisol levels in Nelore (*Bos indicus*) and Caracu (*Bos taurus*) steers submitted to heat stress. Forty Caracu and 37 Nelore steers with 13 months old were evaluated during 2017 (October and December) and 2018 (February and March). In the morning all animals remained in natural habitat, while in the afternoon the steers were separated in two groups: shade and sun, while the rectal temperature (RT) and cortisol were measured. Serum cortisol was analyzed by ELISA method and the relative expression of hsp60, hs70 and hsp90 were determined by real-time PCR. The data were analyzed by MIXED model with least square means. Differences were observed between Nelore and Caracu breeds in RT (38.8°C and 39.2°C), cortisol levels (18.5 and 23.7 ng/mL), expression of hsp60 (2.70 ± 0.12 and 3.05 ± 0.09) and hsp90 (1.54 ± 0.10 and 1.25 ± 0.09). In the morning treatment was observed the lowest cortisol levels (17.2 ng/mL) and RT (38.7°C). The exposure of sun not influenced the relative expression of hsp60, hsp70 and hsp90. Significant changes were observed during the months studied, indicating that the environmental conditions (temperature and humidity) influenced the RT, cortisol levels and expression of HSPs. In March was detected highest cortisol level (24.3 ng/mL) and expression of hsp70 (2.65 ± 0.15), whereby this month the relative humidity was highest. It's known the increase the RT is associated to the activation the HSPs in ruminants, in this study the higher values of RT (39.1°C), expression of hsp60 (3.35 ± 0.15) and hsp90 (1.98 ± 0.13) was observed in October, the month with the smaller relative humidity and high air temperature. The physiological parameters and expression HSPs indicating that Caracu (*Bos taurus)* is less adapted to thermal stress than Nelore breed (*Bos indicus)*.

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mTOR PATHWAY SOMATIC MUTATIONS AND THE MOLECULAR PATHOGENESIS OF HEMIMEGALENCEPHALY: NEW FINDINGS ON *DEPDC5*

Camila A. B. Garcia[1], Simone C.S Carvalho[3], Xiaoxu Yang[2], Laurel L. Ball[2], Renee D. George[2], Kiely N. James[2], Valentina Stanley[2], Martin W. Breuss[2], Marcelo V. Santos[1], Wilson A. Silva Jr [3,4], Joseph G. Gleeson[2], Hélio R. Machado[1].

1Department of Surgery and Anatomy, Ribeirão Preto Medical School, University of São Paulo (FMRP-USP), Brazil. 2Laboratory for Pediatric Brain Disease, Howard Hughes Medical Institute, Department of Neurosciences, University of California, San Diego, La Jolla, CA, USA. 3Department of Genetics, Ribeirão Preto Medical School, University of São Paulo (FMRP-USP), Ribeirão Preto, SP, Brazil. 4Center for Medical Genomics at the University Hospital of Ribeirão Preto Medical School-USP, SP, Brazil

* camila.neurociencias@gmail.com

Keywords: hemimegalencephaly; epilepsy; mTOR.

Focal cortical dysplasias are characterized by malformations of the cerebral cortical tissue and constitute the most common cause of refractory epilepsy in the pediatric population. Hemimegalencephaly (HME), characterized by the distorted and abnormal growth of one of the cerebral hemispheres, is part of this group of malformations. Recently, defects in the protein kinase mTOR (Mammalian Target of Rapamycin) and its associated pathway have been associated with HME. mTOR acts as a central regulator of important physiological cellular functions such as growth and proliferation, metabolism, autophagy, death and survival. Since the genetic basis of HME is still poorly understood, this study was aimed at identifying specific mutations in mTOR signaling pathway genes in patients diagnosed with HME. Using amplicon and whole exome sequencing (WES) of resected brain and paired blood samples from 5 patients with HME, we were able to identify pathogenic mosaic mutations in the mTOR pathway in genes MTOR, PIK3CA and DEPDC5. These results strengthen the hypothesis that somatic mutations of mTOR pathway genes contribute to HME. These findings also provide insights into when in brain development these mutations occurred. An early developmental mutation is expected to affect a larger number of cells and to result in a larger malformation, whereas the same mutation occurring later in development would cause a minor malformation. In the future, numerous somatic mutations in known or new genes will undoubtedly be revealed in resected brain samples, making it possible to draw correlations between genotypes and phenotypes and allow for a genetic clinical diagnosis that may help to predict a patient's outcome.

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ETHANOL AND GENOMIC INSTABILITY: STUDY ON THE EXPERIMENTAL MODEL OF ETHANOL PREFERENCE IN ZEBRAFISH

Izabela Barbosa Moraes[1]; Isadora Marques Paiva[1]; Barbara Miranda Sartori[1]; Luis David Solis Murgas[2], Ana Lucia Brunialti Godard[1]

[1]Laboratório de Genética Animal e Humana - Departamento de Genética, Ecologia e Evolução - Pós-Graduação em Genética - ICB - UFMG, Belo Horizonte, Brazil. [2]Departamento de Medicina Veterinária, Universidade Federal de Lavras, Lavras, Brazil.

*izabela.bmoraes@yahoo.com.br

Keywords: telomere shortening; DNA methylation; alcohol abuse.

Alcohol use disorder is a pathological condition that affects thousands of people around the world. There is a relationship between alterations in the regulation of epigenetic profile and changes in the shortening pattern of telomeres and the use of ethanol. In this context, the objective of this work was to evaluate the genomic instability in ethanol preference phenotypes using a *Danio rerio* (*zebrafish*) model. In a population of 60 adult zebrafish submitted to the behavioral paradigm of Conditioned Place Preference (CPP), different phenotypes were observed in relation to the preference for the ethanol after an acute exposure (20 min). The preference was determined by observing behavior in preference in three different moments: Basal (B), Final (F) and After- Withdrawal (AW). After 15 day, at the end of the experiment, four distinct phenotypes were determined. The first, Light phenotype (L) are animals that had no preference for ethanol in any of the analyzed moments. The second, Heavy phenotype (H), showed preference in F, but in AW they stopped preferring. The third, the Inflexible phenotype (IN), are animals that showed preference for ethanol in F and maintained their preference even after 15 days of withdrawal (AW); and finally, the Negative Reinforcement phenotype (NR) that only prefers alcohol in AW. After euthanasia, the brain was dissected, and DNA and RNA were extracted. For the characterization of this model, behavioral analysis associated with DNA global methylation data and telomere length quantifications were performed. For the evaluation of global DNA methylation, the method of quantification of 5-methyl cytosine by ELISA was used. In order to evaluate the transcription pattern of genes related to the regulation of the DNA global methylation state, the quantification of transcripts of the *dnmt1*, *dnmt3* and *tet1* genes was also performed using qPCR. Telomere length was quantified as the telomere to single copy gene ratio (T/S ratio) using qPCR. Regarding the results, we could observe an increase in the overall DNA methylation pattern in the IN phenotype, whereas there was a hypo-regulation of the *dnmt3* gene in both the IN and NR phenotypes. Telomere shortening was observed in IN and NR phenotypes. In conclusion, our results suggest that exposure to ethanol triggers specific events in the IN and NR phenotypes regarding genomic instability due to increased DNA methylation, decreased *dnmt3* transcripts and shortening of telomeres.

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DUPLICATION AND DIVERSIFICATION OF B-CLASS MADS BOX GENES IN *Passiflora organensis*

Helena Augusto Gioppato[1,2]; Marcelo Carnier Dornelas[2]

[1]Instituto de Biologia, Universidade Estadual de Campinas. [2]Instituto de Biologia, Universidade Estadual de Campinas.

*helena.gioppato@gmail.com

Palavras-chave: floral morphology; MADS-box; Passiflora

Although the molecular and evolutionary mechanisms that gave rise to flowers, the main organs of angiosperms, are still unclear, there is no doubt that some members of the MADS-box gene family play essential roles in floral development processes. The ABC molecular model controls the organ identity of each floral whorl. The genes that take part in this model are members of the MADS-box gene family, except for the A-class gene, *APETALA 2*. Several studies related to the evolution of the MADS-box gene family have uncovered the presence of a considerable amount of duplication events, followed by the divergence of functions in members of this family. Hence, by altering the number of paralogs and possibly their functions, a probable modification of floral morphology is assumed as a result of changes in gene expression pattern and protein interactions. According to the literature, some of these duplications involving B class genes have contributed to the diversification of floral morphology of many angiosperm taxa. The genus *Passiflora* is one of the groups of angiosperms showing complex floral structures, such as the corona filaments, whose origins have not yet been elucidated. Therefore, considering the recent sequencing of the *Passiflora organensis* genome, this work is focused on the identification and analysis of all the members of the MADS-box B class genes in *P. organensis*. Eight class B genes were identified, the double number of members when compared to *Arabidopsis thaliana*. Three of them are *AP3* orthologs, one *TT16 ortholog*, one *GOA ortholog*, one *TM6 ortholog,* and two *PI orthologs*. Analyzes of the differential expression of these genes revealed a potential role in the diversification of floral structures in *Passiflora.*

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FREQUENCY OF PHO-CONSTITUTIVE MUTANTS: THE EFFECTS OF THE SELECTIVE MEDIA AND THE BUFFERS

Gabriella Machado[1]; Beny Spira[1]

[1] Departamento de Microbiologia, Instituto de Ciências Biomédicas, Universidade de São Paulo, São Paulo, SP, Brazil

gabriella.machado@usp.br

Palavras-chave: PHO-constitutive mutants; Tris buffer; mutant frequency

The PHO regulon of Escherichia coli consists of genes responsible for uptake and metabolism of inorganic phosphate (Pi) and other phosphate compounds. The transcription of this regulon occurs when the bacterium is in a environment with concentration of Pi below 4 μM. In this condition, PhoR acts as kinase, phosphorylating the PHO transcription factor, PhoB, which in turn activates the transcription. PHO- constitutive mutants lost this transcriptional regulation and transcribe these genes regardless the Pi concentration. Such mutants are selected in media containing excess Pi, glycerol-2-phosphate (G2P) as single carbon source and Tris buffer. Due to the constitutive expression of alkaline phosphatase in these mutants, G2P can be hydrolyzed, releasing glycerol to sustain the growth in this media. The theoretical frequency of these mutants is approximately 10^{-6} mutants per cell per generation. However, the frequency observed experimentally is smaller, between 10^{-7} and 10^{-8} mutants per cell per generation. Tris buffer decreases the outer membrane permeability of Gram-negative bacteria by breaking its ionic interactions. Therefore, such buffer might be impairing or even preventing the growth of the bacteria, resulting in the observed frequency of PHO-constitutive mutants. To test this hypothesis, the number of viable bacteria exposed to media buffered with Tris and Hepes was assessed through several days. The number of viable bacteria drops markedly in Tris buffered medium while in Hepes buffered medium, which is physiologically inert, this drop was not observed. The effects of other buffers on the dynamics of the emergence of PHO-constitutive mutants were also analyzed. PHO-constitutive mutants were isolated from cultures made in different media prior to the plating on selective media buffered with Tris. The effects of different buffers on the selective media in this isolation was also evaluated. Compared with Tris buffered medium, the frequency of PHO-constitutive mutants are bigger when they growth in a medium buffered with phosphate and smaller when they grow in a Hepes buffered medium. PHO-constitutives mutants are not capable of emerge in a selective medium buffered with phosphate due to partial inhibition of alkaline phosphatase by the high concentration of Pi. This work concludes that Tris buffer is not the main reason for the observed low frequency of PHO-constitutive mutants, since no other physiologically inert medium tested increases the frequency of such mutants.

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APOB GENE STUDY IN PATIENTS WITH FAMILIAL HYPERCHOLESTEROLEMIA

Agatha Ribeiro Mendes[1]; Caio Perez Gomes[1]; Rafael Leite Tavares de Morais[1]; Francisco Antonio Helfenstein Fonseca[2]; Renan Paulo Martin[1]; Maria Cristina de Oliveira Izar[2]; João Bosco Pesquero[1]

[1]Departamento de Biofísica, Universidade Federal de São Paulo – Escola Paulista de Medicina, São Paulo, Brasil. [2]Setor de lípides, aterosclerose e biologia vascular, Universidade Federal de São Paulo – Escola Paulista de Medicina, São Paulo, Brasil.

agathamendes@hotmail.com

Key-words: Familial Hypercholesterolemia; Cardiovascular disease; Apolipoprotein.

Familial Hypercholesterolemia (FH) is a genetic disease characterized by increased circulating LDL cholesterol, being a risk factor for increase in mortality related to the development of cardiovascular diseases, among them atherosclerosis, myocardial infarction and coronary disease. Recent studies show that FH is mainly related to mutations in the *LDLR* gene and, less frequently, to mutations in the genes *APOB*, *PCSK9* and *LDLRAP1*. *APOB* gene encodes the apolipoprotein responsible for LDL binding in its receptor, leading to cholesterol uptake in the cells. Mutations in this gene result in defects in apolipoprotein binding to the receptor, therefore causing accumulation of plasma LDL cholesterol. This apolipoprotein is also known as apolipoprotein B-100 (ApoB-100) and in addition to binding to LDL, it participates in the structure of VLDL, LDL and IDL molecules. APOB also encodes the apolipoprotein B- 48 (ApoB-48) that results after messenger RNA edition. ApoB-48 acts in the absorption of cholesterol from the diet and in the formation and structure of chylomicrons. APOB study is very important, considering the function of its products in the control of the circulating cholesterol associated with the risk of the cardiovascular disease development. This work aimed to investigate alterations present in the *APOB* in the Brazilian population, through genomic DNA sequencing of 102 patients with clinical diagnosis for Familial Hypercholesterolemia. The presence of 19 variants was identified and five of them were never described in the literature. The clinical status and biochemical dosages of the patients were also verified in order to obtain a relation of the variants with their clinical significance. It was possible to observe high levels of lipids in the majority of the patients with the identified variants. Based on these findings, it was possible to conclude that some variants found in the *APOB* may be associated to the development of cardiovascular diseases in patients with FH.

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EFFECT OF ANTIANGIOGENIC TREATMENT IN A HEAD AND NECK CANCER CELL LINE

Juliana Garcia de Oliveira-Cucolo[1]; Gabriela Helena Rodrigues-Flemming[1]; Glaucia Maria de Mendonça Fernandes[1]; Ana Lívia Silva Galbiatti-Dias[1], Mariana Prodóssimo Sant'Anna [1], Erika Cristina Pavarino [1], Eny Maria Goloni-Bertollo [1*]

[1] Faculdade de Medicina de São José do Rio Preto – FAMERP.

* juliana.cucolo@gmail.com

Key-words: Familial Hypercholesterolemia; Cardiovascular disease; Apolipoprotein.

The angiogenesis is known to be pivotal for tumor progression and metastasis and drugs with antiangiogenic effects have been studied in the treatment of various tumor types. However, the chemotherapy treatment for head and neck cancer has shown unfavorable results due to tumoral resistance, and suggest an association with cancer stem cells (CSCs). The vascular endothelial growth factor A (VEGF-A), an angiogenic factor, has been associated with this resistance. The aims of study were to identify CSCs in laryngeal cancer cell line (HEp-2); to compare VEGFA gene and protein expression between CSCs and non-CSCs and evaluate the effectiveness of Paclitaxel and Bevacizumabe anti-cancer drugs in the angiogenesis. Fluorescent-activated cell sorting (FACS) using specific cell surface biomarker combination was performed to isolate CSCs ($CD44^{high}/CD117^{high}/CD133^{high}$) and non- CSCs ($CD44^{low}/CD117^{low}/CD133^{low}$) from cell line HEp-2. After, the subpopulations were cultured and exposed for 24h to treatment with 0.05mg/ml of Paclitaxel, 0.5 mg/ml of Bevacizumab and their associations (Paclitaxel/ Bevacizumab), subsequently the angiogenesis assay was performed by ECMatrixgel (MILLIPORE®). The *VEGFA* gene expression was performed by quantitative Real-Time PCR using *Step One Plus equipment (ThermoFisher*), non-CSCs were considered control. Protein expression was performed by the ELISA technique (*MultisKan FC-ThermoFisher*). The *VEGFA* gene expression was slightly increased in CSCs compared to non- CSCs (RQ = 1.27), as well as the protein expression in CSCs group compared to non-CSCs (p = 0.0493). In the angiogenesis assay, the treatments with Paclitaxel and Paclitaxel/Bevacizumab were more effective in decreasing vessel formation for CSCs (p=0.0178 and p=0.0188, respectively) and non-CSCs (p=0.0178 and p=0.0149, respectively) related with control group. Bevacizumab treatment did not show a statistically significant difference in relation to the control for CSCs and non-CSCs (p=0,0604 and p=0,2357, respectively). Biomarkers alone are not well accepted as CSCs indicator, however, the combination CD44/CD117/CD133 used in this study proved able to identify CSCs. In addition, were demonstrated increased *VEGFA* gene and protein expression in the CSCs related with non-CSCs, as well as, the influence Paclitaxel and Paclitaxel/Bevacizumab drugs decreased vessel formation in the angiogenesis assay. However, more studies are needed to understand the mechanisms that make tumor stem cells more resistant to treatments and better treatment efficacy in head and neck cancer therapy.

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EVALUATION ON OF THE CARCINOGENIC EFFECT OF ECTOPARASITICIDE - AMITRAZ ® BY TEST FOR DETECTION OF EPITHELIAL TUMOR CLONES (*ETT*) IN *DROSOPHILA MELANOGASTER*

Bruna Cristina Silva[1]; Nayane Moreira Machado[1]; Paula Marynella Alves Pereira Lima[1;] CamilaMendes de Deus[1]; Isabella Queiroz[1].

1Laboratório de Citogenética e Mutagênese do Centro Universitário de Patos de Minas – UNIPAM, Patos de Minas – MG.

brunacs@unipam.edu.br

Key-words: Amitraz®; carcinogenesis; *Drosophila melanogaster*.

Antiparasitics encompass most of Brazil's market of veterinary products, and remain one of the therapeutics of choice for mite and tick elimination. External use has the function of combating parasites, but animals are not immune from risks when used improperly and / or in sensitized animals, which can lead to serious intoxications. With the increasing use of these products, it is also developped a concern about the effects on animals, the environment, and humans. Amitraz® is an ectoparasiticide of the formamidines group accessible for use in veterinary medicine. It is found in the form of shampoos and collars for dogs and cats, sprays and concentrated liquid for administration in ruminants and pigs, being totally contraindicated in horses. In view of the above, the present work aims to evaluate the carcinogenic properties of the Amitraz® ectoparasiticide by testing for the detection of epithelial tumor clones (*ETT*) in *Drosophila melanogaster*. For treatment, 72-hour larvae resulting from crossbreeding between virgin females *wts/TM3,Sb1* and males *mwh/mwh* were used. The larvae underwent a chronic treatment with the three different concentrations (12,5; 25,0 and 50 µg/mL) that were selected based on the dose response test. For positive control, doxorubicin (0,4 mM DXR) was used and reverse osmosis water was used for the negative control. At the end of the metamorphosis period, adult individuals were collected and analyzed. The results showed that there was a statistically significant increase ($p<0,05$) in the frequency of tumors in subjects treated with Amitraz® at a concentration of 12,5 µg/mL compared to the negative control. On the other hand, there was no significant statistical difference in the other concentrations (25,0 and 50,0 µg/mL) when compared to the negative control. Therefore, these results demonstrate a dose-dependent, since, as concentrations increased, there was a reduction in the frequency of tumors found. This leads to the possibility of induction of cell apoptosis before phenotypic expression of the tumor. In this sense, the conclusion is that, in the present experimental conditions, Amitraz® presented carcinogenic potential in *Drosophila melanogaster* in the lowest concentration tested.

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Reproductive biology of *Lippia alba* (Verbenaceae)

Victória Rabelo Campos[1]; Rick Goertzen[2]; Timothy F Sharbel[2]; Lyderson Facio Viccini[1]

1Universidade Federal de Juiz de Fora – Juiz de Fora - Minas Gerais - Brazil. 2GIFS (Global Institute for Food Security) – University of Saskatchewan- Saskatoon- Canada.

vicrabelo_2@hotmail.com

Key-words: *Lippia alba*; reproduction; poliploidy

The reproductive biology of a species is closely related to the genetic basis of a population and is one of the main factors related to the dispersion and success of angiosperms. *Lippia alba* (Verbenaceae) is known by several common names such as erva cidreira, falsa melissa, chá de tabuleiro, being widely used in folk medicine mainly due to the components of its essential oil. The species can be easily found from tropical to temperate regions, having broad phenotypic plasticity. Recent studies indicate a possible autopoliploidy origin for the species having diploids (2n = 30), aneuploids (2n = 38), triploids (2n = 45), tetraploids (2n = 60) and hexaploid (2n = 90) individuals. Despite the importance of *Lippia alba*, little information about the species' reproductive system is available. The goal of the present work was to study, by seed flow cytometry, the reproduction strategy of the species to better understand the origin of the five cytotypes as well as to help the elucidation of the genetic basis of the species. Seeds from individuals of different ploidal level and also from different conditions (from isolated buds and non isolated buds) were placed individually along with a bead in each well of a 96- well plate. Then, they were ground for fifteen seconds. Again, they were crushed for another fifteen seconds and centrifuged. 50 Kl of the CyStain UV Precise P buffer was added and then the samples were re-ground. A second centrifugation was performed and then a further 70 Kl of the buffer was added. Samples were stored at 4 ° C for 10 minutes. Subsequently they were filtered and 120 Kl of DAPI was added. The analysis was performed on Cytoflex cytometer. The results showed that individuals from isolated conditions and from all the ploidal levels had a higher rate of graphics without endosperm pick, but when possible to detect, the graphics showed a ratio of sexual reproduction, indicating a possible self-fertilization. It was possible to conclude that despite *Lippia alba* has preferentially an allogamic reproductive behavior, it is possible to detect alternative ways of reproduction that were different between diploids and polyploids individuals. Different strategies of reproduction might help to explain the occurrence of different ploidal level.

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Biological functions associated with prophage density in pathogenic and commensal *Escherichia coli* genomes

Agnello César Rios Picorelli [1], Tarcisio José Domingos Coutinho [2], Glória Regina Franco [3], Francisco Pereira Lobo [1]

1-Laboratório de Algoritmos em Biologia, Departamento de Genética Evolução e Ecologia, Universidade Federal de Minas Gerais, 2-Instituto Federal de Ciência, Tecnologia e Educação do Ceará, 3-Departamento de Bioquímica e Imunologia, Universidade Federal de Minas Gerais.

agpicorelli@gmail.com, franciscolobo@ufmg.br.

Keywords: Comparative genomics; Evolution of bacterial pathogenicity; Prophage biology; Gene Ontology

Bacteriophages are viruses that infect prokaryotic cells. After infection, a bacteriophage genome may integrate into the host DNA, generating a new biological entity known as prophage. Reproduction of prophages may occur through the lytic cycle, where the prophage genome are released from host genome and replicates independently, ending with host cell death through lysis. Another possibility is the lysogenic cycle, where host and viral genomes replicate together during bacterial reproduction. Furthermore, prophages may also bring new genes/biological functions to the bacterial gene repertoire through horizontal gene transfer, which may contribute to bacterial fitness by allowing bacterial adaptation to new ecological niches. Therefore, bacteriophages play an antagonistic role in bacterial evolution, contributing to bacterial fitness but also eventually causing host death. We used a statistical comparative genomics approach to evaluate the association between prophage density in pathogenic and free-living *Escherichia coli* and the biological functions coded in such bacterial genomes, represented by Gene Ontology (GO) categories. After a literature review, we selected 51 pathogenic and 29 non-pathogenic *E. coli* complete genomes from RefSeq. We used PHASTER to detect prophages within host genomes, and computed phage densities (PD) for each strain, defined as the number of detected phages divided by genome length. We used InterProScan to annotate the protein-coding genes to GO terms. We obtained a species tree using a phylogenomics supermatrix approach as follows: 1) OrthoMCL to infer gene homology; 2) muscle to align 1-1 orthologs; 3) TrimAl to trim alignments; 4) *in house* scripts to concatenate trimmed sequences; 5) CodonPhyML to generate a species tree. To take into account phylogenetic relatedness as a confounding factor, we used the species trees to compute phylogenetically independent contrasts, obtained for PD and GO term frequency, and evaluated the association between the contrasts obtained. Data analysis was done using R language, together with the latest version of KOMODO2, to search for GO terms whose frequency is significantly associated with PD (corrected p-values < 0.05 for both Pearson correlation and phylogenetically-aware linear models). We observed that pathogenic lineages have a significantly higher PD than non-pathogenic strains (KS test, p-value=0.01369). For both pathogenic and non-pathogenic bacteria, we found several GO terms important for prophage biology (e.g. “lysozime activity”, and “DNA packaging”) that are also associated with PD. Interestingly, for pathogenic *E. coli*, we also found associated GOs that are known pathogenesis mechanisms, such “catalase activity” and “hemolysis”. Taken together, our analysis suggests that PD in *E. coli* is associated with an increase in biological modules important for prophage biology and, in pathogenic strains, with pathogenicity mechanisms. To the best of our knowledge, this is the first study to report an association between prophage density and biological properties known to contribute to pathogenicity in *E. coli*.

Ackowledgemnts: CNPq, Capes and Fapemig

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EVALUATION OF ROBUST DEVELOPMENT USING FOURIER ELIPTICAL ANALYSIS IN POALES AND ZINGIBERALES STARCH CONTOURS AT DIFFERENT ALTITUDE

Ana Caroline da Silva[1]; Camila Fernandes Carvalho[1]; Danielle Manerich[1]; Indianara de Souza[1]; Isadora Ceratti Foletto[1]; Maria Eduarda dos Santos[1]; Shayane Mendes Gonçalves; Márcio Watanabe[1]; Carlos Geovanni Alves Ledra[1]; Hendrie Ferreira Nunes[1*]

[1] Departamento de Química, Instituto Federal de Santa Catarina, Gaspar, Brazil.

*hendrie.nunes@ifsc.edu.br

Key-words: Morphometry geometrics; *Hedychium coronarium*; *Typha domingensis*;

Stable expression characteristics are more robust in terms of development than phenotypes that are influenced by stochastic or environmental factors. Species that present a greater diversity in a given organ, the development system is not very robust, characteristic of species that diverged earlier. Thus, this work aimed to evaluate the morphological diversity of starch granules in two species that diverged earlier, under different environmental conditions. The morphology of 1,537 starch granules extracted from rhizomes of white ginger lily (*Hedychium coronarium*) and cattail (*Typha domingensis*) collected at different altitudes (0m, 600m, 700m, 800m and 900m) was analyzed. The granular contours were obtained through the Elliptic Fourier Analysis (EFA), of which 80 variables are used for each granule. Then, a Principal Component Analysis (PCA) was performed on the variance-covariance matrix generated by EFA and the first two components (> 81%) were used in the characterization. Starch granules of white ginger lily (*Zingiberales*) presented a high degree of morphological disparity when submitted to different altitudes, whereas the granules of cattail (*Poales*) were more uniform when submitted to the same conditions. Thus, although the robustness of the development of body planes in flowers (e.g. orchids) seems to be a success factor for derived species, the morphology of the granules in divergent species seems to have benefited from their lack of robustness and were able to direct the plants for evolutionary solutions, such as adaptation to different altitudes. Thus, the robustness of the development seems to coexist with the lack of robustness, depending on the phenotypic characteristic that is evaluated, since it was possible to verify differences in the granular morphology of two species that initially diverged.

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A NOVEL PLANT-SPECIFIC SYNTAXIN-6 PROTEIN IS INVOLVED IN INTRACYTOPLASMIC TRANSPORT OF BEGOMOVIRUSES

Bianca Castro Gouveia[1], Laura Gonçalves Costa Martins[1], Maximiller Dal-Bianco[1,2], João Paulo Batista Machado[1,3], José Cleydson Ferreira da Silva[1], Anésia Aparecida dos Santos[1,4], Joseph R Ecker[5], Elizabeth Pacheco Batista Fontes[1,2,3]

[1]National Institute of Science and Technology in Plant-Pest Interactions, Bioagro, Universidade Federal de Viçosa, Viçosa, Minas Gerais, Brazil. [2]Departament of Biochemistry and Molecular Biology, Universidade Federal de Viçosa, Viçosa, Minas Gerais, Brazil. [3]Agronomy Institute, Universidade Federal de Viçosa, Campus Florestal, Florestal, Minas Gerais, Brazil. [4]Departament of General Biology, Universidade Federal de Viçosa, Viçosa, Minas Gerais, Brazil. [5]Howard Hughes Medical Institute and Plant Biology Laboratory, The Salk Institute of Biological Studies, La Jolla, CA 92037, USA

lurcostlin@gmail.com

Key-words: Begomovirus; intracytoplasmic transport; viral DNA.

Due to limited free diffusion in the cytoplasm, viruses must use active and directed transport mechanisms to move intracellularly. Nevertheless, how the plant ssDNA begomovirus hijacks the host intracytoplasmic transport machinery to move from the nucleus to the plasmodesma remains enigmatic. Here, we demonstrated that viralNSP, a facilitator of the nucleocytoplasmic trafficking of viral (v) DNA, associates with a novel vesicle-localized plant-specific syntaxyn-6 protein, designated NISP, and the NSP-NISP complex is formed in trafficking vesicles, likely associated with endosomes. We also showed that begomovirus infection depends on the NISP-NSP interaction; the protein NISP displays a pro-viral function, which was not observed for the highly conserved syntaxin-6 paralog that failed to interact with NSP. Consistent with these findings, the *nisp-1* mutant plants are resistant to begomovirus infection, a phenotype reversed by NISP complementation, whereas overexpressing lines are hypersensitive. Furthermore, NISP interacts with NIG, a pro-viral factor that accessorizes the NSP-vDNA nucleocytoplasmic translocation, and the NISP-NIG interaction is enhanced by NSP. We also showed that NISP associates with vDNA to assemble a NISP-NIG-NSP-vDNA- containing multiprotein complex. The NISP interactions recruited NIG and NSP into trafficking vesicles, providing a mechanism for the directed cytoplasmic translocation of the associated NSP-vDNA complex toward the cell surface.

Funding Agency: CAPES, CNPq, FAPEMIG, INCT.

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INVESTIGATION OF THE INFLUENCE OF POLYMORPHISMS IN THREE TRANSPORTER GENES WITH THE SURVIVAL OF PATIENTS WITH CHRONIC MYELOID LEUKEMIA IN THE NORTHERN REGION OF BRAZIL.

Maria Clara Da Costa Barros[1]; Karla Beatriz Cardias Cereja Pantoja[1]; Darlen Cardoso De Carvalho[1]; Natasha Monte Da Silva[1]; Lucas Favacho Pastana[1]; Lui Wallacy Morikawa Souza Vinagre[1]; Ana Rosa Sales De Freitas[1]; Tereza Azevedo[2]; Marianne Rodrigues Fernandes[3]; Ney Pereira Carneiro Dos Santos[3].

[1]-Universidade Federal do Pará. [2]Hospital Ophir Loyola. [3]Nucleo de Pesquisas em Oncologia.

mariacbarros99@gmail.com

Key-words: Chronic myeloid leucemia; survival; genes.

Chronic myeloid leukemia (CML) is the excessive proliferation of granulocytic lineage cells of hematopoietic system. The main characteristic of CML is the Philadelphia chromosome, which consists of the translocation of chromosomes 9 and 22, resulting in the expression of BCR- ABL with unregulated tyrosine kinase enzymatic activity. The CML main treatment is imatinib (IMB), a selective tyrosine kinase inhibitor. However, literature reports a failure of therapeutic response in 25% of patients during the treatment of CML, which may be associated with individual genetic variability. The ABC and SLC family of genes encode carrier proteins that mediate the transport of substances in cells, including drugs. Genetic polymorphisms that modify the functionality of proteins encoded by these genes are related to the resistance of various drugs used in clinical practice. Besides the resistance, these polymorphisms can also alter the survival of patients, which improved considerably after the implementation of Imatinib, but still there are some cases of adversity in the treatment or even absence of response to the drug. Thus, the study of genes related to this survival in patients with CML is essential for improvement in the treatment of the disease, decreasing the toxicity of the drug and consequently improving the survival of patients. The objetive o four study was to investigate the influence of polymorphisms present in the *ABCC4* (rs4148551), *ABCG2* (rs2231142) and *SLC22A7* (rs4149178) genes on the survival of patients with CML undergoing imatinib- based treatment in the Northern of Brazil. The study population consisted of 105 patients with CML treated with IMB at a reference hospital in oncology in Pará. The genotyping analysis was performed by real-time PCR using QuantStudio ™ 12K Flex Real-Time PCR System (Applied Biosystems®, Foster City, California, USA). A panel of 61 Ancestral Information Markers was used as a method for genomic control. Statistical analyzes were performed in the SPSS 23.0 program. The results showed that there were no statistical signficance between the polymorphisms studied and the response to CML treatment in this study: rs4148551 (p=0.38, HR=0,8129, CI=26.442-10.184), rs2231142 (p=0.89, HR=0,144, CI=22,958-2,007) and rs4149178 (p=0.61, HR=0,387, IC=11,364- 1,910). However, in other research was reported that low *ABCG2* expression levels were associated with a higher risk for death and treatment toxicity, the rs4149178 (*SLC22A7*) was associated with anthracycline-induced cardiotoxicity and in the same population of our study, *ABCC4* were associated with no response during the treatment of LMC. These results vary according to the population studied, as the Brazilian population has a considerable genetic heterogeneity, our results may have a great impact on the specific literature. In conclusion, our data showed that the polymorphisms studied of the *ABCC4*, *ABCG2* and *SLC22A7* genes are not statistically significant.

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SANDY: A straightforward and complete next-generation sequencing read simulator

Thiago Luiz Araujo Miller[1]; José Leonel Lemos Buzzo[1]; Felipe Rodolfo Camargo dos Santos[2]; Helena Beatris Conceição[2]; Rodrigo Barreiro[1]; Gabriela Guardia[3]; Fernanda Orpinelli Ramos do Rego[3]; Pedro Alexandre Favoretto Galante[3*]

[1]Instituto de Química - USP. [2]Programa Interunidades em Bioinformática - USP. Instituto de Ensino e Pesquisa - Hospital Sírio Libanês.

*pgalante@mochsl.org.br

Palavras-chave: Simulation;Next Generation Sequencing;Bioinformatic

Setting pipelines for accurately extracting the abundance of next-generation sequencing (NGS) data is achievable only when well-known controls are available. Here we present SANDY, a straightforward and easy-to-use tool that simulates NGS for genomic and transcriptomic data. SANDY simulates reads for the most popular NGS technologies on the market under a controlled scenario. SANDY is suitable to guide users in assessing and validating computational pipelines, as well as in optimizing time and cost in NGS assays.

MICRORNAS, POTENTIAL COORDINATORS OF THE INSULIN PATHWAY AND THE AGING PROCESS OF *APIS MELLIFERA*

Fábio Oliveira Barbin[1]; Zilá Luz Paulino Simões[2];

[1]Faculdade de Medicina de Ribeirão Preto - USP. [2]Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto - USP.

*fabiobarbin@usp.br

Palavras-chave: miRNA; aging; insulin pathway;

Aging is a progressive and natural decline of all the physiological functions intrinsic to all organisms resulting from genetic and environmental interactions. An important pathway of metabolism is particularly affected by senescence, the insulin-signaling pathway, a highly conserved pathway from less derived to vertebrate organisms. In Apis mellifera, during the adult phase, an aging process occurs: the functions assumed by the young bees are different from those assumed by the older bees, an event known as age polyethism. In the young phase, workers called nurses organize the colony and feed the larvae, but from the second week on, the workers begin to perform activities out of the nest, in search of pollen and nectar, and are now called foragers. A unique characteristic, the phenotypic plasticity, allows changes in this program. Thus, several factors influence this characteristic and, as an example, the demographic distribution of the colony, which, with a lower number of young bees, the foragers assume the status of nurses,returning to previous tasks, such as the synthesis of royal jelly and feeding of larvae. Age polyethism has reflexes not only on the physiology of these bees, but also on their behavior and specific metabolic pathways, such as the insulin signaling pathway and its molecular regulators. MicroRNAs are one of these regulatory molecules. They participate in many systemic signaling pathways, including insulin. However, there are still unanswered questions: how do microRNAs, interacting with insulin and ecdysteroid pathways, interfere with the maturation and aging of bees? The insulin pathway plays an important role in the aging process and A. mellifera bee is a good model for this study. In this work, we obtained, through qRT-PCR, the relative transcription of genes throughout the adult life of the bees in both normal conditions and orphaned conditions, thus suggesting the participation of specific microRNAs, miR-8, miR-14 and miR-34, in the regulation of the pten, DOR, pi3k, chico and eip74ef genes that are modulated in the aging process in many organisms. In addition, we analyzed the morphology of fat body cells in their response to aging and orphanage condition. In the end, we could conclude that regulatory molecules, metabolic pathways and cytoplasmic inclusions, resulting from the metabolic modification, can be indicators of the adult development process in these bees and probably in other organisms.

H19 KNOCKDOWN CELL LINE DEVELOPMENT THROUGH PROMOTER MONOALLELIC DELETION BY CRISPR/CAS9

Santos, Renan da Silva[1]; Rodrigues, Louhanna[2]; Tavares, Kaio[2]; Furtado, Gilvan P[3]; Pessoa, Claudia[1]; Furtado, Cristiana L M[1,*].

[1]Universidade Federal do Ceará. [2]Universidade de Fortaleza. [3]Fundação Oswaldo Cruz/CE.

*clibardim@gmail.com

Palavras-chave: CRISPR/Cas9; Knockdown; *H19;* Cancer.

Target therapy is one of the most promising fields in cancer treatment. In this context, genetically modified cellular models play an essential role in the screening of new anticancer molecules, as they help in understanding the tumorigenesis process. The *H19* long non-coding RNA has a controversial role in cancer initiation and progression, acting both as a tumor suppressor gene and an oncogene, being a potential molecular target for cancer treatment. Thus, we sought to develop a cellular model through *H19* knockdown in murine C2C12 cells via CRISPR/Cas9 for the screening of new anticancer epigenetic drugs. The *in silico* CRISPR design platform was used for the construction of two guide RNAs (gRNA) to edit the promoter region of *H19*. Two independent and functional vectors were constructed to generate double-strand DNA breaks. The molecular cloning was followed by digestion and ligation of the guides to the plasmid px458 and the transfections were followed by electroporation of the vector in C2C12 cells. Genomic DNA was extracted from transfected cells and the restriction enzyme T7 assay was performed. Following the confirmation of the vector functionality, the transfection step was re-done using two vectors simultaneously to isolate isogenic cell colonies. After observing the growth and amplification of the edited region of 76 isogenic colonies, only one presented a distinct cell amplification profile compared to the normal genotype, with a possible deletion of 100 base pairs (bp), indicating a modification in only one allele. The Sanger sequencing confirmed that this deletion was in fact of 80 nucleotides in the edited colony. This deletion seems to reduce the *H19* gene expression in the edited cells (1.00 ± 0.09) when compared to non-edited ones (1.50 ± 0.25). The monoallelic deletion by CRISPR-Cas9 assay reduced the *H19* expression in C2C12 murine cells. The *H19* knockdown cell line can be used to identify the role of this gene in tumorigenesis and for screening of epigenetic drugs in the targeted therapy.

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POLYMORPHISMS IN INFLAMMATORY PATHWAY ASSOCIATED WITH ANTHROPOMETRICAL AND BIOCHEMICAL OUTCOMES OF A PHYSICAL INTERVENTION PROGRAM

Ana Cláudia M. B. Gomes Torres[1*]; Luciane Viater Tureck[1]; Ricardo Lehtonen Rodrigues de Souza[1]; Lupe Furtado-Alle[1]

[1]Department of Genetics, Federal University of Paraná, Curitiba, Paraná, Brazil.

*anaclaudiambraga@gmail.com

Key-words: polymorphisms; inflammatory pathway; physical exercise.

Obesity is a condition characterized by abnormal or excessive fat accumulation. This condition leads to low grade chronic inflammation caused by change of macrophages pattern to M1 subtype related with release to pro-inflammatory cytokines. The aim of this study was to investigate relation between polymorphism in inflammatory pathway genes: *TLR2, TLR4, IL1B, IL6, TNF, NFKB1, NFKBIA, NLRC4, CARD8* and *NEK7* genes and anthropometrical and biochemical outcomes of physical exercise programs. Sample was constituted by 58 children and adolescents (47 obese and 11 overweight) that participated of physical exercise programs during 12 weeks. Children and adolescents were submitted to 3 types of exercise: HIIT (High Intensity Interval Training), land-based aerobic exercise and water walking. Anthropometrical and biochemical parameters were collected before and after of physical intervention program as follows: body mass index (BMI), body fat percentage (fat), LDL cholesterol (LDL-C), HDL cholesterol (HDL-C), triglycerides (TG), total cholesterol (TC), insulin, glucose, HOMA-IR (Homeostatic Model Assessment for Insulin Resistance) and QUICKI (Quantitative Insulin Sensitivity Check Index). Hardy Weinberg equilibrium was tested by chi-square test; means were compared by t test or Mann-Whitney test. Univariate and multivariate linear regression were performed to verify possible associations between polymorphisms and difference (before–after) of anthropometrical and biochemical parameters. We found associations between 14 variants in 10 genes with anthropometrical and biochemical outcomes as follows: AA genotype of rs13105517 (*TLR2*) was associated with lower BMI and improvement of HOMA-IR and QUICKI indexes; CC genotype of rs3804099 (*TLR4)* with higher TG; AA genotype of rs1927911 (*TLR4*) with higher BMI and lower HOMA-IR index; CC genotype of rs1554973 (*TLR4*) with higher BMI, TT genotype of rs3917356 (*IL1B*) with lower fat; AA genotype of rs2069845 (*IL6*) with lower reduction of LDL-C and TC; GG genotype of rs3755867 (*NFKB1*) with lower fat and higher LDL-C; C allele of rs3138053 (*NFKBIA*) with lower glucose; TT genotype of rs385076 (*NLRC4*) and GG genotype of rs455060 (*NLRC4*) with higher fat; G allele of rs6509366 (*CARD8*) with lower TG; G allele of rs7258674 (*CARD8*) with higher HDL-C; GG genotype and G allele of rs6671879 (*NEK7*) with lower glucose and TG respectively. Genotypes related with possible anti- inflammatory profile provide improvement of biochemical and anthropometrical outcomes of physical intervention program.

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CHARSAT-52 IS AN OLD SATELLITE DNA FOUND IN CHARACIFORMES ORDER (VERTEBRATA, TELEOSTEI)

Rodrigo Zeni dos Santos[1]; Pedro Henrique de Mira Rodrigues[2]; Fausto Foresti[2]; Duílio Mazzoni Zerbinato de Andrade Silva[2]; Fábio Porto-Foresti[1]; Ricardo Utsunomia[1,2]

[1]Universidade Estadual Paulista (campus Bauru), Departamento de Ciências Biológicas. [2]Universidade Estadual Paulista (campus Botucatu), Departamento de Morfologia.

rodrigo-zeni@hotmail.com

Palavras-chave: bioinformatics; neotropical fish; concerted evolution

Eukaryotic genomes are mainly composed of repetitive DNA sequences, such as satellite DNAs (satDNAs), known to form long arrays of tandem repeats. In general, these sequences are located in heterochromatic areas and exhibit fairly high evolutionary dynamics, which might be associated with the fact that many of these sequences are species- or genus-specific. For this reason, there are very few cases of conserved satDNAs in the family- or order-levels, which often surpasses millions of years. Recent studies covering the satellitomes of distant species within the Characiformes order (origin at 110mya) revealed the existence of a fairly conserved satDNA. In this context, this study aimed to uncover the origin of this satellite DNA, referred as CharSat-52, as well as to compare the abundance, localization and variation (intra- and intergenomic) at nucleotide and chromosomal levels. For this purpose, several Illumina libraries of multiple Otophysa species (including the orders of Gymnotiformes, Characiformes, Siluriformes and Cypriniformes) were evaluated for the presence of this satDNA. Initially, BLAT searches evidenced the presence of this satDNA exclusively in Characiformes species. Posteriorly, we isolated and aligned CharSat-52 monomers for each species, which evidenced a significant variation in the abundance of this satDNA in several species, although the nucleotide diversity was very low in the between-species level. Fluorescence *in situ* hybridization (FISH) with this probe revealed that this satDNA is clustered in several chromosomes exclusively in a single species, *Characidium gomesi*, while remained scattered and not formed large arrays in all the other species. Our results evidenced that the ChaSat-52 was present in the ancestral genome of Characiformes and point to a frozen satellite DNA in this order. Subsequent diversification, such as accumulation or retraction, seem to be governed by contingent processes.

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TRANSCRIPTOME AND GWAS EVALUATION FOR RESISTANCE TO *Aeromonas hydrophila* IN PACU (*Piaractus mesopotamicus*)

Vito Antonio Mastrochirico-Filho[1]; Raquel Belini Ariede[1]; Milena Vieira de Freitas[1]; Lieschen Valeria Guerra Lira[1]; John Fredy Gomez Agudelo[1]; Natália Jade Mendes[1]; Ricardo Utsunomia[2]; Fabiana Pilarski[1]; José Manuel Yáñez[3]; Carolina Penaloza[4]; Alejandro P. Gutierrez[4]; Diego Robledo[4]; Ross Houston[4]; Diogo Teruo Hashimoto[1*]

[1]Aquaculture Center of Unesp, São Paulo State University (Unesp), Jaboticabal, Brazil. [2]Instituto de Biociências, Universidade Estadual Paulista, Botucatu, Brazil. [3]Facultad de Ciencias Veterinarias y Pecuarias, Universidad de Chile, Chile. [4]The Roslin Institute and Royal (Dick) School of Veterinary Studies, University of Edinburgh, UK

*diogo.hashimoto@unesp.br

Disease outbreaks are considered a major bottleneck for the development of the aquaculture sector. *Aeromonas hydrophila* is a pathogen bacteria widely distributed in freshwater fish farms. Genetic selection directed to disease resistance may represent a sustainable alternative to improve productive performance in culture systems. Pacu (*Piaractus mesopotamicus*) is one of the main farmed freshwater fish species of South America. The aim of this study was: i) to estimate genetic parameters of resistance to *A. hydrophila* in pacu; ii) to identify genes differentially expressed of the immune system related to resistance in pacu by RNA-seq and iii) perform linkage map analysis with genome-wide association studies (GWAS) by RAD-seq. Fifty full-sib families of pacu from the breeding program of Caunesp (Aquaculture Center of the São Paulo State University, Brazil) were used as biological material. In total, about 40 pit-tagged animals were used from each family for the challenge (completely randomized): approximately 30 for infectious treatments (10 in each replicate) and 10 for the control group. Infection was induced by intraperitoneal inoculation of concentration previously determined by LD^{50} ($8x10^7$ CFU ml^{-1}). The traits evaluated for disease resistance were survival rate and time of death. The most susceptible family to infection had a cumulative mortality rate of 64.3% and mean time of death of 5.2 h. Heritabilities (h^2) of resistance to infection were considered low for survival rate (0.15 ± 0.05) and time of death (0.12 ± 0.05). Transcriptome sequencing provided 226,803 unique transcripts and 172,006 putative genes. Functional analyses suggested 2,783 genes related to immune system processes. 6,485 genes were differentially expressed for resistant individuals when compared with susceptible individuals (adjusted p-value < 0.1) with 3,114 upregulated and 3,371 downregulated genes (q-value < 0.1). In relation to the linkage map in pacu, the number of SNPs varied from 186 to 768 in the 27 linkage groups. The length of linkage groups varied from 79.65 to 131.83 cM. The density of linkage groups ranged from 0.15 to 0.44 cM. ssGBLUP analysis suggested that resistance traits have a polygenic architecture affected by multiple loci with small effects on *Aeromonas hydrophila* resistance of pacu. These genetic and genomic data will assist in the selection of superior genotypes for disease resistance in pacu and will serve as framework for other native species of economic interest in South America.

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MOLECULAR IDENTIFICATION OF *Sicalis flaveola* IN SAMPLES COLLECTED IN CAPTIVITY BIRDS

Shirlene Sandoval Arias*; Carolina Barros Machado; Pedro Manoel Galetti Jr.

1 Federal University of São Carlos, São Carlos, SP, Brazil*. 2 Federal University of São Carlos, São Carlos, SP, Brazil. 3 Federal University of São Carlos, São Carlos, SP, Brazil

*monsesandov@gmail.com

Keywords: Passeriformes; illegal traffic; COI.

IBAMA and the "Rede Nacional de Combate ao Tráfico de Animais Silvestres" record that *Sicalis flaveola* is one of the ten species of birds most trafficked illegally in Brazil. Recent study showed that is also the most hunted species in São Paulo state, despite it being breaded in innumerable breeding sites in the state. The genus has a taxonomic uncertainty and depending of the taxonomist a group ranging from eleven to thirteen species. Brazil has four of the thirteen species (*S. flaveola*, *S. luteola*, *S. citrina* and *S. columbiana)* and three of them *(S. flaveola*, *S. luteola* and *S. citrina)* live in the region of São Paulo state. Considering that situation and with the aim to identify a taxon of our samples, we collected ulnar blood from *Sicalis flaveola* birds from eight different breeders in São Paulo state. We analyzed if that samples registered as *Sicalis flaveola* in SISPASS really belong to the reported species using the mitochondrial marker COI. We used three different COI-based methods: the traditional DNA barcoding (based on two thresholds: 2% and the optimum threshold estimated in R using the SPIDER package), GMYC analysis and a Bayesian implementation of PTP. These methods implement different algorithms and strategies of sequence sorting during classification decision. We also added sequences from other *Sicalis* species present in public databases: NCBI and reference sequences from BOLD systems. Our final dataset was composed of 93 sequences from four *Sicalis* species with 578 base pairs. All the methods employed (DNA barcoding, GMYC and bPTP) were congruent, our analyses suggest the presence of four molecular entities (considered "species" for our analysis): *S. flaveola*, *S. luteola*, *S. olivascens* and *S. luteocephala*. Our samples were groupedin *S. flaveola* taxon and we confirmed that the samples are the same specie registered in SISPASS. The results revealed that molecular identification tools were effective to identify correctly the *Sicalis* species and should be employed when the morphological identification is not conclusive

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CARIOTIPICAL SIMILARITY AND GENOMIC AFFINITY BETWEEN THREE SPECIES OF *PIPER* L. NATIVES OF THE AMAZON

Nina Reis Soares[1]; Jhonata Costa da Silva[1], Caio Túlio Rodrigues Correia[1]; Giovana Augusta Torres[1]

[1]Departamento de Biologia, Universidade Federal de Lavras.

*ninareissoares@hotmail.com

Palavras-chave: Piper; FISH; GISH

The Piperaceae family is known to be a producer of essential oils as part of their secondary metabolism, being used in traditional medicine. The genus *Piper* has 1400 species, of which 400 occur in Brazil. The native species of Acre: *P. aduncum, P. hispidinervum* and *P. affinis hispidinervum* are producers of dilapiol, safrol and sarisan, oils of economic importance, having insecticidal, fungicidal and bactericidal activity, as well as being used in the chemical and perfumery industries. The species are morphologically similar, which generates great taxonomic controversy. Some studies classify *P. hispidinervum* and *P. aduncum* as distinct species, others consider *P. hispidinervum* as a chemotype or a variety of *P. aduncum*. The classification of *P. affinis hispidinervum* it is also discussed, being classified in some works as a chemotype of *P. hispidinervum*, while others consider it a hybrid between *P. hispidinervum* and *P. aducum*. The taxonomic definition does not contain information with higher resolution and the study of the chromosomes can help in distinguishing the species, as in the cases of other taxonomic groups. In the present work the native species of Acre were evaluated: *P. aduncum, P. hispidinevum* and *P. affinis hispidinervum*, for their karyotype, fluorescent banding pattern, 45S and 5S ribosomal RNA by Fluorescent in situ hybridization and genomic affinity by means of Genomic in situ hybridization in order to identify the existence of chromosomal polymorphisms, which may contribute to their differentiation. The three species have 2n = 26, x = 13. *P. aduncum* with chromosomes measuring 1.41 to 3.32 Km, *P. hispidinervum* of 1.65 to 3.70 Km and *P. affinis hispidinervum* of 1.60 a 4.22 Km, the morphology of the *P. aduncum* chromosomes is metacentric and that of *P. hispidinervum* and *P. affinis hispidinervum* is composed of metacentric and submetacentric chromosomes. All three species have a genome with a size close to 1 Gb. All presented 1 CMA+ pericentromeric band on one pair of homologous chromosomes. The banding with the fluorochrome DAPI did not produce bands. During the analysis of ribosomal DNA, 1 pair of pericentromeric heteromorphic 45S tags were identified on chromosome 4, which are co-localized with the CMA+ tags, and 1 pair of pericentromeric 5S tags on chromosome 7 of the three species. Genomic affinity through reciprocal hybridizations involving the three species revealed patterns of very similar, predominantly pericentromeric and proximal tags on all chromosomes in all species, revealing the similarity of the repeating fraction to type and localization.

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DOES CENTROMERE DRIVE EXPLAIN HIGH DIVERSIFICATION OF CENH3 CENTROMERE PROTEIN?

João Pedro do Carmo Filgueiras[1]; **Iderval da Silva Júnior Sobrinho**[1]

[1]Universidade Federal de Jataí - GO.

iderval_jr@yahoo.com

Key-words: molecular evolution; histone; positive selection

Centromeres are essential to chromosome segregation during cell division. Centromere formation is epigenetically marked in nucleosomes by the presence of CENH3 histone. Differently from its paralogous copy (histone H3), CENH3 shows a history of rapid evolution inside lineages. This rapid evolution is mainly observed in the Loop-1 and N-terminal tail domain of CENH3 and is explained by the Centromere Drive Hypothesis (CD Hypothesis). According to this hypothesis, CENH3 high evolutionary rate would be triggered by expansions via duplications of centromeric chromatin, which should favor a "competition" among chromosomes to be included in the unique viable egg in asymmetric female meiosis. In this scenario, chances in CENH3 which would enhance the affinity of this protein to the centromeric region would be positively selected, since they would compensate for the meiotic distortion caused by the expansions in the centromeric region. Considering that the core assumption of CD Hypothesis to explain CENH3 rapid diversification is the occurrence of asymmetric meiosis, one way to test the general validity of this theory could be the study of CENH3 evolution in lineages that have symmetric meiosis (zygotic meiosis). Then, this work aims to test the CD Hypothesis as a general hypothesis by studying the evolution of CENH3 in the Apicomplexa, which is a lineage with symmetric meiosis. We collected CENH3 coding sequences from genomes of four Apicomplexa lineages (Cryptosporida, Eimeriidae, Haemosporida e Sarcocystidae) obtained by BLAST software from GenBank. We aligned the sequences using WebSoftware T-Coffee and estimated a Maximum Likelihood phylogeny using MEGA7 software. We tested for signals of diversification by positive selection in the four Apicomplexa lineages using the Branch-site Test implemented in PAML software. In case we would accept CD Hypothesis, we would expect to find a low evolutionary rate in CENH3 in lineages where meiosis is symmetric, like in the Apicomplexa lineage. However, our results evidenced a high evolutionary rate for CENH3, promoted mainly by positive selection. The signals of positive selection were detected in all tested Apicomplexa lineages and were identified in 19 sites, from which nine were found in secondary structures responsible for protein-protein interactions, such as Loop-1, alpha helix-2, and alpha helix-N. We conclude that CD Hypothesis fails as a general explanation of the rapid diversification of centromere protein CENH3. Considering that some diversifying sites were located in protein-protein interaction regions, we propose a new hypothesis in which the rapid diversification of CENH3 is better explained by a coevolution among CENH3 and kinetochore proteins that interact with it.

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Analysis of matri and patrilineages of the quilombola community of Pau D'Arco: a genetic historical study

Allan Ribeiro Reis Scharf Costa[1]; Bárbara Pessoa de Santana[2]; Márcia Danielle dos Santos[2]; Luiz Antonio Ferreira da Silva[2]; Dalmo Almeida de Azevedo[3].

[1]Departamento de Genética, Universidade Federal de Alagoas, Brasil. [2]Departamento de Genética, Universidade Federal de Alagoas, Brasil. [3]Departamento de Genética, Universidade Federal de Alagoas, Brasil.

allanscharf@hotmail.com

Keywords: Quilombolas; Y Chromosome; Mitochondrial DNA.

The Quilombola communities were refuges mainly for Black Slaves and, to a lesser extent, by Amerindians and Whites excluded from society; the three main ethnic groups responsible for the miscegenation of the Brazilian people. Microsatellites and SNPs of the Y chromosome and SNPs of mitochondrial DNA have importance and usefulness in the research of population origin, since they are able to identify and trace a genetic history of the male and female fraction, respectively, of populations. This study aimed to analyze these data in 54 volunteers living in the Quilombola community of Pau D'Arco, near the city of Arapiraca, state of Alagoas; in order to obtain, provide and add molecular information about the ancestors of the current residents of this community. Genotyping was performed with AmpFlSTR YFiller kit for Y STR. Y chromosome and mtDNA SNPs were analysed with SNaPshot. The Pau D'Arco community was then compared with several Quilombola populations, Amerindians and other admixed Brazilian populations. For Y chromosome, a total of 25 haplotypes were observed by STR analysis, and 8 haplogroups by SNPs analysis were identified in 54 individuals. The most common haplogroups were J2 European (39.6%), E1b1a African (24.5%) and Q1a3a Amerindian (1.9%). Female lineages were comprised by 7 mtDNA haplogroups in 53 individuals. The most common were African L3 (41.5%), Amerindian A (17%) and European R(xB) (3.8%). Significant genetic distance was found among all the populations compared, except for the Quilombola population of Vila Santo Antônio, for mtDNA SNPs. The population with the lowest genetic distance was Quilombo, for both the Y chromosome and mtDNA SNPs. The most genetically distant for the Y chromosome SNPs analysis was Vila Santo Antônio and for mtDNA SNPs, Tabuleiro dos Negros. From the verified haplotype frequency, there was no founding effect observed in the community of Pau D'Arco. From the results, a greater contribution of African and Amerindian female ancestors and a greater contribution of European and African male ancestors to the formation of the population were observed.

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OPTIMIZATION OF A DNA EXTRACTION PROTOCOL FOR LARGE-SCALE GENOTYPING OF SHRIMP (*Litopenaeus vannamei*) SAMPLES WITH A FLUIDIGM EP1 PLATFORM

Nayelle Meyre Lisboa Silva[1]; Noeliton Teixeira de Araújo Junior[2]; Gleison Ricardo de Biazio[2]; Alexandre Rodrigues Caetano[2]; Patrícia Ianella[2].

[1]Programa de Pós-Graduação em Ciências Animais, Universidade de Brasília, Brasília, DF, Brazil. [2]Embrapa Recursos Genéticos e Biotecnologia, Brasília, DF, Brazil.

NayelleMeyre@gmail.com

Keyword: extraction of DNA; genotyping; *Litopenaeus vannamei*.

Scalable, low-cost, DNA extraction protocols, which are easy to execute, are of great interest to laboratories performing large-scale molecular biology, genetics and genomics studies involving large numbers of samples. The objective of this study was to adapt and optimize a scalable DNA extraction protocol for Pacific White Shrimp (*Litopenaeus vannamei)* samples, for genotyping Single Nucleotide Polymorphism markers (SNPs) on a Fluidigm EP1 platform. Different amounts of different types of tissues (muscle (M) and pleopod divided into protopodithium (P) and exopodite (E)), as well as volumes of reagents were tested. Tissue samples from eight different individuals obtained in a retail store were submitted to the following treatments: (a) extraction in 1.5ml microtubes with 20mg of muscle (MT) and protopodite medium (P1T) samples; and (b) extraction on 96- well PCR plates: 20mg of muscle (MP), protopodite medium (P1P); exopodite (E1P) and half exopodite (E2P). A total of 100Kl of extraction buffer (50mM KCl, 10mM Tris-HCl pH9,0, 0.1% Triton X-100) and 4Kl of proteinase K (pK, 20mg / ml) were added to each sample in all treatments, except for E2P, where 50Kl of buffer and 2Kl of pK were used. Samples were incubated at 56°C for three hours and at 96°C for 10 minutes for pK inactivation. To verify the efficiency of different DNA extraction treatments, genotyping of all samples, using a customized panel with 96 SNPS, was performed with a Fluidigm EP1 platform, according to the manufacturer's guidelines. Mean proportions of genotyped SNPs (call rates), considering genotype confidence levels >0.98, were calculated for each treatment. Treatments using muscle tissue (MT and MP) showed the lowest average call rates (MT=52.86% and MP=70.31%). Treatments using protopodite samples showed a mean call rates >90% (PT: 90.62%, PP: 91.01%), while treatments using exopod samples showed call rates of 91.53% (E1P) and 92.18% (E2P). Extractions performed in PCR plates generated good quality DNA for genotyping, providing an useful solution for parallel processing of large numbers of samples, while reducing costs and issues with identification of individual tubes. Reducing the amount of buffer and pK (E2P) did not result in significant differences in average call rates, considering treatments P1T, P1P and E1P however, additional overall cost-reductions can there be obtained in addition to the possibility of processing tissue samples of smaller sizes. Observed results indicate that E2P is the most efficient DNA extraction protocol, because it provides good quality DNA for genotyping with call rates> 0,90 and also allows for reduction of costs and use of small portions of tissue.

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THE EVALUATION OF RESISTENCE MUTATIONS TO PROTEASE INHIBITORS IN PATIENTS LIVING WITH HIV/AIDS IN STATE OF PARÁ, BRAZIL IN THE PERIOD OF 2013 TO 2015.

Aline Cecy Rocha de Lima[1*]; Alexandre Augusto Bentaberry Rosa[1]; Jeniffer Ferreira Viana[1]; Maria Amélia de Oliveira da Costa[2]; Rubens Einar Corrêa Dantas[2]; Antonio Carlos Rosário Vallinoto[1]; Rosimar Neris Martins Feitosa[1].

[1]Laboratório de Virologia - ICB, Universidade Federal do Pará, Brazil. [2]Laboratório Central do Estado do Pará, Brazil.

*alinececy@yahoo.com

Key-words: HIV; Mutation, Drug resistance

Introduction: The HIV epidemic is a serious public health problem because of the high mortality rate across all continents. However, with the discovery of antiretrovirals that are used to prevent viral replication in the cell, the level of mortality from this virus has decreased over time, thus contributing to an increase in the life expectancy of people living with HIV mutations to Protease Inhibitors and to relate them with possible resistance to these drugs. **Methods:** This cross-sectional, retrospective-descriptive study included patients living with HIV-1 residing in the state of Pará. Epidemiological and clinical data were obtained by collecting information from genotyping conducted between 2013 to 2015, which were under the custody of the Central Laboratory of Pará. All information was edited, tabulated, quantified and presented in an Excel worksheet. **Results and Discussion:** The majority of the individuals were male (53%) and brown (36%). The group with age between 36 and 45 years was the most prevalent. HIV-1 subtype B was the most frequent in this study. The R41K mutation was the most commonly found when it came to the class of Protease Inhibitors with 52.07%, followed by L63P with 43.20%, with E35D, M36I and 77I being the third mutations with 40.24% each. Mutations 54M; 60N; 63A/T; 63A/V; 63P/T; 63P/Q; 71L; 73A; 73T; 36 I/L; 10F/I/V; 10R; 47A; L10V/L were observed at a lower frequency (0.08% each). The drug Atazanavir (ATV) had the highest resistance index (25.40%); secondly, Nelfinavir was found with 23.87%, followed by drugs: Indinavir (IDV); Atazanavir/Ritanavir (ATV/r); Fosamprenavir (FPV); Fosamprenavir/Ritanavir (FPV/r); Indinavir/Ritanavir (IDV/r) and Saquinavir/ritanavir (SQN/r). The associations of Tipranavir/Ritanavir (TPV/r) and Darunavir/Ritanavir (DRV/r) had the lowest resistance frequency, with 1.06% each. A study carried out in Curitiba, found the mutations described in this study, presenting the L63P mutation with the second highest frequency, however, showed differences in the frequencies for the other mutations. An important factor to be evidenced is the low genetic barrier of this class, which sometimes hinders the appearance of resistance, since a series of mutations to generate resistance is necessary. **Conclusion:** In the present study, the most frequent mutation considering protease inhibitors was R41K, followed by L63P, differing from another study carried out in Pará, in which the L63P mutation was the most observed. Thus, the pharmacological and genotypic monitoring of each patient submitted to antiretroviral therapy is important, aiming at monitoring the appearance of mutations and their correlation with the resistance profiles for both the class of protease inhibitors and for the other classes of antiretrovirals.

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AIRE GENE INFLUENCES mTEC-mTEC CELL AGGREGATION ON A THREE-DIMENSIONAL SPHEROID FORMATION MODEL SYSTEM

Ana Carolina Monteleone Cassiano[1,2,3]; Dimitrius Leonardo Pitol[4], Janaína de Andrea Dernowsek[6], Mayara Cristina Vieira Machado[2,5], Geraldo Aleixo Passos[2,4]

[1]Postgraduate Program in Basic and Applied Immunology – FMRP/USP. [2]Molecular Immunogenetics Group, Department of Genetics, FMRP/USP. [3]Medical School of Ribeirão Preto -FMRP/USP. [4]School of Dentistry of Ribeirão Preto -FORP/USP. [5]Postgraduate Program in Genetics -FMRP/USP. [6]National Institute of Science and Technology in Regenerative Medicine - INCT Regenera

*monteleone@usp.br

Keywords: Aire gene; cell adhesion; spheroids.

The autoimmune regulator (Aire) gene is a transcriptional controller in medullary thymic epithelial cells (mTECs). It controls the expression of a large set of genes encoding peripheral autoantigens (PTAs) involved in the self-representation in the thymus and induction of immunological tolerance. Besides PTAs, Aire also controls expression of genes that encode adhesion molecules. Accordingly, we hypothesized that this gene could influence adhesion between the mTEC cells (mTEC-mTEC adhesion). To test this, we used the loss of function (LOF) strategy using an Aire KO mTEC clone, which had been previously obtained in our laboratory by the Crispr-Cas9 system (mTEC 3.10E6 Aire $^{-/-}$). The mTEC-mTEC adhesion was assessed through 3D cultures for spheroid formation comparing Aire wild-type (WT) (Aire $^{+/+}$) vs Aire KO (Aire $^{-/-}$) cells. We observed the spheroid formation over time and observed that these two cell types show differences in spheroid growth-curve and morphology. Monitoring the spheroid growth has been made though cell counting and time-lapse video captures over 24 h of spheroid cultures by using a CytoSMART ™ System. Aire WT mTEC spheroids were formed after 12 h cultures whereas Aire KO mTEC spheroids showed a delay in the cell-cell aggregation and only after 20 h of culture did the Aire KO cell spheroids consolidate and compact tightly. In order to better characterize the 3D spheroid model system, we constructed a growth curve and quantified the number of cells that form the spheroids at different culture periods (counting cells at 12 h culture interval). We observed that with 12 h culture the Aire KO mTEC spheroids had a cell count greater than that of the WT mTEC spheroids although tightly differed as well as spheroid diameter. In order to observe the pattern of spheroid cellular organization we performed scanning electron microscopy that allow us to study the spheroid surface in detail and observe differences between WT and Aire KO cell spheroids. The WT spheroid show strongly compacted with well defined contour, while Aire KO cell spheroids show irregular surface. These results provide us with a better understanding of the function of Aire in the 3D mTEC-mTEC interaction, whose process is crucial for structuring of the thymus in vivo.

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EXPRESSION OF DNA METHYLTRANSFERASE AND DEMETHYLASE GENES DURING TESTIS DEVELOPMENT

Anaíde Silva Sousa[1]; Jorge Augusto Petroli Marchesi[1*]; Marcia Delfino Hayaxibara[2], Ester Silveira Ramos[1]

[1]Departament of Genetics, Ribeirão Preto Medical School, University of São Paulo. [2]Division of Medical Genetics, Clinical Hospital, Ribeirão Preto Medical School, University of São Paulo.

* jorgea_petroli@hotmail.com

Palavras-chave: DNA methylation, epigenetic reprogramming, animal epigenetics.

DNA methylation is an important epigenetic mechanism involved in the regulation of gene expression. The control of gene activation presents a critical role during gonadogenesis. In mammals, the moment of testis epigenetic reprogramming has already been described after sex determination. In chicken, as in other birds, it is still unknown in which point of development, from the undifferentiated gonad into functional testis this process acts. Our objective was to investigate the expression of genes that are responsible for DNA methylation and demethylation in different stages of testis development. We analyzed chicken fertilized eggs. Testes samples were collected from chicken embryos during different stages of gonadal development (HH32, HH35, HH38, HH41, and HH45). Total RNA was isolated and the cDNA synthesis was performed. The expression of six target genes, three genes that encode DNA methyltransferases (*DNMT1*, *DNMT3A* and *DNMT3B*) and three genes that encode DNA demethylases *Ten-eleven-translocation* (*TET1*, *TET2* and *TET3*), was assessed by RT-qPCR using SYBR™ Green system. We used κ-*actina* and *Ribosomal Protein L5* as reference genes. In the HH38 stage, there was a significant increase of *DNMT1*, *DNMT3B* and *TET2* gene expression. The *DNMT3b* gene encodes a methyltransferase that acts in de novo methylation, and the *DNMT1*, in the maintenance of methylation. *TET2* gene plays a key role in active DNA demethylation. In mammals, testis differentiation requires specific demethylation events, and *TET2* gene may be involved in the erasure of acquired epigenetic information, while *DNMT3B,* in the establishment of a new methylation pattern during chicken testis development. Our results suggest that testis epigenetic reprogramming may occurs around the HH38 stage, and that the *DNMT3B* and *TET2* genes play a relevant role in this mechanism throughout chicken gonadal development.

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Rare genetic variants in a patient with Autistic Spectrum Disorder reveal interconnection between intracellular signaling pathways in an oligogenic pattern of inheritance

André Luiz Teles e Silva[1,2]; Juliana da Cruz Corrêa Velloso [3], Talita Glaser[3], Karina Griesi-Oliveira[2], Alexander Henning Ulrich[3], Maria Rita Passos Bueno[1], Andrea Laurato Sertié[2].

[1] Instituto de Biociências. Universidade de São Paulo - USP. [2] Hospital Israelita Albert Einstein – Centro de Pesquisa Experimental. [3] Instituto de Química. Universidade de São Paulo - USP.

andre.luiztelles@hotmail.com

Palavras-chave: ASD, *CACNA1H* gene, *RELN* gene, mTORC1 signaling.

Recentgenomicstudieshaveidentifiedalargenumberofrareandpotentiallydeleterious variants in Autism Spectrum Disorder (ASD), most of them rare and private. One of the major current challenge is to determine which of these variants are involved in the etiologyofthediseaseandhowmanyvariantsarerequiredforthecompletepenetrance of the disorder in each patient. Recently, we performed whole-exome sequencing in a subgroup of ASD patients, in whom we found mTORC1 signaling hyperfunction, and identified in one of the patients, referred to as F2688, rare and potentially pathogenic variants in the *RELN* and *CACNA1H* genes. The *RELN* gene encodes Reelin, a large secreted glycoprotein that controls neuronal migration and plasticity of synapses. The *CACNA1H* gene encodes the α1-subunit of the T-type low voltage dependent calcium (Ca^{2+}) channel Cav3.2. Using iPSC-derived neural progenitor cells (NPCs) from patient F2688, we have recently shown that the variants identified in the *RELN* gene are deleterious and lead to diminished Reelin secretion and impaired Reelin signal transduction.Also,ourresultssuggestthatmTORC1signalingisoveractivatedinF2688 NPCs and function as a "second hit" event contributing to downregulation of the Reelin cascade. We do not yet know whether the *CACNA1H* variant identified in this patient contributes to overactivation of the mTOR pathway and to downregulation of the Reelin signaling. Theaimsofthisstudyaretoevaluatewhethertherarevariantidentifiedinthe *CACNA1H* gene in patient F2688 are functional, affects Ca^{2+} influx, alters the activity of mTORC1 and Reelin signaling pathways and the migration of NPCs derived from this patient. In order to achieve this, we used as experimental models HEK293 cells overexpressing wild-type and mutated *CACNA1H*, as well as NPCs from patient F2688 and from control individuals. We observed that HEK293 cells overexpressing mutated *CACNA1H* show significantly increased Ca^{2+} influx and hyperfunctional mTORC1 signaling, which were rescued when cells were cultivated with a blocker of T-type Ca^{2+} channels (NNC55-0396 dihydrochloride). Concordantly, NPCs from F2688 patient exhibitincreasedCa^{2+}influx,andtrea tmentofthesecellswithNNC55-0396rescuedboth hyperfunctionalmTORC1signalingandimpairedReelincascade,suggestingacrosstalk between*CACNA1H*andReelinpathwaysthroughmTORC1signaling.Finally,wefound that F2688 NPCs show altered migration patterns, which were also rescued byblocking T-type Ca^{2+} channels. Taken together, our results suggest that the variant identified in *CACNA1H*isfunctionallyrelevant,increasesCa2+influxinneuralcellsderivedfromthe patient, which leads to enhanced mTORC1 signaling activity and, in turn, contributes to impaired Reelin signaling and abnormal migration of the cells. This is the first study to show the interconnection between rare genetic variants acting in an oligogenic mode of inheritance in ASD.

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THE GENETICS OF THE MARATHON: GENETIC DIFFERENTIATION IN EAST AFRICAN ETHNICITIES AND ITS RELATIONSHIP WITH ENDURANCE RUNNING SUCCESS

André Luís da Silva Zani[1*]; Mateus Henrique Gouveia[2]; Marla Mendes de Aquino[2]; Nelson Jurandi Rosa Fagundes[1]

[1]Departamento de Genética, Instituto de Biociências, Universidade Federal do Rio Grande do Sul, Porto Alegre, Rio Grande do Sul, Brazil.
[2]Departamento de Genética, Ecologia e Evolução, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, Belo Horizonte, Minas Gerais, Brazil.

*zani.andre@yahoo.com.br

Key-words: endurance runnig; East A

Elite athletes competing in long-distance events accomplish impressive feats, such as running 42.195 km in just over two hours in the Marathon. Since the 1960s, East African athletes manly from Kenya and Ethiopia have been dominating these events. Demographic studies have shown that in each of these countries, two ethnic groups are overrepresented among elite endurance runners: the Kalenjin, from Kenya, and the Oromo, from Ethiopia. While both biological and cultural issues may play a part in explaining such success, no simple explanation has emerged by looking at life history or at simple genetic loci associated to athletic performance. The aim of this study is to contribute to a better understanding of the molecular and physiological processes that ultimately result in long distance running success. We started by hypothesizing that multiple genetic factors predispose individuals of Kalenjin and Oromo ethnic groups to be overrepresented among elite athletes of long distance running events. Next, we looked for genomic peaks of genetic differentiation in these two ethnic groups using public allele frequency data for 1,152,000 single nucleotide polymorphisms (SNPs) obtained from the African Genome Variation Project (AGVP) and the 1000 Genomes Project. For each focal population we scanned the genome using the normalized version of the Population Branch Statistics (PBSn1) in sliding-windows of 20 SNPs with an overlap of 15 SNPs among windows. PBS calculation requires two reference populations in addition to the focal population. We used the Luhya, from Kenya and the Amhara, from Ethiopia as local counterparts, and two composite populations representing West Africa and Eurasia, as distantly related populations. For each comparison, we retained for later analyses the 0.1% windows with the highest PBSn1 values, and annotated the genes associated with these windows around a 5 kbp neighborhood. The full lists of genes were submitted to gene-set analysis using the Functional Mapping and Annotation of Genome- Wide Association Studies (FUMA-GWAS) platform to recover biological processes (GO Biological Processes) and phenotypes (GWAS category) that may be associated with endurance running. So far, we found four genes that are functionally associated with calcium regulation in striated muscle and that may be associated with endurance running: RYR2, GSTO1, PDE4D and CALM2. RYR2 encodes for the Ryanodine receptor 2, which forms a calcium channel in the sarcoplasmic reticulum affecting heart muscle contraction. Proteins encoded by GSTO1 and PDE4D have already been demonstrated as regulators of the activity of ryanodine receptors in heart and skeletal muscle. Finally, CALM2 codes for calmodulin, a calcium binding protein which has been implicated in congenital heart arrhythmias. These results indicate the importance of calcium homeostasis in distance running performance.

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Impact of the enhancer SNP rs5758550 on *CYP2D6* haplotypes and inferred metabolizer phenotypes

Anna Beatriz Ribeiro Elias[1], Gilderlanio Santana de Araújo[2], Sandro José de Souza[3], Guilherme Suarez-Kurtz[1,4]

[1]Coordenação de Pesquisa, Instituto Nacional do Câncer, Rio de Janeiro, Brazil, [2]Instituto de Ciências Biológicas, Universidade do Pará, Pará, Brazil, [3]Instituto do Cérebro, UFRN, Rio Grande do Norte, Brazil, [4] Rede Nacional de Farmacogenética, Rio de Janeiro, Brazil

annaribeiro.elias@gmail.com

Keywords: pharmacogenetics, CYP2D6, metabolic phenotypes

Cytochrome P-450 2D6 (CYP2D6) metabolizes ~25% of currently used medications. The encoding gene, *CYP2D6,* is highly polymorphic, which accounts for large interindividual variability in enzyme activity, affecting drug dosage, efficacy and adverse effects. A single nucleotide polymorphism (SNP), rs5758550, in a critical enhancer region downstream of the *CYP2D6* promoter was recently shown to be a major determinant of CYP2D6 activity, depending on its linkage disequilibrium (LD) with the common *CYP2D6* SNP, rs16947. The extent of LD varies considerably across the 1000 Genomes superpopulations. We extended the analysis of LD between rs5758550 and rs16947 to: i) the overall 1000 Genomes Admixed American superpopulation (AMR, n=347); ii), the four AMR subpopulations, namely CLM, MXL, PEL and PUR; iii) two AMR subcohorts comprising 80 individuals each with the highest proportions of either European (average 0.79) or Native (0.87) ancestry; iv) an admixed Brazilian cohort (n=242) of self-reported White, Brown and Black subjects. The 1000 Genomes data phase III was downloaded and individual proportions of Native, European and African ancestry in AMR were estimated by ADMIXTURE analysis. Individual ancestry and distribution of *CYP2D6* SNPs in the Brazilian cohort were derived from our previous studies. A Taqman probe was used for allele discrimination of rs5758550. The R^2 for LD between rs5758550 and rs16947 ranges from 0.38 (PUR) to 0.88 (CLM) among AMR, is higher in the Native (0.87) than in the European (0.59) AMR subcohorts, and higher in Brazilians with >90% European ancestry (0.52) compared to those with >70% African ancestry (0.16). The variable extent of LD between rs5758550 and rs16947 impacts the inference of CYP2D6 phenotypes based on *CYP2D6* diplotypes. For example, the Clinical Pharmacogenetics Implementation Consortium (CPIC) guidelines list the common *CYP2D6*2* variant haplotype allele, which comprises rs16947, as conveying normal enzymatic activity. However, rs16947 *per se* is a reduced activity allele, and the normal activity conveyed by *CYP2D6*2* results from the LD of rs16947 with the enhancer rs5758550. In the absence of rs5758550, carriers of *CYP2D6*2* have reduced metabolic activity, which is in conflict with the CPIC guidelines. Among Latin Americans, the risk of false prediction of *CYP2D6*2* phenotype is greater in individuals of predominant African ancestry, compared to European or Native ancestry. It is suggested that *CYP2D6* genotyping panels intended for use in Latin American populations should incorporate the enhancer rs5758550 SNP, for adequate inference of CYP2D6 metabolizer phenotype.

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IDENTIFICATION AND INITIAL CHARACTERIZATION OF A HYPOTHETICAL PROTEIN OVEREXPRESSED BY *Trichoderma harzianum* UNDER BIOMASS DEGRADATION

Anna Carolina Emi de Lima Tanada[1]; Maria Lorenza Leal Motta[1]; Jaire Alves Ferreira-Filho[1]; Ricardo Rodrigues de Melo[2]; Letícia Maria Zanphorlin[2]; Anete Pereira de Souza[1]; Clelton Aparecido dos Santos[1, *]

[1]Centro de Biologia Molecular e Engenharia Genética, Universidade Estadual de Campinas, Campinas, São Paulo, Brasil. [2]Laboratório Nacional de Biorrenováveis, Centro Nacional de Pesquisa em Energia e Materiais, Campinas, São Paulo, Brasil.

*clelton@pq.cnpq.br

Keywords: Hypothetical protein; Saccharification; Biofuels.

The conversion of biomass in fermentable sugars via enzymatic hydrolysis for biofuel production is a challenge. To increase the efficiency of the saccharification of cellulosic substrates, enzymes with promising features for industrial applications are continually sought. In particular, proteins that are heavily used by microorganisms under specific conditions are promising targets for practical applications. With advances in RNA sequencing technology, the transcriptional profiles of *Trichoderma harzianum*, an important fungus with an efficient cellulase machinery, and other cellulolytic microorganisms are currently available. Thus, this technology can facilitate the discovery of the differentially expressed genes under specific metabolic conditions. However, several of the targets found during these analyses are in general annotated as gene uncharacterized or hypotheticals and, the role of these genes remains unknown. Here, we report the identification and initial characterization of a hypothetical protein from *T. harzianum* (ThHyp) that was successfully produced using *Escherichia coli* as a host. Initially, transcriptome and secretome data were used to compare *hyp* gene expression and the detection of secreted ThHyp among *T. harzianum* spp. Sequence-based prediction of protein domains showed the presence of a carbohydrate-binding module (CBM) in the ThHyp. Recombinant ThHyp was purified at a high yield and with high purity and showed secondary folding by circular dichroism technique. The tertiary folding of the recombinant purified protein was assessed by size-exclusion chromatography, revealing a monomer monodisperse in solution. Initial screening for enzymatic activity was evaluated employing fourteen different artificial substrates, widely used for the characterization of glycoside hydrolases. Additionally, the ability of the ThHyp to binding to lignocellulosic substrates via CBM was investigated experimentally, revealing its strong affinity by components of the plant biomass matrix, including Avicel and Xylan. The role and mechanism of action of the hypothetical proteins remain to be investigated. In the present study, we produced a recombinant hypothetical protein from *T. harzianum* using a prokaryotic host and confirmed its ability to binding to cellulose. Our work paves the way for further studies evaluating the structure and function of this protein, especially regarding its role in plant biomass degradation.

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CYTOGENETIC CHARACTERIZATION OF PUERARIA PHASEOLOIDES (FABACEAE): CHROMOSOME BANDING, FISH MAPPING AND DNA CONTENT

Aryane C. Reis[1]; Elisa G. Cabral[1]; Lyderson, F. Viccini[1]; Saulo, M. Sousa[1]

[1]Universidade Federal de Juiz de Fora, UFJF.

saulo_marcal@yahoo.com.br

.**Key-words:** Flow cytometric; forage; karyogram

The genus *Pueraria* (Fabaceae) is composed by 19 native species of Southeast Asia with wide distribution in tropical regions around the World. The identification of some species is unclear and the group is considered taxonomically controversial. Several species are used in traditional medicinal and in the textile, paper and timber industries. Among the species of the genus, *P. phaseoloides* consists in an interesting species, once it is widely used in tropical regions as forage. Even though its economic importance and several problems involving its classification, there are few studies about cytogenetic characterization for the species. Thus, we proposed to describe a detailed karyotype for *P.phaseoloides* through chromosomal markers (FISH and chromosome banding) and to estimate the DNA content by flow cytometry. For this, slides were prepared from young root tips pretreated with 8-hydroxyquinoline 0.003 mol/L at 4ºC for 9 h and then fixed in 3:1 v/v ethanol–acetic acid for 24h at -20ºC. Slides with spread metaphases were hybridized with rDNA probes (5S and 45S). Chromosome banding was preformed through fluorochomes DAPI and CMA. The DNA content was estimated though chopping of fresh leaf tissue in order to result in a nuclei suspension. The histograms were analyzed using CytExpert 2.0 software. *P.phaseoloides* presents symmetrical karyotype with 2n=22 chromosomes (2 pairs median and 9 pairs submedian chromosomes). Chromosome banding revealed the occurrence of CMA^+ pericentromeric sites in all chromosomes of set. FISH mapping of rDNA 5S revealed a subterminal site on chromosome IX while 45S sites was located on pericentromeric region on chromosome VIII. The 2C value found was 3.20 pg. These results enhance the karyotype description for *P.phaseoloides* and allow a better characterization of the species for future breeding programs.

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THE INTERPLAY BETWEEN MGMT AND NRF2 IN TEMOZOLOMIDE RESISTANCE

André Uchimura Bastos[1]; Clarissa Ribeiro Reily Rocha[1,2]; Carlos Frederico Martins Menck[1]

[1]Instituto de Ciências Biomédicas, Universidade de São Paulo, São Paulo, Brazil. [2]Departamento de Oncologia Clínica e Experimental, Universidade Federal de São Paulo, São Paulo, Brazil.

* uchibastos@yahoo.com.br

Palavras-chave: Glioblastoma, Temozolomide; MGMT; NRF2; oxidative stress.

MGMT (O-6-Methylguanine-DNA Methyltransferase) is a DNA repair enzyme that is not only an important prognostic factor in gliomas, but also a strong predictor of success in therapy with alkylating agents, such as temozolomide (TMZ). Although TMZ treatment granted improvements in patients' survival, several mechanisms of resistance lead to patients' complications and death. As expected, one of the main mechanisms is the ability of MGMT to directly reverse the DNA lesions caused by TMZ, however several additional mechanisms were identified. Our group showed that glutathione, a peptide that plays a crucial role in the maintenance of redox cell homeostasis, prevents cell death caused by TMZ by a mechanism mediated by the transcription factor NRF2. However, it is unclear how NRF2 modulates TMZ cytotoxicity. Thus, by over- expressing MGMT in glioma cell lines, our aim was to investigate the role of MGMT and NRF2 resistance mechanisms in the TMZ induced cell death. In this work, U138- MG and U251-MG glioma cell lines were transduced with a MGMT expressing vector. Cell viability after TMZ treatment was assessed by XTT assay. MGMT and NRF2 expression were determined by Western blot. Flow cytometry was used to investigate cell cycle information, the levels of active caspase 3 and γH2AX-induction. Surprisingly, when we compared cells expressing MGMT to cells not expressing MGMT, no significant difference in cell viability was observed and a small differential increase in active caspase 3 was found at 24 h in the cell line lacking MGMT. Active caspase 3 levels were higher after 72 h, but no difference were observed between cells. Importantly, TMZ leads to G2 arrest in the cells, even the ones expressing MGMT, however this arrest is accompanied by an increase in subG1 cells in the cell line lacking MGMT. Additionally, only cells not expressing MGMT showed high NRF2 induction after treatment with TMZ. Interestingly, when cells were treated with N- acetylcysteine, an important activator of glutathione pathway, only the cells expressing MGMT did not show cell cycle arrest anymore. Thus, we propose that, although MGMT can provide cell resistance to TMZ treatment, NRF2 plays a crucial role on cell survival upon TMZ treatment.

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CITOTOXIC AND GENOTOXIC EVALUATION OF BOTTOM ASHES LEACHATE FROM CROMATATED COPPER ARSENIATE TREATED WOOD COMBUSTION

Claus Tröger Pich[1*], Rahisa Scussel[1,2]; Nathalia Coral Galvani[2]; Ana Carolina Feltrin[2]; Bárbara Queiroz[1]; Paulo Emilio Feuser[1,2]; Elidio Angioletto[2]; Ricardo Andrez Machado de Ávila[2].

[1]Universidade Federal de Santa Catarina. [2]Universidade do Extremo Sul Catarinense.

*claus.pich@ufsc.br

Keywords: Chromated copper arsenate; Wood ashes leachate; Genotoxic evaluation.

Wood have been widely used for construction, fuel and others. In order to increase its durability preservative treatments are used. Chromated copper arsenate (CCA) is one of the most common. Its composition includes copper (CuO), chromium (CrO_5), and arsenic (As_2O_5). The toxic potential of burned CCA-treated wood can be high, because there are emissions of significant quantities of these components that are known as toxic and carcinogenic. In this way, the objective of our work was to evaluate the cytotoxic and genotoxic effect of bottom ash leachate originated from the combustion of CCA-treated wood compared to untreated one. Ashes were obtained by the combustion of CCA-treated wood and untreated wood and the two leachates were produced using ultrapure water (5g/100ml). Analysis of metals was performed by flame atomic absorption spectroscopy. NIH3T3 cells were used to evaluate the cytotoxicity of leachates by MTT assay. Plasmid DNA cleavage test and comet assay were performed for genotoxic evaluation. Arsenic and chromium concentrations were 59.45 and 54.28 mg/L in treated wood ashes leachate (TWAL) and 0.70 mg/L, 0.30 mg/L, were found in untreated wood ashes leachate (UWAL), respectively. Leachates presented no significant amount of copper. In MTT test the UWAL presented no significant result when compared to the control group. However the TWAL presented results significantly different at concentrations of 2, 5, 10, 25 and 50% when compared to the control group. The viability decreased in an inverse relation to the leachate concentration being 50% the most cytotoxic. In the comet assay UWAL did not present statistically significant difference when compared the control group at the concentrations tested (2, 5 and 10%). At the concentrations of 2 and 5% TWAL presented statistically significant increase of damage index and damage frequency when compared to the control group and when compared to UWAL at the same concentrations. At concentration of 10% of TWAL the quantification was not possible and there were indications of apoptosis and necrosis. These results corroborate to the ones obtained in the MTT test, in which 10% of TWAL had an average viability of 50%. Regarding the results of plasmid DNA cleavage test, none of leachates presented statistically significant difference in relation to the control group showing that the results of comet assay could be due to metabolic alterations. As conclusion there is evidence that TWAL causes cell death and indirect damage to the DNA, which can progress to diseases and complications in cellular systems. These effects are verified even at low concentrations, while UWAL did not demonstrate them.

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Lignans of *Piper nigrum* modulate cell proliferation, migration and citotoxicity in head and neck carcinoma cell line

Stefanie Oliveira de Sousa[1-2]; Luana Pereira Cardoso[2]; Mayra Carolina da Silva Ferreira[2]; Flávia Cristina Rodrigues Lisoni[2]

[1] São Paulo State University (UNESP), Institute of Bioscience, Humanities and Exact Science (IBILCE), Department of Biology, São José do Rio Preto, SP, Brazil. [2] São Paulo State University (UNESP), Faculty of Engineering Ilha Solteira (FEIS), Department of Biology and Animal Science, Ilha Solteira, SP, Brazil.

steh.sousa4@gmail.com

Keywords: cell culture; natural compound; alternative therapy.

The head and neck squamous cell carcinoma includes neoplasms appearing at the oral cavity, pharynx and larynx, it is the sixth most common kind of cancer worldwide and the most of them are from squamous histological type. Because of the fact that chemotherapy causes side effects and functional impairment, there is an interest the research for a treatment less invasive and more effective, such as phytotherapy. The *Piper nigrum* is one of them and it is getting the attention of the scientific community thanks to its antitumorigenic, anti-inflammatory, anti-hypertensive, antioxydant, antimicrobial and antidepressive effects. According to the importance of the antitumor activity of *Piper nigrum*, including their chemical elements, it was proposed the present study that had as objective to evaluate the changes in the morphology, proliferation, cytotoxicity and migration by the action of *Piper nigrum* leaf full extract, in a human head and neck carcinoma cell line (Hep-2) to explore the antitumorigenic potential. For this, we used the Hep-2 treated with total extract of the *Piper nigrum* leaf, in three different concentrations (10, 50 and 100µg/mL) for 4, 24, 48 and 72 hours. The morphology, the growth and the migration of the cells were evaluated daily using the inverted phase- contrast microscope Olympus CKX41. The cytotoxicity of the lignans was measured by MTS assay. For statistical analysis, we used the GraphPad Prism 6.0. The morphology of the Hep-2 cells was characterized of a monolayer of nucleate cells in the control group, without changes after the treatment with the extract. In the experiment of the cellular proliferation, it was possible to observe the inhibitory effect of cellular growth caused by the action of the total extract referring to the cell control. The cytotoxicity was observed after the treatment for different concentrations and times and the extract altered cell viability but was not cytotoxic. It seems that, if used, the lignans of extract total, work on the tumorigenic cells, causing cell death and achieving a decrease of the growth of those, although it has not reduced cell migration. These data are the starting point for genetic studies, such as modulation of gene expression or functional assays. The results increase our understanding of how lignans of the Piper nigrum participate in the tumorigenic processes and open new possibilities for the alternative therapy on head and neck cancer.

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Análise de variantes no gene *SLC44A2* associadas à Doença de Ménière

Luis Gustavo Lantin[1,2]; Giselle Bianco Bortoletto[2,3]; Karen de Carvalho Lopes[4]; Fernando Freitas Ganança[4], Edi Lúcia Sartorato[2*]

[1]UNIARARAS - Fundação Hermínio Ometto. [2] Laboratório de Genética Humana, Centro de Biologia Molecular e Engenharia Genética, Universidade Estadual de Campinas (UNICAMP), Campinas-SP, Brasil. [3] Faculdade de Ciências Médicas, Universidade Estadual de Campinas (UNICAMP), Campinas-SP, Brasil. [4]Universidade Federal do Estado de São Paulo (UNIFESP), São Paulo-SP, Brasil.

gustavolantin67@gmail.com.

Palavras-chave: Hidropsia Endolinfática; Perda Auditiva; *SLC44A2*.

A doença de Ménière (DM) é um distúrbio que acomete o labirinto do ouvido interno, um sistema de canais e cavidades que afetam diretamente a audição e o equilíbrio. Os sintomas são caracterizados pela tétrade sintomática: perda de audição, vertigem, plenitude aural e zumbido associados também à hidropsia endolinfática. A frequência da DM varia, prevalecendo em adultos entre 30-50 anos com incidência de 46 a 200 casos em cada 100 mil indivíduos, preferivelmente caucasianos. Não há dados desta prevalência no Brasil, porém é pouco comum em jovens e crianças. Não foi reportada a diferença distributiva entre os sexos nos pacientes acometidos pela DM. Apesar de ser facilmente diagnosticada, sua etiologia ainda é desconhecida, mas é sabido que resulte de fatores ambientais e genéticos (multifatorial). Vários genes relacionados a homeostase da orelha interna têm sido estudados, entre eles o gene *SLC44A2*, no qual já foram descritas duas variantes associadas a DM. Conhecendo a alta incidência da DM e seu impacto na qualidade de vida dos pacientes, é importante o estudo de sua etiologia para um melhor entendimento da doença, que por sua vez pode permitir estratégias terapêuticas, beneficiando os pacientes. O objetivo deste trabalho foi analisar as variantes do gene *SLC44A2* (rs3087969 no éxon 4 e rs228890 no éxon 7) em 28 pacientes com diagnóstico clínico definido de doença de Ménière. Essa análise foi realizada por meio da técnica de Sequenciamento Automático de Sanger, usando o *Sequencer ABI PRISM® 3700 DNA Analyzer*. O gene *GJB2* envolvido na etiologia da perda auditiva, foi rastreado como critério de exclusão. A variante rs3087969 (c.204C>T) foi encontrada em todos os pacientes, sendo 17 em homozigose e 11 em heterozigose. Essa é uma variante sinônima (p.Asp66=), mas estudos de predição *in sílico* mostraram que ela altera o sítio de *splicing*, potencialmente alterando a expressão do gene. Já a variante rs228890 (c.461A> G) codifica a troca de aminoácido na proteína (p.Gln154Arg) alterando sua estrutura. Dezessete dos 28 pacientes apresentaram a variante em homozigose, 7 em heterozigose e 4 pacientes não apresentaram essa alteração. O *SLC44A2* é um gene que codifica um antígeno expresso nas células de suporte da orelha interna. A ausência de sua função leva à morte de células pilosas auditivas, impossibilitando que as ondas sonoras sejam convertidas em impulsos elétricos para o cérebro. Estudos já mostraram que as variantes rs3087969 e rs2288904 podem estar associadas com a DM intratável comparada com a forma menos grave e controles normais.

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DESICCATED *Panagrolaimussuperbus* TOLERATESIMMERSIONWITHINLIQUID GALLIUM METAL: DISTINCT CHARACTERISTICS OF METALLOTHIONEINS FROM ANHYDROBIOTIC SPECIES

Danyel Contiliani[1,2]; Tiago Campos Pereira[1,2]

[1]Departamento de Biologia – Faculdade de Ciências e Letras de Ribeirão Preto – USP, Brasil. [2]Programa de Pós-graduação em Genética, Faculdade de Medicina de Ribeirão Preto – USP, Brasil.

danyel.contiliani@usp.br

Keywords: anhydrobiosis, nematode, metallothionein, metal ion, gallium.

In nature, some organisms display the ability to enter into a true state of suspended animation (no detectable metabolism), when exposed to extreme desiccation. This phenomenon is designated anhydrobiosis, meaning "life without water". During the dry state, these organisms exhibit tolerance to a variety of stresses, such as extremes of temperature, hydrostatic pressure, ultracentrifugation, ionizing radiation and vacuum. Metallothioneins (MTs) constitute a family of small, taxonomically-widespread, cysteine- rich metal-binding proteins that are responsible for heavy metal homeostasis and detoxification through the interactions of their cysteine thiol groups with physiological and xenobiotic metal ions. Considering that dry soils present high concentrations of metal ions, it is argued that anhydrobiotic organisms have developed strategies for the maintenance of ionic homeostasis, thus, they would tolerate extreme conditions of ionic imbalance. Therefore, the purpose of this study was to perform in silico analyses of MT protein, searching for its role in extreme desiccation conditions, and to investigate the tolerance of the anhydrobiotic nematode Panagrolaimus superbus completely immersed in metal Gallium (Ga) matrix (a condition of hypoxia/anoxia) as a means to assess its ametabolic status. MT-1 homologues in anhydrobiotic and non-anhydrobiotic organisms were identified by BLASTP tool, multiple sequences alignments via Clustal Omega, conserved sites and residue composition analyses via BioEdit v7.0.5, metal ion binding sites prediction via IonCom and alignment of MT α-chain tertiary structures from Protein Data Bank (PDB) by using PyMOL. Desiccated worms (n=600) were maintained immersed inside Ga matrix for seven days and viability and population growth analyses were carried out. Although residue composition did not show a significant difference in the number of cysteine residues between MTs, the multiple sequence analysis among 15 homologues (for each group) showed a higher number of conserved cysteines (183%) in anhydrobiotic organisms. Anhydrobiotic and non-anhydrobiotic MTs showed a higher affinity to Zn2+ ions than Cu2+ and Fe2+ in IonCom analyses. Alignments of tertiary structures of MT α-chain showed structural differences between non-anhydrobiotic and anhydrobiotic species (RMSD = 5.308), but not between non-anhydrobiotic organisms. On the other hand, the nematode P. superbus showed high viability (~70%) in Ga matrix, independently of external relative humidity. Also, gallium treatment did not show population growth differences in 28 days after Ga exposure. These results show differential aspects of MTs between anhydrobiotic and non-anhydrobiotic organisms, suggesting a protective role of these proteins in extreme desiccation scenarios. Also, we show for the first time that an anhydrobiotic organism is able to tolerate hypoxia promoted by a metallic isolating structure (Ga matrix), which might also be used for organ structural preservation in the dry state.

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COMPARATIVE TRANSCRIPTOMICS ANALYSIS OF CASTE- AND SEX-SPECIFIC GONAD DEVELOPMENT IN BEES

Denyse C. Lago[1*]; Martin Hasselmann[2]; Klaus Hartfelder[3]

[1]Department of Genetics - Ribeirão Preto School of Medicine (FMRP) – University of São Paulo (USP), Ribeirão Preto, SP, Brazil. [2]Department of Livestock Population Genomics – Institute of Animal Science – University of Hohenheim, Stuttgart, Germany. [3]Department of Cell and Molecular Biology and Pathogenic Bioagents - Ribeirão Preto School of Medicine (FMRP) – University of São Paulo (USP), Ribeirão PretoSP, Brazil.

*denysecl@usp.br; denyse_cavalcante@hotmail.com.

Keywords: Social insect; ovary development; gonad evolution.

Phenotypic plasticity associated with a complex social lifestyle makes the honey bee, *Apis mellifera*, an interesting model for evolutionary biology studies. Adult honey bee queens and workers strongly differ in ovary size. Queens have exceptionally large gonads composed of hundreds of serial units (ovarioles), while in the workers 95-99% of the worker ovarioles degenerate during larval development, as result of massive programmed cell death, turning them facultatively sterile. Strikingly, the testes of *A. mellifera* drones are also composed of hundreds of serial units (testiolar tubules). These extraordinary large gonads in both sexes sets queens and drones of the genus *Apis* apart from the females and males of all other bees, whose gonads are typically made up of only 3-4 ovarioles or testioles, respectively. Another major difference between the genus *Apis* and all other bees concerns the mating system. A honey bee queen is highly polyandric, typically mating with 15 and more males, while all the other bees are mainly monandric. Hence, we hypothesized that certain gene regulatory modules underlying gonad development in the honey bee may be shared among the sexes, but differ from those of other bees. To gain insights into the molecular architecture and possible evolutionary events underlying the development of the highly derived *A. mellifera* gonad phenotype, we used Illumina sequencing for comparative transcriptome analyses on gonads. First, we studied the transcriptomes of early fifth instar queen, worker, and drone larvae. Different from the expected in our hypotheses, a similar expression pattern was observed between queens and workers, while the comparisons with drones revealed a high number of differentially expressed genes (DEGs). A gene ontology (GO) analysis of DEGs from all contrasts of the honey bee comparisons revealed enrichment for categories related to binding and oxidoreductase activity. As a next step we asked whether and how the distinct gonad morphology of honey bees may be related with their highly polyandric mating system. The reads counts of DEGs with relevant GO attributes in *A. mellifera* were compared with their orthologs in *Bombus terrestris* gonads transcriptomes. This made it possible to identify clusters and reveal a set of candidate genes that can now be investigated in depth to see whether their coding sequences reveal evolutionary signatures. Our data provides new insights into potential gene regulatory networks underlying the development of the divergent gonadal morphologies in social bees, and on how these may be linked to the evolution of the respective different mating strategies.

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DISTRIBUTION OF GENE VARIANTS ASSOCIATED WITH DEMENTIAS IN A RANDOM SAMPLE POPULATION

Maette APC[1,4], Cavalcanti JL[2], Brindeiro R. M[3], Silva R[1], Moura-Neto RS[4]

[1]Instituto de Biofísica Carlos Chagas Filho da UFRJ. [2]Instituto de Neurologia Deolindo Couto da UFRJ. [3]Laboratório de Virologia Molecular, Instituto de Biologia da UFRJ. [4]Laboratório de Biologia Molecular Forense, Instituto de Biologia da UFRJ.

anamaette@gmail.com

Palavras-chave: Neurodegenerative diseases; Genetic diagnosis.

INTRODUCTION: Neurodegenerative diseases are a number of neuronal deficiencies related to massive neuronal loss in the last stages of the disease, including Alzheimer's disease, Parkinson's disease, Huntington's disease and Lou Gehrig's disease (amyotrophic lateral sclerosis). Genetic diagnosis of inherited dementias currently depends on the sequencing of these genes, by the Sanger method, on a clinical basis. The process is expensive, time-consuming, and the testing is widely available, contributing to the determination of hereditary dementia in the population. The genes PRNP, PSEN1, PSEN2, APP, GRN, MAPT, TREM2, CHMP2B, CSF1R, FUS, ITM2B, NOTCH3, SERPINI1, TARDBP, TYROBP e VCP has been documented in the literature and associated with neurodegenerative diseases. Despite that, the distribution of gene variants was not established in our genetic prevalence strategies, statistically significant in groups of patients, with access to data on predictive factors in the clinical evaluation. **OBJECTIVES:** (1) investigate genes of interest, (2) investigate a possibility of prediction others results, and (3) to test on a part of the patients, in which it was used for the method of prediction, if there are a correlation between genotype and phenotype. **METHODOLOGY AND RESULTS:** Initially, 12 variables were chosen from five genes of interest: Microtubule-Associated Protein TAU (MAPT), Charged Multivesicular Body Protein 2B (CHMP2B), TAR DNA Binding Protein (TARDBP), Triggering Receptor Expressed on Myeloid Cells 2 (TREM2) and Granulin Precursor (GRN). Through the database, the frequencies of the alleles were compared between the four communities: African, European, Eastern Asia and Native Americans. In order to compare in the future with the population of Rio de Janeiro. **CONCLUSION:** We hope that the results of genotyping can contribute to the creation of a genetic database of patients with the same injury and facilitate the early treatment of the diseases.

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EPIGENETIC REGULATION CONTROL OF THE LEFT-RIGHT OVARIAN ASYMMETRY IN BIRDS

Jorge Augusto Petroli Marchesi[1*]; Reginaldo Aparecido Vila[1]; Ester Silveira Ramos[1]

[1]Departament of Genetics, Ribeirão Preto Medical School, University of São Paulo.

*jorgea_petroli@hotmail.com

Key-words: DNA methyltransferases; epigenetic programming; DNA methylation.

Most birds, including chickens, have reproductive organs with unique characteristics. In female embryos, the gonads develop asymmetrically and only the left gonad generates a functional ovary. The development and function of the left ovary requires accurate gene transcription control. Epigenetic mechanisms, including DNA methylation, are critically important in mediating the precise gene expression, and may be involved in ovary gene regulation. We aimed to verify if epigenetic mechanisms are involved in the ovarian asymmetry in birds. In this study, we used chicken fertilized eggs and the ovary (right and left) samples were collected from the different embryonic development stages HH32, HH35, HH38, HH41 and HH45. Total RNA was isolated and the cDNA synthesis was performed. The qPCR analysis was performed using Power SYBR® Green reagent. The genes analyzed were three DNA methyltransferases (*DNMT1*, *DNMT3A,* and *DNMT3B),* three *Ten-eleven-translocation* (*TET*) genes (*TET1*, *TET2* and *TET3*), involved in demethylation, and the *Ribosomal Protein L5* and the β-actin as reference genes. In the HH32 stage, all genes presented similar expression in the left and right gonads. All studied genes had an increase in their expression from HH38 stage, except for the *DNMT3B*, which had its expression reduced 1.5 times throughout development. In this stage (HH38), the *TET1* gene, that plays a key role in active DNA demethylation, had an increased expression in the left gonad, followed by an overexpression peak of the *DNMT3A* gene, responsible for the *de novo* methylation. From the HH38 stage until hatching, an increase in the expression of the *DMNT1* gene was observed only in the left gonad. The *DNMT1* gene is responsible for maintenance of the DNA methylation and may be involved in the maintenance of the new ovarian specific reprogrammed epigenome in the HH38 stage. Morphological and immunofluorescence data show that the HH38 stage is an important point of the differentiation of the left gonad in a functional ovary in chickens because, at this stage, the germ cells undergo a meiotic wave in the left cortex. Our study confirms that the epigenetic mechanisms are important in the regulation process of left-right ovarian asymmetry in birds, and that the HH38 stage is probably the establishment point of a specific ovary epigenetic programming in the gonad cells that will develop into a functional ovary.

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New SHANK3 mutation in a complex case of Phelan-McDermind syndrome

João Paulo Kazmierczak de Camargo[1]; Angelica Beate Winter Boldt[1]; Roberto Rosati[2]; Alessando Max[1].

[1]Departamento de genética, Universidade Federal do Paraná, Curitiba, Brasil. [2]Instituto Pelé Pequeno príncipe, Curitiba, Brasil.

jpkcamargo@gmail.com

Keywords: autism; *SHANK3; NLGN4X; CHD8;* Phelan-McDermind syndrome; epilepsy; mental retardation; copy number variation

The essential characteristics of autism spectrum disorder (ASD) are described as persistent impairment in reciprocal social communication and social interaction, and restricted and repetitive patterns of behavior, interests, or activities. The etiology of this disease is consistently related to a multiplicity of genes acting in conjunction with non- genetic factors. In this case study, we searched for underlying genetic variations responsible for the phenotype of the autistic spectrum disorder of a girl, also affected with epilepsy and mental retardation, whose parents and three older sisters were healthly. During her pregnancy (just after her last deliverance three months before), the mother reported sleep and eating disturbances. At birth, the proband presented hypertrichosis lanuginosa and refused milk (she was not breastfed). She soon presented sleep disturbances and restlessness, showing repetitive movements. Her first convulsive episode occurred at 11 months of age and needed hospitalization. She is now 15 years old and presents hyperactivity, delayed bone development, lactose intolerance, frequent bladder and ear infections, intense touch sensibility, vitamin D and Iron deficiencies, anemia and considerable difficulty to socialize. She has no gross chromosomal abnormalities. We evaluated microalterations through genomic comparative hybridization (CGH) (Genetika laboratory and counseling center), and evaluated rare genetic variants by the exomiser through exome sequencing on the IonProton platform, using Ampliseq for library preparation and TMAP for alignment and mapping. Variants were selected with the Exomiser program v.8.0.0 using identifiers of the Human Phenotype Ontology database and excluding synonymous variants and those in regulatory and/or intergenic regions, or with frequency of the less common allele, higher than 1%. CGH revealed 5 translocations, 2 deletions (124.6 kb on 8p11.23 and 539.5 kb on 14q11.2) and 3 duplications (36.6 kb on 1q44 and two fragments of 137.9 kb and 538.3 kb on Xp22.31), all heterozygous. One of the Xp22.31 duplication affected the *NLGN4X* gene (neuroglin 4), whose overexpression seems detrimental to neuronal development, being associated with ASD and Asperger's Syndrome. The 14q11.2 deletion contained the *CHD8* gene (chromodomain helicase DNA-binding protein), whose haploinsufficiency in mice caused ASD-like behavior. Exome analysis revealed a new mutation in the *SHANK3* gene (SH3 and multiple ankyrin repeat domains 3), whose product plays an important role in synapse formation and dendritic spine maturation. The *c.80A>C*:p.Tyr27Ser mutation occurs in a highly conserved sequence, next to a CpG site, which is methylated in the brain (ENCODE data). Mutations in this gene cause Phelan-McDermid syndrome, which almost perfectly fits her clinical frame. The described mutations do possibly interact to produce the observed ASD phenotype and should be further evaluated in functional assays.

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PHYLOGENY SHOWS CUTICULAR HYDROCARBONS DIMORFISM IN DROSOPHILA

Marcelo Wilson Aparecido Moretto[1]; Maura Helena Manfrin[1,2]

[1]Pós-graduação em Biologia Comparada - FFCLRP - Universidade de São Paulo, [2]Departamento de Biologia – FFCLRP – Universidade de São Paulo. Ribeirão Preto, São Paulo, Brazil.

*marcelomoretto@usp.br

Palavras-chave: Drosophila, CHCs, sexual dimorphim.

The cuticular hydrocarbons (CHCs) are organic molecules presentes in epicutilar layer of *Drosophila*. These molecules are involved in mating recognition beyond another factors. Therefore there are many studies showing that these molecules are variable between male and female. Using character states as presence/absence of CHCs in a phylogenetic analysis, we aim to investigate if the CHC pattern differs between males and females. We analyzed the seven species from the Drosophila buzzatii cluster, a group of cactophilic sibiling species. We build three matrices – one matrice representing the species and other two matrices with sexes separately. *Drosophila mojavensis* e *Drosophila hydei* were used as external groups. In all matrices the CHC compounds were plotted as character state. Our data came from the literature. Concerning the species tree our results show that the cluster is a monophyletic group corroborating the results of mitochondrial and nuclear DNA sequences, however with a different arrangement of the ramifications in the relations intra-cluster. Our hypothesis shows D. antonietae and D. serido as sister taxa in agreement to the hypothesis based on period gene sequences. Concerning the hypothesis based on quantitative CHCs data our results disagreed, because CHCs quantitative analyses has a different arrangement of intra-cluster relationship, however it has higher values of bootstrap than our results. Concerning the sex, our results show that there are strongly dimorphisms between the sexes. The males tree shows a polytomy with six of the internal taxa, while the females tree shows that there is a polytomy with three of the seven internal taxa. Some molecules are exclusive of the males and other are exclusive of the females. For example, we found (Z)-5- hentriacontene in males of D.serido and (Z)-14-; (Z)-12-pentatriacontene in *D.buzzatii* females, what can explain the observed dimorphism. Our results show that the males tree have a poor phylogenetic signal in comparison to females tree, which confirms the results of other works. This difference could be explained by natural selection as CHCs may evolve faster than other traits and their diversity could be associated to adaptation to dry climates and in mating recognition. We conclude that the present qualitative method of CHC analysis in phylogenetic context can recover relationships in the cluster and can show sexual dimorphism.

TWO BETA-LACTAMASES OF DISTINCT CLASSES CONTRIBUTE TO THE HIGH BETA-LACTAM ANTIBIOTIC RESISTANCE IN *Chromobacterium violaceum*

Carlos Eduardo Milani Neme; José Freire da Silva Neto

Departamento de Biologia Celular e Molecular e Bioagentes Patogênicos, Faculdade de Medicina de Ribeirão Preto, Universidade de São Paulo, Ribeirão Preto, SP, Brazil

jfsneto@usp.br

Keywords: antibiotic resistance; beta-lactamases; *Chromobacterium violaceum*

Antibiotic resistance is spreading among bacteria on a global scale. Bacteria can become resistant to beta-lactam antibiotics by many mechanisms, such as expressing beta- lactamases, enzymes that degrade the beta-lactam ring of these antibiotics. *Chromobacterium violaceum*, a Gram-negative bacterial pathogen, is intrinsically resistant to beta-lactam antibiotics, but the reasons are still unknown. In this work, we discovered that two chromosomal genes encoding beta-lactamase enzymes are key determinants of beta-lactam resistance in *C. violaceum*. *In silico* analyses detected two chromosomal genes, here named *ampC* and *cphA*, in the *C. violaceum* genome. We constructed null in frame mutant strains deleted of each or both genes (Δ*ampC*, Δ*cphA* or ΔampC/*cphA*), as well as, their complemented strains. Susceptibility tests (MIC and disc diffusion assays) using beta-lactam antibiotics of three classes revealed that the Δ*ampC* mutant was more susceptible to penicillins and cephalosporins, and the Δ*cphA* was more susceptible to carbapenems relative to the wild type strain; the ΔampC/*cphA* double mutant showed a susceptibility pattern similar to each of the single mutants. The complemented strains, containing each gene cloned in a low-copy plasmid, had the phenotypes rescued and were even more resistant than the wild-type strain. Moreover, the *C. violaceum ampC* and *cphA* genes introduced in *Escherichia coli* make it more resistant to beta-lactams. Sequence analyses and susceptibility to EDTA indicated that CphA is a class B metallo-beta-lactamase, while AmpC is a class C beta-lactamase. Together, our data indicate that *C. violaceum* is highly resistant to beta-lactam antibiotics by expressing AmpC, a broad-spectrum beta-lactamase (penicillinase and cephalosporinase), and CphA, a specific carbapenemase. Our work offers new insights into the occurrence of beta-lactamases in an environmental bacterial pathogen and open perspectives to improve the treatment of *C. violaceum* infections.

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UNDERSTANDING THE EVOLUTION OF ACQUIRED RESISTANCE TO CISPLATIN IN ORAL CANCER

Julia L Oliveira[1*]; Thaís M Milan[1]; Rayana L Bighetti[1]; Roger R Fernandes[1], Luciana O Almeida[1]

[1]Laboratório de Cultura de Células. Departamento de Biologia Básica e Oral. Faculdade de Odontologia de Ribeirão Preto - Universidade de São Paulo.

*juc_lima.oliveira@hotmail.com

Palavras-chave: Oral Cancer; Cisplatin; Cancer Stem Cells; EMT

Acquired resistance is a serious issue in cancer treatment. Over 7.5 million people die every year because of failures in the therapy due chemoresistance. Cisplatin is the one of the most used therapeutic drug in cancer treatment, including oral cancer, and several patients develop resistance, responding well in the beginning (over 70% of tumor volume reduction), however, tumor reduction diminishes to 15-20% with the treatment course. Regarding oral cancers, few improvements in the treatment have been observed in the last 3 decades and the 5 years survival rates are below 50%. Here we established cell lines resistant to cisplatin aiming to better understanding biological differences driving the progression of oral cancers after treatment. Two oral cancer cell lines, CAL27 and SCC9 were assessed to establish cisplatin-resistant cell lines through the exposure to different concentrations and time courses of cisplatin. IC25 and IC50 (Inhibitory Concentrations of 25% and 50%) doses of cisplatin were defined using MTS assay. Cellular proliferation was determined through immunofluorescence using KI67 antibody and MTS assay. Potential of migration was investigated using scratch assay and the clonogenic potential by colony formation assay. Epithelial to mesenchymal transition (EMT) was assessed by immunofluorescence using E-cadherin, Vimentin and Pan-keratin antibodies and the accumulation of cancer stem cells (CSC) was analyzed by flow cytometry. CAL27 and SCC9 cell lines were treated with IC25 and IC50 doses of cisplatin for 72 hours and left to recover for 45 days to establish CAL27-IC25, CAL27-IC50, SCC9-IC25 and SCC9- IC50. To establish CAL27-CisR and SCC9-CisR, IC50 cell lines were submitted to a new exposure to cisplatin for additional 45 days. We demonstrated that cellular proliferation, migration and clonogenic capability significantly increased with the levels of resistance, wherein the most significant increases were observed in CAL27-CisR and SCC9-CisR, suggesting that prolonged exposure to cisplatin increases tumor aggressiveness. In addition, cisplatin administration had not effect over the viability of any of the resistant cell lines. EMT phenotype was observed by loss of E-cadherin, gain of Vimentin and by the conversion from a cuboidal epithelial structure into an elongated mesenchymal shape as evidenced by pan-keratin staining. Surprisingly, increasing of Vimentin was more evident in IC25 and IC50 than CisR cell lines suggesting the activation of EMT is an early event during the resistance process. CSC accumulation was more evident in CAL27-CisR and SCC9-CisR than IC25 and IC50 cell lines. Recent studies have highlighted a link between EMT and CSC accumulation. EMT is relevant to the acquisition and maintenance of CSC and is sufficient to endow differentiated cancer cells with stem cell properties. This relationship between EMT and CSC might have implications in tumor progression, aggressiveness and resistance to therapy.

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GENOME-WIDE ASSOCIATION IN REPRODUTIVE TRAITS IN WATER BUFFALO

Luciana Takada[1*]; Francisco Ribeiro de Araujo Neto[2]; Daniel Jordan Abreu dos Santos[3]; Rusbel Raul Aspilcueta-Borquis[1]; André Vieira do Nascimento[1]; Danielly Beraldo dos Santos Silva[1]; Henrique Nunes de Oliveira[1]; Humberto Tonhati[1]

1Faculdade de Ciências Agrárias e Veterinárias, FCAV/UNESP (Campus de Jaboticabal), SP– Brazil. 2Instituto Federal Goiano, Campus Rio Verde, GO-Brazil. 3University of Maryland, College Park MD, EUA

*utakada@gmail.com

Key Words: *Bubalus bubalis*; *GWAS;* Reproduction

Buffaloes have been intensively used in Brazil to produce mozzarella cheese, so that the main selection criterion used is the estimated cheese yield. However, in view of the importance of reproductive traits in production systems, this work was developed with the objective of using techniques to prospect genomic regions for reproductive traits in buffaloes, and to select candidate genes. Phenotypic records for age at first calving (AFC) and calving interval (CI) were used for the study, and genotypes were obtained using 90 K Axiom® Buffalo Genotyping array. For the analyzes, the single-step GBLUP method was used, and windows of SNPs with 1MB of length were constructed. For the windows that explained a greater proportion of the additive variance, the genes were identified using the UOA_WB_1 assembly as a reference. For the two traits, the candidate regions were identified on the chromosomes BBU 3, 4, 6, 7, 8, 9, 12, 14, 15, 19, 21 and 22. Among the genes with the greatest contribution to the additive variance, the CNGA1, ROCK2 and PMVK genes and, for CI, the genes ADCY2, PPP2R2A and MAP2K6 are considered candidates for AFC. These results may contribute to future applications in animal breeding programs, study to identify causal polymorphisms through sequencing or gene expression analysis in the species, reducing the cost of investigating heifers, improving the reproductive efficiency of buffaloes.

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DIFFERENTIAL EXPRESSION OF *ANK1* GENE IN NORMAL AND BACTERIAL CHONDRONECROSIS WITH OSTEOMYELITIS- AFFECTED BROILERS

Ludmila Mudri Hul[1]; Adriana Mércia Guaratini Ibelli[1,2]; Jane de Oliveira Peixoto[1,2]; Mateus Tremea[3], Mayla Regina Silva[4], Igor Ricardo Savoldi[4], Débora Ester Petry Marcelino[5], Marina Eduarda Auler[6], Mônica Corrêa Ledur[2,4].

[1]Programa de Pós-Graduação em Medicina Veterinária, Universidade Estadual do Centro Oeste do Paraná- UNICENTRO, campus Guarapuava-PR; [2]Embrapa Suínos e Aves, Concórdia, SC, Brasil; [3]Universidade Federal de Santa Maria, campus Palmeira das Missões-RS;[4]Programa de Pós-Graduação em Zootecnia, Universidade do Estado de Santa Catarina – UDESC Oeste, Chapecó, SC;[5]Faculdade de Concórdia FACC, Concórdia-SC;[6]Universidade do Contestado, Concórdia- SC.

*ludhul@yahoo.com.br.

Keywords: Poultry; Gene expression; Cytoskeleton.

Bacterial chondronecrosis with osteomyelitis (BCO) is a pathology characterized by necrotic degeneration and microbial infection, primarily within the proximal head (articular cartilage, growth plate) of the femur and tibiotarsus. The high prevalence of this defect has been one of the main concerns of the poultry industry, due to its impact on production and welfare. Therefore, this study aims to improve the understanding of the molecular mechanisms involved with BCO. Thus, the femoral cartilage of 10 normal and 10 BCO-affected broilers with 35 days of age was used. The collected samples were frozen and subjected to the extraction of total RNA and cDNA synthesis. The *ANK1* (Ankyrin1) candidate gene was selected for validation with quantitative PCR (qPCR) based on a previous RNA-Seq study which identified BCO-related genes. The qPCR reactions were performed and amplification cycle values were obtained. The relative expression was analyzed using the REST® software, with *RPL5* and *RPLP1* as reference genes. The *ANK1* was 5.45 fold upregulated in the affected compared to the normal broilers ($p < 0.05$). The *ANK1* gene encodes the Ankyrin1 protein which is related to the structural binding of the constituents of the cytoskeleton, such as proteins that aid in the fixation of other membrane proteins to the actin-spectrin cytoskeleton. The positive regulation of *ANK1* in the affected chickens indicates its involvement with the BCO since its expression is induced as a consequence of cell damage. *ANK1* acts as a modulator of signaling pathways for actin remodeling and its high expression can alter the structure of actin cytoskeleton affecting the structural integrity of the femur articular cartilage.

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AN OVERVIEW OF THE *HLA-G* AND *HLA-A* GENETIC DIVERSITY AND SIGNATURES OF NATURAL SELECTION IN AN ADMIXED BRAZILIAN SAMPLE

Marília R. S. Passos[1*], Thálitta H. A. Lima[2], Andreia S. Souza[2], Nayane S. B. Silva[1], Heloisa S. Andrade[2], Camila F. B. Castro[3], Celso T. Mendes-Junior[4], Erick C. Castelli[1]

[1]Universidade Estadual Paulista (UNESP), Faculdade de Medicina de Botucatu, Brasil. [2]Universidade Estadual Paulista (UNESP), Instituto de Biociências de Botucatu, Brasil. [3]Centro Universitário Sudoeste Paulista – UniFSP. [4]Departamento de Química, Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto, Universidade de São Paulo, Ribeirão Preto, SP, Brasil.

The authors declare no conflict of interest.

marilia.passos@unesp.br, erick.castelli@unesp.br

Key words: Natural selection; *HLA-G*; *HLA-A*

The Major Histocompatibility Complex (MHC) is located on the short arm of chromosome 6 in a segment of approximately 3.5 Mb, with about 224 genes most of them related to the immune system. Some genes of this complex are highly polymorphic and directly involved in the presentation of antigens to T lymphocytes, such as *HLA-A*. Also in the MHC, the *HLA-G* gene encodes an immunomodulatory molecule expressed mainly in the trophoblast and other non-physiological situations, such as tumors. In contrast to *HLA-A*, *HLA-G* presents only five frequent coding sequences detected worldwide. Balancing selection was previously documented for the *HLA-A* coding and regulatory segments (mainly the coding region), and also for the *HLA-G* regulatory segments. Here we assessed *HLA-G* and *HLA-A* complete genetic variability, together with an Alu element (AluYHG) encoded between these two loci, in 365 Brazilian samples. *HLA-G* and *HLA-A* were characterized by using massively parallel sequencing and bioinformatics workflow that optimizes read mapping in HLA genes and defines complete sequences using either the phase among variable sites directly observed in sequencing data and probabilistic models. We also evaluated extended haplotypes. We observed a strong Linkage Disequilibrium (LD) among *HLA- G*, *HLA-A*, and AluYHG, encompassing a genomic segment of 111Kb. Some *HLA-G* alleles are exclusively associated with specific *HLA-A* alleles and vice versa, although we observed recombination events. For instance, all copies of the allele G*01:05N follows the absence of the AluYHG element and A*30:01, while the AluYHG element marks the presence of alleles G*01:01:01:01 and A*02:01:01:01. Considering the complete sequences of *HLA-G* and *HLA-A* (promoters, exons, and introns) we detected high and significant Tajima's D (3.015 and 3.415, respectively), indicating a signature of balancing selection in both these genes. However, as expected, the strongest signal came from the *HLA-A* coding sequence. Balancing selection at the *HLA-A* coding sequence is related to its antigen presentation feature. The complete *HLA-G* sequence is associated with high Tajima's D, but this scenario changes when we consider only the exonic sequences. In fact, *HLA-G* presented evidences of purifying selection (dS-dN = 2.803, P= 0.003). *HLA-G* nucleotide diversity is much higher in intronic and regulatory segments, and these variants are in LD with the *HLA-A* coding region. Thus, the Tajima's D observed for *HLA-G* in the complete coding region may reflect its intronic diversity, and also reflect a hitchhiking effect because of *HLA-A*. Many previous studies have indicated balancing selection for *HLA-G* at its regulatory segments. Balancing selection operating at the *HLA-A* coding region might have shaped *HLA-G* promoter and intronic variants frequencies, downplaying the direct role of balancing selection on *HLA-G*.

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Molecular alterations of the gene *ND1* within mitochondrial DNA in dogs affected by breast cancer

Deyse Dayane Chaves Borges[1,2*], Caio Dantas Alves[1,2], Ana Carolina Pinheiro[1,2]; Anna Carolina Lima Rodrigues[1], Brenda Suelen Jardim Oliveira[1], Raissa Melo de Sousa[1], Rommel Rodríguez Burbano[3], Danilo do Rosário Pinheiro[1,2], Bárbara do Nascimento Borges[1]

[1]Laboratory of Molecular Biology, Institute of Biological Sciences, Federal University of Pará, Belém, Pará, Brasil. [2]UNAMA, Belém, Pará, Brasil. [3]Federal Rural University of Amazonia, Belém, Pará, Brasil. [4]Molecular Biology Laboratory, Ophir Loyola Hospital, Belém, Pará, Brasil.

deysemedvet@gmail.com

Key-words: Cancer, mtDNA, polymorphisms.

Mammary neoplasia in dogs is a reality that has been gaining increasing prominence within veterinary medicine, considering that this pathology is the second tumor with higher incidence in canids, specifically in females. It is also worth noting that these tumors can more frequently affect middle-aged and elderly animals, which are associated with reproductive status, progestogen use and episodes of pseudocyesis. In addition, studies demonstrate that the carcinogenesis process may be related to changes in the mitochondrial DNA that cause a dysfunction of the electron transport chain, interfering in cellular energy metabolism. Thus, this work aims to identify the genetic alterations present in the *ND1* region of mitochondrial DNA in samples of canine breast tumors. The materials and methods count on the analysis of 43 samples of tumors collected from animals submitted to mastectomy at the veterinary hospital of the Federal Rural University of Amazônia. Samples collected between the years of 2013 and 2015 were used, with neoplastic and non-neoplastic tissue fixed in formalin and paraffin for histopathological analyzes, while another portion was stored in microtubes containing solution of RNA Later until the extraction of nucleic acids. DNA was obtained with the aid of a commercial kit, and subsequently the regions of interest were amplified using canine mtDNA specific primers. The amplified fragments were submitted to automatic sequencing on ABI 3130 apparatus. Sequences were aligned with the aid of BioEdit software and the identified changes were related to the histopathological and clinical data of the patients using the Exato Fisher, Odds Ratio and Kruskal -Wallis in the BioEstat 5.0 program (AYRES et al., 2007), where values were considered statistically significant when $p < 0.05$. This work was approved by the Ethics Committee on Animal Use of Federal Rural University of Amazônia (001/2013 and 037/2015). The following changes were identified in the analyzed samples: six polymorphisms: (C2962T, A3028C, T3195C, T3259C, C3365T, T3373C) and a cytosine insert at position 3340 which results in a change from arginine to asparagine followed by threonine to asparagine resulting in a termination codon. This may influence the translation of the protein making it incomplete and consequently non-functional. To date, there have been some studies on the relationship between mutations and polymorphisms with carcinogenesis in canine mtDNA, however, the changes described in the literature occur in positions different from those found in this study. None of the alterations found were statistically correlated with the clinical and histological data of the patients, however, this result may be due to the low sample size. Therefore, it is necessary the analysis of a larger sample number to characterize the real role of the ND1 gene alterations in mammary tumorigenesis.

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IDENTIFICATION OF CELL SURFACE PROTEINS AS POTENTIAL IMMUNOTHERAPY TARGETS IN ADRENOCORTICAL CARCINOMA

Milena Ramos[1,2], Heitor Castanha[1], Luiz Gonzaga Tone[1] and Kleiton Silva Borges[1]

[1]Ribeirão Preto Medical School - University of São Paulo. [2]University of Franca.

milenasilvaramos@gmail.com

Keywords: targets; immunotherapy; ACC.

Despite being a rare tumor the adrenocortical carcinoma (ACC) exhibits greater incidence in South and Southwest regions of Brazil due to a specific mutation in TP53 gene, which makes it an important target of research. The affected patients are often diagnosed late and present low survival rates due to treatment inefficiency, and even those diagnosed in early stages show considerable rates of tumor recurrence. Recently, the immune system role in cancer was discovered to be crucial to avoid neoplasms and extinguish mutated cells. In many other cancer types, it was already demonstrated that differences in immune infiltrate profile are strongly correlated with prognostic and treatment response. Consequently, immunotherapies were developed in order to enhance the immune function in tumor, with great success. These strategies however have not shown significant results in ACC. The identification of surface proteins may advance rapidly the production of antibody-derived therapeutics targeting cell surface proteins. Here, we investigated the expression of genes that code for surface proteins in ACC patients in order to find new potential therapeutic targets to treat this tumor. There was analyzed data from the The Cancer Genome Atlas Program (TCGA) on the UALCAN platform from 79 ACC adult patients. We focused on genes that have its expression associated with poor patient survival. In this manner we generated a list of potential immune targets expressed on the cell surface, such as: *ICAM1, IRAK1, CD46, TLR5* and *THBS1*. These targets have been identified at the mRNA level and are yet to be validated at the protein level. The safety of targeting these antigens has yet to be demonstrated and therefore the identified transcripts should be considered preliminary candidates for new therapeutic antibody targets. Prospective candidate targets will be evaluated by proteomic analysis including Westerns and immunohistochemistry of a transgenic mouse model that develops ACC spontaneously.

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COMPARATIVE ANALYSIS OF THE TRANSCRIPTIONAL PROFILE OF THE SUGARCANE SMUT DISEASE PATHOGEN DURING THE INTERACTION WITH RESISTANT AND SUSCEPTIBLE GENOTYPES

Renato Gustavo Hoffmann Bombardelli[1]; Hugo Rody Vianna Silva[1]; Claudia Barros Monteiro-Vitorello[1].

[1]Universidade de São Paulo, Departamento de Genética, Escola Superior de Agricultura "Luiz de Queiroz", Piracicaba, São Paulo, Brazil.

rghbombardelli@usp.br

Key-words: RNA-Seq; *Saccharum* spp.; *Sporisorium scitamineum*

The fungal pathogen *Sporisorium scitamineum* causes sugarcane smut disease. The characteristic symptom of the disease is the development of a whip-like structure from the apex of the plant responsible for fungal sporogenesis and dissemination. The fungus can colonize resistant plants but forming no whips. This work aimed to compare the transcriptional profiles (RNA-Seq) of the fungus infecting plants of two different sugarcane genotypes (resistant and susceptible) 48 hours after inoculation. A total of 211,940,408 sequencing reads (207,043,218 after trimming) obtained of the interaction resulted in the alignment of 275,486 reads to 73.65% of the total fungal coding sequences (4,918 in a total of 6,677 genes encoding proteins). Differential expressed genes and GO term enrichment were conducted using Cutadapt v1.18; Hisat2 v2.1.0; FeatureCounts v1.6.0; EdgeR; Blast2GO v5.2.5 and ErmineJ v3.1.2. A total of 498 genes using a cutoff set to a p-value < 0.05 were found differentially expressed (364 up-regulated and 134 down-regulated). In the GO enrichment analysis, 12 terms were identified, among them were transmembrane transport (GO:0055085), oxidation- reduction process (GO:0055114) and hydrolase activity (GO:0016787). We also identified 21 genes expressed only in planta, sixteen of them exclusively represented in the resistant genotype (most of them were hypothetical proteins, but also an aldehyde dehydrogenase, a retrovirus-related POL polyprotein, and an HMG-box Hmg3-related protein); one identified in the susceptible genotype (sugar transporter); and four detected in both genotypes (zinc finger transporter, two Dik6-associated proteins, and a glycosyltransferase). Among candidate effectors differentially expressed, were found orthologs of effectors characterized in *Ustilago maydis* (Corn smut): Rsp3 (g3970_chr10_Ss) and Pit2 (g2337_chr05_Ss); and other 5 candidates (g6293_chr21_Ss; g1450_chr03_Ss; g3224_chr07_Ss; g2_chr01_Ss; g5200_chr15_Ss). Collectively our results suggest that the fungus is using different strategies to colonize resistant and susceptible genotypes. The set of differentially expressed effectors identified here are good candidates for further functional characterization.

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EPIGENETIC MODIFICATIONS ASSOCIATED TO CANCER STEM CELLS ACCUMULATION AND CHEMORESISTANCE OF HEAD AND NECK CARCINOMAS

Thaís M Milan[1] *; Julia L Oliveira[1]; Rayana L Bighetti[1]; Roger R Fernandes[1], Luciana O Almeida[1]

[1]Laboratório de Cultura de Células. Departamento de Biologia Básica e Oral. Faculdade de Odontologia de Ribeirão Preto - Universidade de São Paulo.

*thaismmilan@gmail.com

Palavras-chave: HNSCC, epigenetics, chemoresistance, Cancer Stem Cells

Head and Neck Squamous Cells Carcinoma (HNSCC) are a heterogeneous group of tumors characterized by lesions in oral cavity, larynx, pharynx, salivary glands and thyroid. HNSCC is the sixth most prevalent cancer worldwide, with proximately 780,000 new cases and 350,000 deaths annually. Recurrence affects about 60% of the patients and metastasis are reported in 20% of the cases and both are associated with poor response to the treatment. Epigenetic modifications contribute to the cellular plasticity during the tumor evolution. Aberrant epigenetic alterations may transform normal stem cells into cancer stem cells (CSC) with self-renewal ability and the capability to generate all cell types that constitute the whole tumor. CSC can block differentiation by suppressing gene expression through epigenetic reprogramming, altering the profile of histone modifications and DNA methylation. Differentiated cells are responsive to conventional chemotherapy, whereas CSC remain intact and will eventually repopulate the tumor. Here, we aimed to explore epigenetic modifications of HNSCC promoted by Cisplatin treatment and its association with CSC accumulation and chemoresistance. HNSCC cell lines CAL-27 and SCC9 were treated with Cisplatin and the gene expression was investigated using pPCR for histone deacetilases (HDAC1, HDAC2, SIRT1 and MTA1), lysine acetyltransferases (KAT6A and KAT6B) and histone methyltransferases (EZH2, EHMT2 and SETDB1). Protein accumulation of acetyl- H3K9, acetyl-H3K27, acetyl-H4K5, acetyl-H4K8, dimethyl-H3K9, trimethyl-H3K27, HDAC1 and EZH2 were assessed by western blot. CSC levels were evaluated by spheres formation assay and proliferation was determined using MTS assay. Our results indicated that Cisplatin treatment increased the expression levels of all genes analyzed for both CAL-27 and SCC9 cell lines, except EHMT2, which was decreased after treatment. In agreement to gene expression, EZH2 and HDAC1 protein levels were also higher in cell lines treated with Cisplatin. Tri-methylation of Histone 3 (trimethyl-H3K27), which is a target of EZH2, was increased after Cisplatin administration and di-methylation of Histone 3 (dimethyl-H3K9), which is a target of EHMT2, was decreased after Cisplatin administration. Surprisingly, despite the increase of histone deacetylases, acetylation of the histones H3K9, H3K27, H4K5 and H4K8 were higher in the cell lines treated with Cisplatin, probably driven by the accumulation of lysine acetyltransferases KAT6A and KAT6B, which have preference by the acetylation of Histones H3 and H4 and they are also associated with increasing of CSC (according to the literature). Interestingly, here, we also observed the accumulation of CSC after Cisplatin administration and these cell lines demonstrated chemoresistance after a subsequent treatment with Cisplatin. Together, our results suggest that Cisplatin may be inducing alterations in the epigenetic signaling, which promotes CSC accumulation driving to the tumor progression and the resistance to the treatments currently available.

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POTION2: a computational pipeline for genomic-scale search of positive selection in protein-coding genes in user-defined taxa

Thieres Tayroni Martins da Silva[1*]; Leonardo Vinicius Dias da Silva[1]; Francsico Pereira Lobo[1]

[1]IDepartamento de Genética, Ecologia e Evolução, Universidade Federal de Minas Gerais.

*thierestayroni@gmail.com

Keywords: positive selection; bioinformatics; comparative genomics;

Nowadays, detection of genes evolving under positive Darwinian evolution in genome- scale data is a prevailing strategy in comparative genomics studies to identify genes potentially involved in adaption processes and to infer gene function. We previously developed POTION, an open source, modular and end-to-end software for genomic- scale detection of positive selection in groups of homologous coding sequences. POTION greatly reduces analytical errors due automation of processing steps (filtering, alignments, trimming, phylogenetic tree reconstruction and positive selection detection) and computation time due to a heavily parallelized design. However, POTION currently only supports site-model analysis, where all sites (homologous codons from different species) are evaluated for signs of positive selection. Another major mode for positive selection search not currently implemented in POTION is the branch-model, where, in a species tree, a monophyletic group genomes (e.g. primates) is evaluated for positive selection when compared with the other genomes in the phylogeny (e.g. non-primate mammals). We report POTION2, a major new version of POTION that supports branch- model analysis, therefore allowing users to survey specific taxa in a phylogeny for adaptive genes. POTION2 also supports new software for sequence alignment, trimming and phylogenetic tree reconstruction. Regarding positive selection itself, POTION2 now supports FastcodeML, an alternative up to 10 times faster than codeml, currently the state-of-the-art software for positive selection detection. We validate POTION2 using two set of datasets: we select 12 yeast genomess from two species (7 *Saccharomyces cerevisiae* and 5 of its non-domesticated ancestor, *S. paradoxus*) to detect possible adaptive genes involved in domestication, finding 13 groups of fast- evolving genes in *S. cerevisiae*. Several of those genes are involved in biological processes important for fermentation, such as redox stress response (GRX7), cell wall processes (DAN1), double-strand DNA breaks repair (YKU80) and alcohol tolerance (AGA1). We also found genes with unknown function that may be interesting targets to be further evaluated for possible roles in fermentation. A second validation was made comparing the evolution of protein-coding genes in 10 primates when compared with 10 non-primate mammals. The analysis of 16892 1-1 orthologs, selected after POTION2 filtering steps, found 698 groups of homologous genes with evidence of positive selection in the Primate lineage. Gene enrichment analysis found these genes to be enriched in the following several categories of neuronal processes, such as GABA-ergic synapse (12 genes, FDR=1,7E-04);synaptic membrane (30 genes, FDR=5,2E-04); neuron part (89 genes, FDR=1,3E-06); neuron projection (67 genes, FDR=6,7E-05), suggesting these biological processes may play a role in primate evolution. In conclusion, we demonstrated that POTION2 can search for positive selection in specific lineages in a phylogeny, being able to find biologically relevant genes evolving under positive selection. POTION2 is freely available at https://github.com/franciscolobo/POTION2/.

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PROSPECTION OF FUNCTIONAL VARIANTS ASSOCIATED WITH SUBFERTILITY IN GIR BREED THROUGH WHOLE- GENOME SEQUENCING

Carolina Guimarães Ramos Matosinho[1]; Pablo Augusto de Souza Fonseca[2]; Francislon Silva Oliveira[1,3]; Marcos Vinicius Gualberto Barbosa da Silva[4]; Vicente Ribeiro do Vale Filho[5]; Venício Andrade[5]; Guilherme Silva Moura[5]; Izinara Cruz Rosse[6]; Maria Raquel Santos Carvalho[1*]

[1]Departamento de Genética, Ecologia e Evolução, UFMG. [2]Department of Animal Biosciences, Centre for Genetic Improvement of Livestock, University of Guelph. [3]Grupo de Genômica e Biologia Computacional, Centro de Pesquisas René Rachou, FIOCRUZ-Minas. [4]EMBRAPA Gado de Leite –MG. [5]Escola de Veterinária, UFMG. [6]Departamento de Farmácia, UFOP.

*ma.raquel.carvalho@gmail.com

Key-words: gir breed; subfertility; whole-genome sequencing.

Brazil is the fifth largest milk producer worldwide, yielding approximately 34 billion kg milk/year. Brazilian herds consist of taurine breeds (*Bos taurus*), indicine breeds (*Bos indicus*) and their crossbreeds. Gir and its crossbreed with Holstein (Girolando) are responsible for most of the milk production in Brazil. Consequently, Gir breed is under a constant demand for genetic material for breeding schemes. Intensive selection for dairy production has been associated with reproductive problems affecting also the bulls. High frequency of subfertile bulls have been reported in some Gir herds. The main causes of subfertility or infertility in bulls from Brazilian herds are testicular degen- eration or hypoplasia, delayed sexual maturity and abnormalities in spermiogenesis and spermatogenesis. Bovine subfertility results in significant economic impacts. In a previous study, we investigated the presence of testis and sperm abnormalities in a sample of 311 Gir bulls, including 30 sib-pairs or half-sib pairs, composed of one af- fected and one unaffected bulls. From this sample, we selected one subfertile bull for the search of potentially pathogenic mutations. Next-generation, whole-genome se- quencing (NGS) was performed using the HiSeq 2500 platform. Quality control was performed using FastQC tool. Reads were trimmed using the Trimmomatic tool and mapped to the references (UMD3.1 and ARS-UCD1.2 assemblies) using BWA men software. Variants were identified using Bcftools in the mpileup parameter and were confirmed with GATK. After the quality filter, variants were annotated using SnpEff and SnpSift. Missense and stop codons gain variants were investigated for colocation with reproduction QTLs using an in-house developed script. As a result, 99.5% of reads were mapped and 17,051,946 variations (SNVs and INDELs) were identified. These variants map to introns (47%), intergenic regions (44%), downstream and upstream re- gions (4%, each). Only 1% of the variants discovered were located in exons, 3'UTR, 5'UTR, and splice site regions. According to the effects, variants detected were mis- sense (46,997 variants), stop codon gain (644), stop codon loss (121) or map to the 5'-UTR (21,405). Among these, 13 potentially pathogenic variants were identified, distrib- uted in BTAs 4, 8, 9, 10, 14, 16, 19, 20, 21, 22, 26 and 28. Of them, eight potentially pathogenic mutations (present in *DNAI1*, *SPAG8*, *DNAH5*, *CYP17A1*, *SHBG*, *DGAT1*, *INHBA* and *ACTN1* genes) were selected for an association study, using the 311 Gir bulls sample referred above. For that, a target sequencing multiplex system is under construction. The results obtained here may help to better understand the biological processes associated with the development of fertility problems in bull. Subsequently, helping in the development of breeding strategies to reduce fertility problems in ze- buine Brazilian herds.

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COMPARATIVE *IN SILICO* ANALYSES OF LIPID BINDING PROTEIN 3 (LBP-3) AMONG ANHYDROBIOTIC AND NON- ANHYDROBIOTIC SPECIES

Vitor Nolasco de Moraes[1,2]; Danyel Contiliani[1,2]; Yasmin de Araújo Ribeiro[1,2]; Tiago Campos Pereira[1,2]

[1]Depto de Biologia, FFCLRP – Universidade de São Paulo. [2]Programa de Pós-Graduação em Genética, FMRP – Universidade de São Paulo.

vitor.moraes@usp.br

Keywords: Anhydrobiosis; Fatty acid binding protein, *Caenorhabditis elegans*

Anhydrobiosis is defined as an ametabolic state into which some organisms enter via extreme desiccation; biological activity is resumed by rehydration. Lipid binding protein homolog 3 (LBP-3) is a member of fatty acid binding proteins family (FABPs), which is hypothesized to be associated with anhydrobiosis, since this protein is involved with *dauer* formation (an alternative development stage of the nematode *Caenorhabditis elegans* which is desiccation tolerant) and longevity. The LBPs family is a member of the calycins superfamily, which is widely expressed among the animal kingdom. Nonetheless, little is known about LBP-3 conservation anhydrobionts and non- anhydrobionts species . Thus, the aim of this present investigation is to verify theLBP-3 protein structure conservation in anhydrobiotic versus non-anhydrobiotic species, as well as its tertiary structure similarities. First, we used BLASTp tool to search homologues of *Homo sapiens* FABP3 protein (GenBank: CAG33148.1) in anhydrobiotic and non- anhydrobiotic species. Homologues were aligned in Clustal Omega and analyzed using the Jalview software. *Ab initio* protein structure modeling was performed using the I- TASSER server to generate a predicted tertiary structure of the LBP-3 protein from *C. elegans*. FABP3 protein structure from *H. sapiens* was obtained from Protein Data Bank (PDB). PyMOL software was used to compare protein structures. We observedvariable conservation levels among anhydrobiotic and non-anhydrobiotic species, with identity percentages ranging from 41% to 86%. However, all homologues of human FABP3 presented all the three conservated domains (FATTYACIDBP 1-3). Additionally, we were not able to identify homologues in plants and fungi. We observed that the anhydrobiont *C. elegans* LBP-3 three-dimensional structural model (GenBank: CAG33148.1) and the non-anhydrobiont *H. sapiens* LBP-3 (PBD ID: 3RSW) share a 10 stranded antiparallel β-barrel structure containing a ligand-binding pocket molded by an helix-turn-helix motif on the N-terminal side which is thought to act allowing fatty acid entrance and exit, and they also present a good overlapping score (RMSD = 0,772). We conclude that although the animal homologues of FABP3 vary concerning their primary structures, their functional domains and tertiary structures are conserved between anhydrobiotic and non-anhydrobiotic species.

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Gene silencing by RNAi of the Ras family-gene in *Diaphorina citri*, vector of Huanglongbing of citrus

Vitória Bergamo Rodrigues[1]; Diogo Manzano Galdeano[2]; Marcos Antonio Machado[2]

[1]Centro Universitário Hermínio Ometto – FHO|UNIARARAS, Araras, São Paulo. [2]Instituto Agronômico de Campinas – Centro de Citricultura Sylvio Moreira, Cordeirópolis, São Paulo.

bergamovitoria@gmail.com

Key-words: RNAi; *GTPases*; Asian citrus psyllid

Currently, *Diaphorina citri* is one of the world's leading agricultural pests and is responsible for the transmission of phytopathogenic bacteria of the genus *Candidatus* Liberibacter, associated with one of the most devastating diseases of citrus, Huanglongbing (HLB). As an alternative form of HLB management, gene silencing by interference RNA (RNAi) is one of the biotechnological promises used to control pests from specific target genes. The aim of the present study was to evaluate the silencing of the *Rab-6* gene responsible for the regulation of the transport of proteins from the Golgi complex to the endoplasmic reticulum. For gene silencing, dsRNA solutions of the target gene were used at concentrations of 500 ng.μL^{-1} and 1000 ng.μL^{-1}, applied topically to the ventral region of the thorax of 4th instar nymphs of *D. citri* with a 10 µL microsyringe (Hamilton). After total sucking of the drop by the stylus of the nymphs, they were transferred to fresh orange (*Citrus sinensis* [L.] Osbeck, var. Pera-Rio) saplings containing young leaves. Each treatment consisted of 10 nymphs in five plants. The insects were kept confined to the seedlings in voil and were evaluated for 10 days regarding development, mortality and morphological changes. In addition, the same experiment was performed at three distinct times (6 hours, 24 hours and 48 hours) to analyze the expression levels of the target gene. In this case, after the established time, the insects were collected for extraction of total RNA and analysis of gene expression in quantitative PCR. Solutions containing GFP dsRNA and distilled water were used as negative controls. The results demonstrated a reduction in the *Rab-6* gene expression after 24 hours after the topical application of the dsRNA solution, in addition to the increase of nymphs mortality and developmental delay. Therefore, gene silencing was effective for the target gene and corroborates as an important tool for the technological advances of the agricultural control, besides being an interesting component for integrated pest management, because it presents high specificity.

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CIRCULATION OF CHIKUNGUNYA VIRUS EAST/CENTRAL/SOUTH AFRICAN LINEAGE IN RIO DE JANEIRO, BRAZIL

Joilson Xavier[1,3]; Marta Giovanetti[1,2]; Vagner Fonseca[1,4]; Julien Thezé[5]; Tiago Gräf[6]; Allison Fabri[2,6]; Jaqueline Goes[3]; Marcos Mendonça[2]; Cintia Damasceno[2]; Maria Mares-Guia[2]; Carolina Cardoso[2]; Stephane Tosta[1]; Darlan Candido[5]; Rita Nogueira[2]; André Abreu[7]; Wanderson Oliveira[7]; Carlos Albuquerque[8]; Alexandre Chieppe[9]; Tulio de Oliveira[4]; Patrícia Brasil[10]; Guilherme Calvet[10]; Patrícia Sequeira[2]; Nuno Faria[5]; Ana Maria Bispo de Filippis[2]; Luiz Carlos Junior Alcantara[1,2].

[1]Laboratório de Genética Celular e Molecular, Universidade Federal de Minas Gerais, Belo Horizonte, Brazil. [2]Laboratório de Flavivírus, Instituto Oswaldo Cruz, Rio de Janeiro, Brazil. [3]Laboratório de Patologia Experimental, Instituto Gonçalo Moniz/Fiocruz, Bahia, Brazil. [4]KwaZulu-Natal Research Innovation and Sequencing Platform, University of KwaZulu-Natal, South Africa. [5]Department of Zoology, University of Oxford, United Kingdom. [6]Departamento de Genética, Universidade Federal do Rio de Janeiro, Brazil. [7]Secretaria de Vigilância em Saúde, Ministério da Saúde, Brasília, Brazil. [8]Organização Pan-Americana da Saúde/Organização Mundial da Saúde, Brasília, Brazil. [9]Superintendência de Vigilância do Estado, Rio de Janeiro, Brazil. [10]Instituto Nacional de Infectologia Evandro Chagas, Rio de Janeiro, Brazil.

joilsonxavier@ufmg.br

Key-words: chikungunya virus; Rio de Janeiro; genomic epidemiology.

Chikungunya fever is an infectious disease caused by chikungunya virus (CHIKV), which is transmitted by the bite of infected hematophagous mosquitoes of the genus *Aedes*. The state of Rio de Janeiro experienced a CHIKV outbreak in 2016, reporting 18,516 probable cases. The emergence of CHIKV has raised concerns due to the rapid spread of the virus into new geographic areas and the clinical features associated with the infection. Thus, we aim to investigate the genomic epidemiology of chikungunya virus circulating in the state of Rio de Janeiro. We used the portable nanopore sequencing approach to successfully generate 11 new CHIKV genomic sequences from serum samples, collected between 2016-2018 from symptomatic patients mostly residing (n = 6) in the city of Rio de Janeiro. Our phylogenetic reconstructions indicated the circulation of the East-Central-South-African (ECSA) lineage in Rio de Janeiro. Time-measured phylogenetic reconstructions combined with CHIKV notified case numbers revealed the ECSA lineage was introduced into Rio de Janeiro state around June 2015 (95% Bayesian credible interval: May to July 2015) indicating the virus was circulating unnoticed for 5 months before the first reports of CHIKV autochthonous transmissions in Rio de Janeiro, in November 2015. Moreover, both epidemiological and phylogenetic data suggested that ECSA lineage strains were introduced into Rio de Janeiro from at least two distinct CHIKV dispersion events from Northeast region of Brazil, where this lineage was first detected in late 2014.

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THE RHO GTPASE PATHWAY: A NEW P53-DEPENDENT ROAD FOR DNA REPAIR AND IR-RESISTANCE OF GLIOBLASTOMAS?

Yuli Thamires Magalhães[1] **e Fábio Luís Forti**[1]

[1]Departamento de Bioquímica, Instituto de Química - Universidade de São Paulo, São Paulo, Brasil.

*yuli.magalhaes@usp.br

Key-words: Rho GTPases signaling; Glioblastoma resistance; DNA repair

Glioblastoma is the most frequent brain tumor in adults and despite aggressive therapies with combined surgery and radio-chemotherapy, an effective prognosis of treatment remains obscure, with a two-year survival rate. The increased cell migration and invasiveness of these tumors are characteristic of their aggressiveness, which has been linked to Rho GTPase pathways. We proposed that Rho GTPase is pro-acting to genomic stability of glioblastoma cells, and here we explored the effects of Rho pathway inhibition on glioblastoma with different p53 status in the resistance to γ- radiation, commonly used in clinical practice. U87-MG (p53 wild-type) and T98G (p53 mutated) cells were subjected to modulation of Rho pathway signaling by either the Rho inhibition by C3 toxin, the knockdown of downstream Rho pathway components mDia-1, Profilin-1 and the myosin- binding subunit MYPT1 or the inhibition of ROCK by Y-27632 inhibitor. From alkaline comet assays the Rho pathway disturbance by C3 toxin or knockdown of mDia-1 and Profilin-1 led to increased DNA damage breaks and a delayed capacity of DNA repair after γ- radiation in U87-MG cells, with no effect in T98G cells. The knockdown of MYPT1 reduced the amount of DNA breaks and increased the repair in both cells. However the inhibition of ROCK sensitized the U87-MG cells while increased the resistance of T98G cells to IR. Immunofluorescence assays showed that foci formation of γH2AX are compromised by Rho pathway inhibition and enhanced by Rho pathway activation by MYPT1 knockdown, as well as the 53BP1 foci formation, with a pronounced effect in p53 wild-type cells. The impairment of Rho pathway increases the levels of phospho-Chk2 after irradiation, as demonstrated by immunobloting and immunofluorescence analysis. Functional assays for the analysis of HR and NHEJ repair pathways showed that the impairment of Rho signaling decreases the DSBs repair by HR only in U87-MG cells, while affects the NHEJ repair in both cells. In the other hand, the knockdown of MYPT1 and ROCK inhibition increased the repair by NHEJ in p53 mutated cells. These results indicate a striking regulatory relationship between Rho GTPase and DNA damage response (DDR) and HR/NHEJ repair pathways. Bioinformatics analysis indicate that high expression of RHOA and RHOC, as well as some of its downstream components, is related to the higher level and aggressiveness of gliomas and a shorter patient survival. Comparative analysis of Rho activity in cells expressing wild-type or mutated p53 showed that p53 wild-type cells are more susceptible to the effects of Rho inhibition in response to IR. This work thereby shows that the Rho pathway might be a fragile point in the resistance of gliomas against the usual therapies, being this effect dependent on p53 transcriptional activities.

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IDENTIFICATION OF X-LINKED CAUSES OF INTELLECTUAL DISABILITY IN FEMALES THROUGH X-CHROMOSOME INACTIVATION SKEWING

Evelyn Quintanilha Vianna[1]; Andressa Pereira Gonçalves[1]; Rafael Mina Piergiorge[1]; Jussara Mendonça dos Santos[1]; Veluma Calassara[1]; Carla Rosenberg[2]; Ana Cristina Victorino Krepischi[2]; Raquel Tavares Boy da Silva[3]; Márcia Gonçalves Ribeiro[4]; Suely Rodrigues dos Santos[5]; Filipe Brum Machado[6]; Enrique Medina-Acosta[7]; Márcia Mattos Gonçalves Pimentel[1], Cíntia Barros Santos-Rebouças

[1]Department of Genetics, State University of Rio de Janeiro, Rio de Janeiro, Brazil. [2]Departament of Genetis and Evolutive Biology, University of São Paulo, São Paulo, Brazil. [3]Pedro Ernesto University Hospital, State University of Rio de Janeiro, Rio de Janeiro, Brazil. [4]Clinical Genetics Service, IPPMG, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil. [5]Gaffrée and Guinle University Hospital, Federal University of Rio de Janeiro State, Rio de Janeiro, Brazil. [6]Department of Biological Sciences, Minas Gerais State University, Minas Gerais, Brazil.[7]Laboratory of Biotechnology, State University of Northern Rio de Janeiro Darcy Ribeiro, Rio de Janeiro, Brazil.

cbs@uerj.br

Key-Words: Intellectual disability; X-chromosome inactivation; whole exome sequencing.

Intellectual disability (ID), characterized by limitations on both intellectual functioning and adaptive behavior, affects 30% more males than females. This unbalanced sex ratio can be greatly attributed to the recognized excess of genes on chromosome X playing important roles on central nervous system development and function and their hemizygous state on males. Moreover, as a result of X-chromosome inactivation (XCI), most genes on one of the two X chromosomes in female somatic cells are epigenetically silenced, so that females carrying mutations on this chromosome will not be so severely affected by ID as males. Consequently, studies on X-linked ID (XLID) including females with no obvious clinical manifestations are rare and the knowledge about genes on the X chromosome causing ID in females is scarce. In this study, we aimed to identify under-detected genetic causes of ID in females using XCI extreme skewing patterns as an indicator of the presence of mutations in an X-linked gene. XCI profiles from 53 females with idiopathic ID were estimated by determining the active X / inactive X ratios in genomic DNA from peripheral blood and buccal mucosa using a methylation-sensitive *AR*/*RP2* biplex assay. DNA samples with extreme XCI skewing (≥ 90%) were submitted to array-comparative genomic hybridization (array-CGH) to exclude pathogenic copy number variations (CNVs). Females with no pathogenic structural rearrangement were subsequently analyzed by whole exome sequencing. All potential pathogenic variants filtered by our pipeline were validated by Sanger sequencing, followed by segregation analysis and genotype-phenotype correlation. Seven females exhibited extreme XCI skewing (13.2%), a percentage significantly higher than expected for healthy females in our population ($p<0,001$, $x^2=18,61$). XLID- related changes were identified in five of the seven patients with extreme XCI skewing, including one pathogenic structural rearrangement (der(2) chromosome from a t(2;X)) and four pathogenic point mutations in *HDAC8*, *NLGN4X*, *TAF1* and *USP9X* genes, two of them novel. All pathogenic variants were *de novo* and two of them affect XCI escape genes (*NLGN4X* and *USP9X*). In conclusion, XCI skewing proved to be an excellent tool for the characterization of new molecular mechanisms associated with XLID in females, allowing the identification of previously unrecognized alterations responsible for ID and XCI skewing. Furthermore, our study uncovers new insights into the involvement of XCI escape genes on XLID.

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GENETIC CHARACTERIZATION OF MARANHÃO USING 25 Y- STR MARKERS

Julyana Ribeiro[1], Juliana Jannuzzi[1], Rossana Azulay[1], Marília Gomes[1], Elizeu Carvalho[1], Dayse Aparecida[1], Leonor Gusmão[1]

[1]Laboratório de Diagnóstico por DNA (LDD), Universidade do Estado do Rio de Janeiro, Rio de Janeiro, Brasil

julyanaribeiro@ymail.com

Palavras-chave: Short tandem repeats; Y Chromosome

Y chromosome-specific STR markers are widely used in population genetics to infer paternal ancestry, migratory movements, and genetic differences between populations. The YfilerPlus kit has been shown to be a good tool in population genetic studies and forensic identification, due to the high discrimination power attributed to markers with high mutation rates. The Brazilian population is well-known for a genetic heterogeneity, resulting from the miscegenation among Europeans, Native Americans and Africans. i) The aim of this study was to characterize 25 Y-STR markers in a population sample from Maranhão. Therefore, 84 samples of unrelated males, were genotyped using the YfilerPlus® kit, following manufacturer's instructions. The values of haplotype diversities (HD) and genetic distances (F_{ST}) were calculated using Arlequin software v3.5.1.2. A HD of 1.0000 ± 0.0018 was observed, and no shared haplotypes were found in the Maranhão sample. This value is higher than that previously reported for a sample of 114 individuals from Maranhão (HD = 99.992). When comparing the present sample with that available in YHRD (YA004348) from Maranhão, no statistically significant differences were found (F_{ST} = 0.00012, with a non-differentiation p-value of 0.40165, for 50.000 permutations), pointing to genetic homogeneity in the population of Maranhão. The analysis of genetic distances (F_{ST}) between Maranhão and four other populations of Brazilian states revealed no population substructure (F_{ST} ≤ 0.0014, non-differentiation p-values for 50,000 permutations ≥0.1096). Other studies based on 23 and 25 Y-STRs in Brazilian populations presented similar results, in which non-significant genetic distances pointed to a homogeneity among the majority of the populations of the five regions. The increase in the population sample of Maranhão contributed to a better representation of this population in the database, which can be used in forensic investigations and kinship analyzes. No substructure was detected, after comparing the five Brazilian states, supporting that a single database can be representative of admixed populations from Brazil.

Funding Agency: CAPES; CNPq; FAPERJ.

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MOLECULAR ALTERATIONS IN THE EXON 5 OF *TP53* GENE IN CANINE MAMMARY CANCER

Ana Carolina Pinheiro[1,2], Anna Carolina Lima Rodrigues[2]; Brenda Suelen Jardim de Oliveira[1,], Caio Dantas Alves[1,2], Deyse Dayane Chaves Borges[1,2], Danilo do Rosário Pinheiro[1,2], Rommel Rodriguez Burbano[3], Bárbara do Nascimento Borges[1]

1Laboratory of Molecular Biology, Institute of Biological Sciences, Federal University of Pará, Belém, PA. 2University of Amazon, Belém, PA, [3]Federal University of Pará, Belém, PA. *Correspondence: Laboratory of Molecular Biology, Institute of Biological Sciences, Federal University of Pará. R. Augusto Correa,01. Guamá, Belém-PA.

*acarolinapinheiro94@gmail.com.

Keywords: p53, dogs, mammary cancer

Mammary cancer is the second most frequent tumor in canine females, and thus of great relevance in Veterinary Medicine. The *TP53* gene codifies a protein responsible for the regulation of the cell cycle and the maintenance of the genome integrity and is strictly involved in the tumorigenesis process, being frequently altered during the neoplastic development. The literature shows that exons 5 to 8 are mutational hotspots in this gene, and alterations in these regions, can disrupt the regular cell process, contributing to cancer proliferation. Considering this, the aim of this study was to analyze molecular alterations in the exon 5 of the *TP53* gene in canine mammary tumors. Samples of neoplastic and non-neoplastic tissues were collected at the Veterinary Hospital of the Universidade Federal Rural da Amazônia, in Belém, Pará, Brazil. The DNA was obtained using phenol-chloroform method. A fragment of 300 base pairs of the exon 5 was amplified using conventional PCR approach and the amplicons sequenced on ABI 3130. Samples were analyzed using BioEdit and Swiss Model softwares. Thirty-one samples (16 non-tumoral and 15 tumoral) were analyzed and in 100% it was identified the presence of a heterozygosity (G/C), resulting in an amino acid change (Arginine → Proline) on the position 162 (175 in humans) of the protein, corresponding to the DNA binding domain, a hotspot region in humans. This alteration leads to a change in the twist angle (0.71 → 0.95) and in the solvation (-1.37 → -1.26) of the protein, which may alter its conformation, resulting in a protein disfunction or inactivation, due to an interference in the stability of peptide bonds. The presence of this alteration may represent a predisposition risk of mammary cancer development, as both neoplastic and non- neoplastic tissues present this alteration.

Funding Agency: CNPq, UFPA

INVESTIGATION OF SINGLE BASE POLYMORPHISMS (SNPS) ON *ND1* AND *ND3* GENES IN LUNG CANCER

Brenda Suelen Jardim de Oliveira[1]; Deyse Dayane Chaves Borges[1,2], Anna Carolina Lima Rodrigues[1]; Caio Dantas Alves[1,2]; Ana Carolina Pinheiro[1,2]; Francisco Canindé Ferreira de Luna[1]; Lorena Duarte Fernandes[1]; Danilo do Rosário Pinheiro[1,2]; Rommel Mario Rodríguez Burbano[3]; Bárbara do Nascimento Borges[1]

[1]Laboratory of Molecular Biology, Institute of Biological Sciences, Federal University of Pará, Belém, Pará; [2] UNAMA, Belém, Pará; [3]Molecular Biology Laboratory, Ophir Loyola Hospital, Belém, Pará. *Correspondence: Laboratory of Molecular Biology, Institute of Biological Sciences, Federal University of Pará. R. Augusto Correa, 01. Guamá, Belém, Pará.

*brenda.suelenoliveira@yahoo.com.br.

Keywords: mitochondrial DNA, lung cancer; complex I.

Lung cancer is the most common cause of cancer-related deaths worldwide, and excluding non-melanoma skin cancer, is the second most common in men and women in Brazil. Mitochondria are important organelles for the generation of ATP through oxidative phosphorylation, and have their own genome, the mitochondrial DNA (mtDNA), which includes the complex I (*ND1, ND2, ND3, ND4L, ND4, ND5* and *ND6*), complex III (*CYTB*), complex IV (*COI, COII* and *COIII*) and complex V (*ATP6* and *ATP8*). The *ND1* and *ND3* takes part in the first initial step of the electron transport chain of the mitochondrial energy-generating pathway. These mitochondrial genes are often mutated and/or repressed in various human cancers, including lung cancer. Thus, this study aimed to analyze the molecular alterations in genes coding for mitochondrial DNA Complex I subunits in non-small cell lung cancer**.** A total of 80 samples (cases and controls) of lung cancer were obtained from Ophir Loyola Hospital, and the DNA was obtained with commercial kit. Subsequently the target fragments were amplified by standard PCR and sequenced on the ABI 3130 apparatus. Sequences were aligned with the BioEdit software. Four alterations in the gene *ND1* (C3553T, T3552A, G3666A and C3595ins) and two alterations in the gene *ND3* (A10398G and C10400T) were identified. No statistical significance was observed when comparing the frequency of alterations found with the clinical and histopathological data of patients with lung cancer. However, the presence of the A allele in T3552A was significantly associated with a protective effect on the development of lung cancer (A allele frequencies of 0.1131 in cases and 0.4167 in controls). In the *ND3* gene, the allelic frequencies for both SNPs were significant different when comparing cases and controls and were significantly associated with lung cancer. Our results suggest these SNPs could be used as markers in patients with non-small cell lung cancer.

Funding Agency: CAPES, UFPA

THE ROLE OF DUSP8 IN PDAC PROGRESSION AND CHEMORESISTANCE

Bruno Mari Fredi[1]; Gabriela Maciel Vieira[1]; Vanessa da Silva Silveira[1]

[1] Departamento de Genética, Faculdade de Medicina de Ribeirão Preto - Universidade de São Paulo.

vsilveira@fmrp.usp.br

Palavras-chave: PDAC; dual-specificity-phosphatase-8; chemoresistance

Background: Pancreatic Ductal Adenocarcinoma (PDAC) is the most frequent (corresponding to more than 95% of cases) and aggressive type of pancreatic cancer and affects the cells of the exocrine portion of the pancreas, more specifically pancreatic duct cells. KRAS mutation is the most frequent mutation, being present in more than 95% of the patients. This mutation results in constitutive activation of several downstream pathways, such as the pathway of MAPKs (mitogen-activated protein kinases) related to processes of increased cell proliferation, chemoresistance, and decrease of apoptosis. DUSP (dual-specificity phosphatases) family constitutes one of the major classes of phosphatases involved in MAPK regulation. However, their role in PDAC is still very unclear in the literature. Here we investigated the DUSPs expression on PDAC and the impact of DUSP8 in chemoresistance of PDAC cell lines. **Methods**: RNAseq data from 178 PDAC samples from the *TCGA Reseach Network* database were analyzed using UALCAN and GEPIA and the gene expression profile of several DUSPs were correlated to the clinical stages of cancer development and the survival of patients. In addition, we performed a 4-day MTT cell viability assay after inhibition of DUSP8 by CRISPR/Cas9 in combination with gemcitabine treatment in PDAC cell lines (MIA PaCa-2 and PANC-1). **Results:** In silico analysis demonstrated that DUSP8 has a higher expression in patients with PDAC when compared to patients without tumor development, although it was not statistically significant. ($p = 4.37e-01$). The DUSP8 expression was statistically different between PDAC patients with different tumor grades: Grade 1 vs Grade 2 ($p = 2.49e-02$) and Grade 1 vs Grade 3 (1.04e-02). In addition, there is also a correlation between the expression of DUSP8 and event free survival. The data reveal that a high expression of DUSP8 is associated with an increase of event free survival ($p = 0.014$). In vitro cell viability assay has shown that DUSP8 inhibition significantly increased gemcitabine chemosensitivity in both cell lines. Altogether, these data suggest that DUSP8 may play an important role on PDAC chemoresistance.

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RATIONAL ENGINEERING OF METAL HOMEOSTASIS IN *SACCHAROMYCES CEREVISIAE* FOR EFFICIENT 2G ETHANOL PRODUCTION

Gisele Cristina de Lima[1]; João Gabriel Ribeiro Bueno[1]; Leandro Vieira dos Santos[1*].

[1]Brazilian Biorenewables National Laboratory (LNBR), Brazilian Center for Research in Energy and Materials (CNPEM), Zip Code 13083-970, Campinas, Sao Paulo, Brazil.

*leandro.santos@lnbr.cnpem.br

Keywords: 2G etanol; *Saccharomyces cerevisiae;* xylose

The use of carbon-based fossil fuels is the main cause of greenhouse gas emissions that impacts climate change. The replacement of fossil fuels by renewable sources, such as the second-generation (2G) ethanol is essential to mitigate global warming and is the basis for the development of biorefineries. The cost-effective production of 2G ethanol from biomass implies the development of engineered strains of *Saccharomyces cerevisiae* with efficient conversion of sugars from cellulosic and hemicellulosic chains. Conversion of xylose-to-ethanol remains an industrial challenge and is a key target for a feasible scalable production of biofuels from renewable biomass. The integration of the complete metabolic pathway in yeast to produce 2G ethanol requires at least 10 genetic modifications, including the heterologous expression of xylose isomerase enzyme (*xylA*) that allows a one-step isomerization of xylose to xylulose. However, the *xylA* enzyme show a very low activity in *S. cerevisiae* strains, impairing fermentation. Since xylose isomerase is a metalloenzyme which requires divalent cations as cofactors to catalyze its reaction, the rational engineering of metabolic pathways involved in metal homeostasis can increase specific metal ions availability, boosting the *xylA* enzyme activity and increasing the 2G ethanol productivity. In this work, based on an interactome regulatory network, 10 target genes related to cellular metal homeostasis were rationally selected and evaluated on an engineered yeast strain containing the *xylA* from *Orpinomyces* sp. Through modifications of intracellular levels of iron and manganese, two mutants showed a significant improvement in xylose consumption rate and 2G ethanol yield under semi-anaerobic conditions. One of the prominent targets is a vacuolar Fe^{2+}/Mn^{2+} importer and the second one is a protein involved in heavy metal ion homeostasis, responsible for vacuolar degradation of membrane metal transporters. Interestingly, deletion of genes that decreased cytosolic metals' levels by impairing mechanisms responsible to provide these elements to cytosol have developed strains with inferior fermentative performance when compared to the parental strain. Combined, these results confirm that the fine-tuning adjustment of divalent cations levels and intracellular cofactor availability could be rationally engineered to improve xylose fermentation, providing promising new genetic targets for metabolic engineering procedures aiming 2G ethanol production.

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THE CROSSTALK OF THE RECEPTOR-LIKE KINASE NIK1 IN PLANT IMMUNITY AGAINST VIRUS AND BACTERIA

Marco Aurelio Ferreira[1, 2]; Ruan Maloni Teixeira[1,2]; Bo Li[3,4]; Paola de Avelar Carpinetti[1,2]; Elizabeth Pacheco B. Fontes[1, 2*]

[1]Department of Biochemistry and Molecular Biology, Universidade Federal de Viçosa, Minas Gerais, 36570.000, Brazil; [2]National Institute of Science and Technology in Plant- Pest Interactions, Bioagro, Viçosa, Minas Gerais, 36570.000, Brazil; [3]State Key Laboratory of Agricultural Microbiology, Huazhong Agricultural University, Wuhan, Hubei, 430070, China; [4]The Provincial Key Lab of Plant Pathology of Hubei Province, College of Plant Science and Technology, Huazhong Agricultural University, Wuhan, Hubei, 430070, China

Key-words: pathogen, immunity, receptor-like kinases

Receptor-like kinases (RLKs), represented by PAMP recognition receptors (PRRs), are the first class of membrane receptors that recognize conserved structural motifs of pathogens (PAMPs). The perception of PAMPs by PRRs activates PAMP-triggered immunity (PTI), which inhibits a broad spectrum of potential pathogens, including bacteria, viruses, fungi and oomycetes. The RLK NIK1 (NUCLEAR SHUTTLE PROTEIN- INTERACTING KINASE 1) triggers an antiviral defense mechanism by activating its kinase domain that in turn mediates RPL10 phosphorylation at Ser-104. Phosphorylated RPL10 is then targeted to the nucleus and interacts with the LIMYB transcription factor, mediating host translation suppression by down-regulating genes from the translational machinery. In this investigation, we analysed the role of NIK1 in the regulation of anti- bacterial immunity induced by flg22 treatment and initiated by FLS22-BAK1 complex formation. The interation of NIK1 with BAK1 or FLS2 was monitored by BiFC in tobacco, which demonstrated that the interactions between these receptor-like kinases occurred in the plasma membrane. We also used Co-IP assays and showed that NIK1 interactions with FLS22 and BAK1 are enhanced by flg22 treatment and inhibit antibacterial immunity. To directly assess whether the antibacterial PAMP flg22 also activates the NIK1-mediated antiviral signaling, we used RD-RPL10-GFP-overexpressing transgenic lines and transient expression of RPL10-GFP in protopolasts of *Arabidopsis thaliana*, ecotype Columbia (Col-0), and nik1, bak1-4, fls2 mutants. Treatment of transgenic lines with flg22 derived from bacterial flagellin at 100 nM for 3h promoted RPL10 phosphorylation to the same extent as expression of the phosphomimetic NIK1-T474D mutant. This was monitored by immunoprecipitating RPL10-GFP with α-GFP antibody followed by immunoblotting with α-phosphoserine antibody. The flg22-induced phosphorylation of RPL10 was not only dependent on the NIK1 function but also on the formation of an active FLS22-BAK1 immune complex, because flg22 did not mediate RPL10 phosphorylation in the knockout lines. We further analysed flg22-mediated NIK1 activation by monitoring the expression of antiviral immunity-associated marker genes. Ribosomal protein gene expression was verified by qRT-PCR in WT plants, NIK1T474D- expressing lines, in the mutants nik1, bak1-4, fls2 and in the double mutant nik1-bak1-4, which were grown in vitro and treated or not with flg22 for 3h. Like RPL10 phosphorylation, flg22-mediated downregulation of *RP13A, RPL28A, RPS13B* and *RPS25B* required not only NIK1 but also the functions of FLS2 and BAK1. However, the expression of NIK1-T474D in bak1-4 and fls2 knockouts represses the expression of the ribosomal genes, confirming that flg22-induced phosphorylation of NIK1 is downstream of BAK1-FLS2 complex formation. Taken together, these results indicate that NIK1 inversely modulates antiviral and antibacterial immunity in plants.

COMPREHENSIVE ANALYSIS OF LNCRNAS IN CANINE INVASIVE UROTHELIAL CARCINOMA

Nayra Valle[1]*, Flavia Lopes[1]

[1]São Paulo State University (Unesp), School of Veterinary Medicine.

*nayra.valle@unesp.br

Keywords: epigenetics; cancer; dog

Urothelial carcinoma is the most prevalent lower urinary tract cancer in dogs, the invasive subtype is predominant and linked to higher metastatic rates and worst prognosis. Frequently, diagnosis is delayed; increasing incidence of metastasis and therapeutic protocols are rarely curative. Characterizing oncogenesis can lead to identification of biomarkers, early diagnosis and development of novel therapies. Epigenetic mechanisms control gene expression pre and post-transcription and are largely associated to the development and progression of tumors. Long non-coding RNAs (lncRNAs) mediate one of those mechanisms and their implications on the etiology and pathogenesis of cancer has been experimentally observed. On the present study, our goal was to characterize the expression profile of lncRNAs on invasive urothelial carcinoma in dogs. RNA-seq data of tumor samples from dogs with naturally occurring urothelial carcinoma (n=11) and normal bladder tissues from five healthy dogs were obtained from a previous study (Sequence Read Archive, DRA005844, National Center for Biotechnology Information). Reads were aligned (HISAT2) to the dog reference genome (CanFam 3.1.72), transcripts were assembled (StringTie) and *in silico* analysis was performed to identify known and predicted lncRNAs (FEELnc). Following read counts, differential expression was determined by DESeq2 (Fold Change ≥±2; FDR-adjusted p≤0.01). A total of 34764 lncRNAs were identified on tumor samples and 27095 on control. Of those, 1012 were differentially expressed. Given that canine lncRNA function is largely uncharacterized, orthology to human lncRNAs was performed, and we highlight lncRNA MEG3, known as a tumor suppressor, downregulated on different types of human cancers, as well as in our study. Our results suggest a role for lncRNAs on invasive urothelial carcinoma in dogs. Supported by grant 2018/16621-8, São Paulo Research Foundation (FAPESP).

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ANALYSIS OF CIS-ACTING ELEMENTS OF THI1 GENE IN *Arabidopsis Thaliana* AND *Physcomitrella patens* PROVIDE CLUES ABOUT THEIR FUNCTIONS

Henrique Moura Dias[1]; **Marie-Anne Van Sluys**[1]

[1]Genomics and Transposable Elements Laboratory, Department of Botany, Institute of Bioscience, University of São Paulo.

diash@usp.br & mavsluys@usp.br

Keywords: Promoter analysis; Functional gene; Plant biology

The thi1 (thiamine thiazole synthase) gene encodes an essential protein that takes part of several metabolic pathways, such as carbohydrate and amino acid metabolisms. However, little is known about the regulatory mechanisms of thi1 expression in plants. One possible approach to better understand the regulation of eukaryotic gene expression is the analysis of Cis-Acting Elements (CAEs), which control and take part of all stages of development and is directly associated with transcription factors (TF) in complex signalling networks. In this work, we performed an *in silico* analysis to look for motifs that could indicate the mechanisms of thi1 gene expression in plants. For that, we selected a genomic fragment of 5 kbp located upstream of the start codon of each thi1 occurrence in *Arabidopsis thaliana* and *Physcomitrella patens*, sequences that were extracted from the PHYTOZOME database. Motif prediction and comparison was performed by using the PlantCARE tool and database. A total of 301 CAEs were identified in the promoter region of the *A. thaliana* thi1 gene (AtThi1) and on average about 396 CAEs in *P.patens* Thi1 copies (PpThi1). We detected CAEs distributed throughout the upstream region of the gene with sizes ranging from 4 to 12 bp, whereas most CAEs were 4-9 bp long, grouped into four different functional categories (light response, elements associated with developmental pathways, stress e others), that were equally present in both plant species. Although the functional groups found were the same in *A. thaliana* and *P.patens*, they were differentially distributed. The central elements: TATA and CAAT motifs were found very close to the translation starting site (ATG) in most thi1 genes, in both species. However, the number of occurrences of these motifs was higher in thi1 copies of *P.patens* when compared to the *A. thaliana* gene. These results corroborate with data previously published by our research group, as thi1 gene responds to light and plays an important role in plant development at different stages.

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Setaria sphacelata: a polyploid complex?

Ana Luiza Franco[1]; Marina Arantes[1]; Thiago Angelo Cruz[2]; Elyabe Monteiro de Matos[1]; Ana Luisa Sousa Azevedo[2]; Antônio Vander Pereira[2]; Lyderson Facio Viccini[1]

[1]Universidade Federal de Juiz de Fora. [2] Embrapa Gado de Leite.

*luiza.francojf@hotmail.com

Palavras-chave: polyloidy; forage plants; Setaria

Brazil is one of the top milk and beef producing countries in the world and major efforts have to be made to increase the forage productivity. *Setaria* Beauv. (Poaceae) is a promising genus regarding the forage production and *Setaria sphacelata* remains poorly studied. The knowledge about the genome organization is a fundamental step to design breeding strategies. In the present study, we analyze the genome of *S. sphacelata* populations focusing on DNA content, chromosome number and karyotypic data. Seventy two populations collected around the world were gently provided by USDA-EUA and cultivated at the experimental area of Embrapa Gado de Leite, Coronel Pacheco, MG. The genome size was accessed by flow cytometry. Chromosome counting and fluorescent *in situ* hybridization were also performed on selected individuals that represent the genome size variation. Most populations showed DNA contents ranging from 3 to 3.7 picograms. However, populations 8, 9 and 78 presented lower values, ranging from 1.6 to 2.7pg. On the other hand, populations 33 and 20 showed around 4.5pg, whereas in population 54, it was possible to observe 5.8 pg of DNA. Five different chromosome numbers was observed. Considering x=9 as the basic chromosome number of the species, we observed diploids (2n=18), tetraploids (2n=36), pentaploids (2n=45), hexaploids (2n=54) and octaploids (2n=72 ca). Regarding the mapping of ribosomal genes, the number of 5S and 18S marks increase as the chromosome number increased reaching, respectively, 8 and 14 signals in the octaploid. The results emphasized the wide intraspecific variation with five ploidal levels and two new chromosome numbers, giving evidences that support the occurrence of an interesting polyploid complex. The data contribute to provide evolutionary insigths about the species and represents a valuable pre-breeding information.

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DETECTION OF AGR LOCUS AND VIRULENCE GENES IN STAPHYLOCOCCUS AUREUS ISOLATED FROM ARTISANAL CHEESE FROM SERRA DA CANASTRA, BRAZIL.

Ana Paulina Arellano Pineda[1]; Gabriela Zampieri Campos[1]; Ruy Chacón[2]; Nathalia Cristina Cirone Silva [3]; Uelinton Manoel Pinto[1,4]

[1]Food Research Center; University of São Paulo, São Paulo 05508-000, Brazil [2] Departament of Pathology, School or Veterinary Medicine, University of São Paulo, São Paulo 05508-270, Brazil [3] Faculty of Food Engineer, University of Campinas, São Paulo 13083-000. [4]Present address: Harvard Medical School (340 Thier Builiding, 50 Blossom St. 02114, Boston - MA, USA).

anapaulinarellano@usp.br

Keywords: *Staphylococcus aureus*; virulence factors; *agr* locus.

Serra da Canastra's Minas Artesanal cheese is one of the most important traditional cheeses produced in Brazil. Manufacture involves the inoculation of endogenous starter cultures called *pingo*, which are collected from previous lots, in freshly collected raw milk contributing with the fermentation process. Some studies have shown the presence of *Staphylococcus aureus* in cheeses made with raw milk which may cause food poisoning due to consumption of *Staphylococcus* enterotoxins (SEs). *S. aureus* is one of the main causes of mastitis, which is a common disease in dairy cows affecting the quality of milk and resulting in contaminated food. Other sources of contamination may be handling practices, milking equipment, the environment, and the skin of dairy animals. The expression of more than 70 genes that encode for virulence factors in *S. aureus* is controlled by a quorum sensing regulatory locus called accessory gene regulator (*agr*). The aim of this study is to find the presence of virulence factors in different types of *agr* locus *S. aureus* strains isolated from cheese produced in *Serra da Canastra*. 161 *Staphylococcus aureus* strains were isolated from cheeses collected from 83 producers and submitted to biochemical tests for species confirmation, such as coagulase, catalase, termonuclease, cumpling factor and Gram staining. The strains were analyzed by polymerase chain reaction (PCR), to confirm the presence of the genes *nuc*, *coa* and *femA*. Furthermore, classical enterotoxins (*sea-see*), enterotoxins-like (*sem, seo*), new enterotoxin (*seg*), toxic shock syndrome toxin 1 (*tsst*), fibronectin-binding protein (*fnbB*) and the accessory gene regulator alleles (*agr*) were evaluated. Strains with methicillin resistance (MRSA) were investigated by amplification of the *mecA* gene. The clonal profile of the isolates was characterized by *agr* typing. The results showed that the most frequent enterotoxin genes were *sea* (1,8%), *seg* (19,3%), *seo* (19,3%) and the fibronectin-binding protein was found in 24,2% of strains. The agr groups were determined by multiplex PCR and distributed as follows: *agr* I (21%), *agr* II (25%), agr III (24%), *agr* IV (0%), while 29% did not present an *agr* type. The *mecA* and *tsst* genes were not detected in this study. The results show that most of the detected virulence genes are present in *agr* type II strains, showing a greater virulence potential in this group. The isolates of *agr* type II carry genes *seg* and *seo*. These findings may serve as a baseline study and further investigations are needed to determine the pathogenic potential of these strains as well as the risks associated with consumption of cheese containing these bacteria.

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IDENTIFICATION AND PHYLOGENETIC ANALYSIS OF *MARINERS* ELEMENTS IN THE GENOME OF *EUCHROMA GIGANTEA* (BUPRESTIDAE)

Catarine A. A. Mello[1]; Igor C. Amorim[1]; Crislaine Xavier[1]; Gabriel L. Wallau[2]; Rita C. Moura[1]

[1]Laboratório de Biodiversidade e Genética de Insetos – Instituto de Ciências Biológicas, Universidade de Pernambuco, Recife, PE – Brasil. [2]Departamento de Entomologia, Instituto Aggeu Magalhães – FIOCRUZ, Recife, PE, Brasil.

ritamoura.upe@gmail.com

Key-words: Transposable elements,horizontal transfer, protein similarity.

Transposable elements (TEs) are repetitive and dispersed sequences, which are characterized by mobility in the genome. TEs can be originated in the genome both by vertical inheritance or horizontal transfer (HTT). Among the superfamilies of the elements, *Tc1-Mariner* is remarkable for its diversity and for presenting the largest number of horizontal transfer events reported, especially those belonging to the *Mariner* family. The purpose of this work was to characterize the *Mariner* elements of *Euchroma gigantea* (Buprestidae) genome and evaluate their mode of transmission. For this, a genomic sequencing was initially performed on the Illumina platform of an individual from the population of Recife, Pernambuco, and later the elements were characterized by two different approaches. On the first approach, the sequences were analyzed in dna-PipeTE, which assembly the repetitive sequences using two or more Trinity interactions, and, finally, the annotation of the assembled contigs. On the second, the sequences were clustered by RepeatExplorer, resulting in different contigs. The resulting contigs from the two analysis were reassembled in CAP3. From this analysis, the sequences obtained were submitted to ORFfinder, followed by Blastp (NCBI) and Censor (Repbase), in order to identify homologous elements. Phylogenetic trees were reconstructed using the maximum likelihood method. The results revealed the presence of 43 elements belonging to the *Mariner* family, of which 32 were obtained in the first approach and 11 in the second. These elements are structurally conserved, with intact domains including HTH, integrase, transposase and / or DDE catalytic domain. Phylogenetic analysis revealed evidence of HTT, since the incongruence between the phylogenies of the elements and those of the hosts was observed. In these phylogenies, the elements of *E. gigantea* were monstly grouped with elements found in the genomes species from the Hymenoptera and Diptera orders, even in the presence of elements from other Coleoptera species. In relation to the protein similarity, the TEs of *E. gigantea* have high similarity with TEs of phylogenetically distant species. The highest percentages were observed with the ants *Acromyrmex echinatior* (96.61%), *Harpegnathos saltator* (96.4%) *Linepithema humile* (95.73%), *Camponotus floridanus* (95%) and the bee *Apis mellifera* (96,36%). These species have indirect ecological relations with *E. gigantea*, since they are all parasites or plant pollinators of the Malvaceae family. The characterization of TEs *Mariner* by different approaches allowed to identify several elements in *E. gigantea*, which are structurally conserved and are probably originated from HTT events between different species of insects.

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INVESTIGATION OF GENETIC CAUSES IN WOMEN WITH ENDOMETRIAL CANCER PROFICIENT FOR THE MISMATCH REPAIR SYSTEM.

Jessica Oliveira de Santis[1*]; Reginaldo Cruz Alves Rosa[1]; Jennifer Thalita Targino dos Santos[2]; Victor Evangelista de Faria Ferraz[1]

[1]Department of Genetics at Ribeirão Preto Medical School – University of São Paulo. [2]Ribeirão Preto College of Nursing - University of São Paulo.

*jolisantis@gmail.com

Key-words: Germline mutations; Endometrial carcinoma; Next generation sequencing

Background: Germline mutations harbored by endometrial cancer patients can be passed on through generations. Endometrial carcinoma (EC) is related to hereditary predisposition in 5% cases. Of those, and in most cases, the familial risk for EC is related to pathogenic variants of genes of the mismatch repair (MMR) system, PTEN and STK11. Although several studies have been done on association between EC and cancer predisposition syndromes, a significant percentage of patients cannot be diagnosed. **Objective:** Thus, the aim of this project is to identify germline mutations in coding and regulatory regions of cancer predisposing genes in women with endometrial cancer proficient for the MMR system (MSS), who have a familial and/or personal history of cancer. **Methods:** The casuist includes eight women with endometrial carcinoma classified as MSS, whose tumor was previously triad from a universal unsorted cohort of women with EC. Following, each patient was classified, based on personal and/or familial history of cancer, for risk of cancer predisposition according to modified criteria from Society of Gynecologic Oncology (SGO). After, the cases were sequenced through a 63 genes Next Generation Sequencing (NGS) panel methodology, which includes repair genes, genes of stress signaling pathways, oncogenes and tumor suppressor genes. The variants were annotated according to its pathogenicity based on ACMG (American College of Medical Genetics and Genomics) guidelines. **Results:** All women were classified as high risk for cancer according to history of cancer, whereas 1/8 woman through personal history only, 4/8 had both personal and family history, and other 3/8 had family history only. Of the variants found, two were classified as pathogenic: BRCA1 (c.178C>T, p.Q60X) and MUTYH (c.230G>A, p.G77D), both in heterozygosis. Ten variants of uncertain significance (VUS) were found in the genes AMT, BMPR1A, BRIP1, EGFR, GREM1, MUTYH, RINT1 and RAD51D. **Discussion:** Both pathogenic mutations have controversial reports about its association with EC in the literature. BRCA1 mutations have strong correlation to breast and ovarian cancers, with EC, association studies mostly relate the increase risk with tamoxifen use. MUTYH gene has strong association to carcinogenesis, the majority in cases of biallelic variants. However, studies have reported cases of monoallelic variants doubling the risk for endometrial cancer. The VUS found in the EGFR gene has not been associated with cancer susceptibility phenotype. **Conclusion:** Evidenced by the results, the conclusion promotes the importance of studying the etiology of familial cancer. This project introduces the concept that women with MSS endometrial carcinoma who met high risk criteria for cancer predisposition should be considered for genetic investigation.

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INVESTIGATION OF VARIANTS IN *CASP9* GENE IN ASSOCIATION TO GASTRIC CANCER

Giovanna Chaves Cavalcante[1],*; Milene Raiol de Moraes[1]; Cristina Maria Duarte Valente[1]; Caio Santos Silva[1]; Antônio André Conde Modesto[2]; Paula Baraúna de Assumpção[2]; André M. Ribeiro-dos-Santos[1,2]; Sidney Santos[1,2]; Paulo Pimentel de Assumpção[2]; Ândrea Ribeiro-dos-Santos[1,2]

[1]Laboratório de Genética Humana e Médica – Programa de Pós-Graduação em Genética e Biologia Molecular, Universidade Federal do Pará, Belém, Pará, Brazil, 66075-970; [2]Programa de Pós-Graduação em Oncologia e Ciências Médicas – Núcleo de Pesquisas em Oncologia, Universidade Federal do Pará, Belém, Pará, Brazil, 66073- 005

*giovannaccavalcante@gmail.com

Keywords: apoptosis; *CASP9*; gastric cancer.

Caspases are enzymes responsible for different pathways in apoptosis, a regulated type of cell death. Caspase-9 (encoded by *CASP9* gene) is a caspase that plays a central role in the intrinsic pathway of apoptosis, leading to activation of effector caspases in the final phase of this mechanism. Evasion of apoptosis is a fundamental process in carcinogenesis, so that genetic variants in apoptosis-related genes could affect cancer development. In this context, it is important to study gastric cancer (GC), one of the most incident and aggressive types of cancer worldwide. In northern Brazil, it is the second most incident type of cancer among men and the fourth among women. Currently, there are not many studies regarding *CASP9* gene variants in association to GC. Therefore, here, we analyzed the genotypic distribution of two variants in *CASP9* gene (rs4645982 and rs61079693) in GC patients (Case) and cancer-free individuals (Control) from northern Brazil. These variants were genotyped by multiplex PCR in samples from case (N=93) and control (N=98) groups. A previously developed panel of 61 Ancestry Informative Markers (AIM) was also employed to avoid misinterpretation due to substructure in this admixed population. Amplification reaction was done using Veriti thermal cycler and DNA fragments were separated and analyzed using ABI PRISM 3130 genetic analyzer and GeneMapper ID v.3.2 software. Statistical tests were carried out using JASP software v. 0.9.2.0. In the three performed analyses (DEL/DEL *vs.* others, INS/DEL *vs.* others and INS/INS *vs.* others), we did not find a statistically significant difference between case and control groups regarding distribution of rs4645982 (P 0.897, 0.583 and 0.535) and rs61079693 (P 0.418, 0.765 and 0.621) genotypes. Thus, it is likely that these variants do not play a relevant part in GC development, suggesting that other genetic variants in *CASP9* might be involved in this process considering the important role this gene performs in apoptosis. Further studies are recommended to strengthen our findings, but this work contributes to a better comprehension of the influence of variants in *CASP9* gene to gastric cancer.

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IDENTIFICATION OF REFERENCE GENES FOR IMMUNE RELATED GENES EXPRESSION IN BLOOD SAMPLES OF CATTLE INFECTED WITH *Babesia bovis* AND *Babesia bigemina*

Hayala Caroline Silva Ferreira Gomes[1*]; César Cristiano Bassetto[1,2]; Maria Fernanda Tonelli[3]; Henrique Nunes de Oliveira[1]; Márcia Cristina Sena de Oliveira[2]; Cintia Hiromi Okino[2]

[1]School of Agricultural and Veterinary Studies (FCAV), UNESP, Jaboticabal, SP, Brazil. [2]Embrapa Southeast Livestock (CPPSE), São Carlos, SP, Brazil. [3]University Center Central Paulista (UNICEP), São Carlos, SP, Brazil.

[*]hayala_caroline@hotmail.com

Keywords: Reference gene, RT-qPCR, stability test

Bovine babesiosis is an infectious disease caused by the protozoas *Babesia bovis* and *Babesia bigemina*, which are intra-erythrocytic parasites responsible by intravascular hemolytic anemia. The occurrence of babesiosis follows the dispersion of the tick *Rhipicephalus microplus*, considered the unique vector responsible by transmission and dissemination of the etiological agents in the herds. It is considered an endemic disease and causes significant economic losses due to high rates of mortality and morbidity. Therefore, the characterization of the immune response in infected animals may allow the selection of resistant animals. Among the available methods for the evaluation of immune responses, quantification of gene expression by RT-qPCR is an option characterized by high reproducibility and fastness. However, to reach accurate results, some variables, individual or cumulative, must be considered, such as RNA quantity and quality, reverse transcription efficiency, presence of reaction inhibitors, the primers efficiency, among others factors. Besides, for normalization of these variables, it is recommended the use of one or more reference genes, characterized by high stability. In this context, the present work aimed to evaluate the stability of five reference candidate genes: ACTB, B2M, GAPDH, PPIA and YWHAZ. Blood samples from 48 calves were sampled during one year, in a total of 16 samples, the complementary DNA (cDNA) was synthesized from 1000 ng of total RNA extracted from the animals' blood, using the High-Capacity kit cDNA Reverse Transcription (Applied BiosystemsTM, Cat. 4368814) and Oligo dT primers (Sigma). Two computational software were used to evaluate the results for the selection of the reference gene. The BestKeeper © - version 1 software , the highest stable genes were B2M and PPIA, presenting higher correlation coefficients (0.750 and 0.753, respectively), and lower values of the coefficient of variation (2.44 and 2.29, respectively). The results of the Normfinder software pointed out B2M as more stable, with a stability value of 0.131 and the best combination of genes was the association of B2M and PPIA, with a stability value of 0.098. Thus, the B2M and PPIA genes could be used as reference genes in the study of immune related genes expression in cattle blood.

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ASSOCIATION BETWEEN HLA-G (14PB INS/DEL) AND PRIMARY FEMALE INFERTILITY

Thaiany Luz[1*]; Júlia Pereira[1]; Rebeca Calasans[1]; Fernanda Garcia[1]; Raquel Dettogni[1]; Raquel Reis[1]; Layza Borges[2]; Iúri Louro[1]

[1]Universidade Federal do Espírito Santo. [2]Hospital Universitário Cassiano Antônio Moraes.

*thaianyluzm@gmail.com

Key words: HLA-G; Polimorfism; Infertility

Infertility is defined as "no pregnancy after one year of regular intercourse and no use of contraception" and affects about 20% of couples of childbearing age worldwide. Approximately 10% of the cases have no known motivation and, in these situations, immunological causes can be attributed, such as maternal intolerance to the embryo. Correct implantation of the embryo to the mother is related to the binding of Human Leukocyte Antigen - G (HLA-G) molecules, expressed by trophoblast cells, to the inhibitory receptors of uterine Natural Killers (uNK's) cells present in the endometrium. The differences in expression and interaction of these molecules are due to polymorphisms in their respective coding genes, which may be associated with gestational complications, implantation failure and recurrent spontaneous abortion, as observed in the 14 bp Ins-Del polymorphism in the HLA-G gene. This study aimed to perform a population characterization, determining the allelic and genotype frequencies of the 14pb ins/del polymorphism of the HLA-G gene in the population of Espírito Santo (ES), and through a case-control study to relate the polymorphism in question with primary infertility. The general population was composed of 100 healthy individuals, randomly selected and the control case was formed by 26 women with primary infertility (case) and 26 women with 2 or more pregnancies with no history of gestational complications (control). Samples for the population characterization were with the genetic material already isolated, while the samples for the control case were collected by FTA paper and/or Swab and phenol-chloroform extracted, then the samples from both studies were submitted to the Reaction in Polymerase Chain and visualized in vertical electrophoresis. The frequencies found for the general population were 58 individuals with heterozygous Ins-Del genotypes, 15 homozygous Ins-Ins and 33 Del-Del homozygotes, this population is outside the Hardy-Weinberg equilibrium. The frequencies were compared with those of other 12 populations, 10 of them with similar values to the ES population. Analyzing the case-control groups, the case group consisting of 3 samples for Ins/Ins, 9 for Del/Del and 14 for Ins/Del, and the control group presented 4 for Ins/Ins, 9 for Del/Del and 13 for Ins/Del. Populations were tested for Hardy-Weinberg equilibrium, demonstrating that the population is within the equilibrium patterns. The results were also submitted to the Fisher Exact test, evidencing no statistically significant values, and it is not possible to infer an association between primary infertility and the polymorphism in question in the studied population. These results can have as interference the low sample number, being necessary more studies with a larger population.

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CHARACTERIZATION OF SNPs IN HIGH DENSITY FOR THE PRE-BREEDING PROGRAM IN TAMBAQUI *Colossoma macropomum*

John F. Gomez[1], Raquel B. Ariede[1], Vito A. Mastrochirico-Filho[1], Diogo T. Hashimoto[1]

[1]Centro de Aquicultura, Universidade Estadual Paulista, Jaboticabal, Brazil.

jhnpnk@hotmail.com

Keywords: ddRAD-seq; genetic diversity; variability

The South America aquaculture has experienced significant growth in recent years. However, the potential of production will certainly be better explored with the use of products resulting from breeding programs. The tambaqui, *Colossoma macropomum* (Characiformes, Serrasalmidae), is a fish from the Amazon basin and is considered the main native species used in aquaculture production in South America (*e.g.*, Brazil, Peru and Colombia). The use of molecular markers in fish farming has been shown to be essential, especially for genetic monitoring of populations and pre-breeding programs. In general, lack of knowledge on genetic variability of stocks can result in inbreeding and fixation of deleterious genes, reduced growth rates, disease resistance problems and reduced ability to adapt to new environments. The objective of this study was to characterize SNPs (single nucleotide polymorphisms) in high density by ddRAD-seq (double-digest RAD-sequencing) for the pre- breeding program in tambaqui *Colossoma macropomum*. The base population from the breeding program of Caunesp (Aquaculture Center of the São Paulo State University, Brazil) were used as biological material. For the library construction, the genomic DNA was extracted from 18 individuals of tambaqui, and then digested with the restriction enzymes SpHI and MlucI. Posteriorly, the digested DNA was ligated to specific adapters of each enzyme, including individual barcodes. The library was sequenced on the Illumina Hiseq platform, with average of 2.5 million reads per individual. Bioinformatics analyzes were performed by Stacks software. Initially, low quality reads were eliminated for the assembly of SNP catalogs. A total of 243,687 loci were identified, of which 51,813 loci were maintained after filter steps, presenting 16,438 SNPs. We randomly selected 500 SNPs, of which 13 presented Hardy-Weinberg disequilibrium (p-value <0.0027). The observed heterozygosity values were between 0.056 and 1, with a mean of 0.420; while the expected heterozygosity values were between 0.272 and 0.519, with a mean value of 0.415. The coefficient of inbreeding (F_{IS}) ranged from -0.766 to 0.866. The SNPs discovered in this study can be used as the framework for the genetic management of breeding stocks, which might provide a basis for a genetic pre-breeding program in tambaqui.

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OXIDATIVE STRESS AND MITOCHONDRIAL ALTERATIONS IN PATIENTS WITH MILD COGNITIVE IMPAIRMENT

Laís Bianchoni Manoel[1*]; Johnathan de Andrade Vieira[2]; Elza Tiemi Sakamoto Hojo[1].

[1]Faculty of Philosophy, Sciences and Letters of Ribeirão Preto, University of São Paulo, Ribeirão Preto, São Paulo, Brazil. [2]Department of Genetics, University of São Paulo – Faculty of Medicine of Ribeirão Preto, Ribeirão Preto, São Paulo, Brazil.

*lais.manoel@usp.br

Key-words: TP53 protein; SOD1 protein; mitochondrial alterations.

Mild cognitive impairment (MCI) is a disease that confers a risk factor for the development of dementia, such as Alzheimer's disease (AD). Although MCI patients show cognitive decline more than expected, daily activities remain intact or only slightly altered. The objective of this work was to study possible molecular and mitochondrial alterations related to MCI in individuals diagnosed with this disease, compared to healthy individuals. Blood samples were taken from patients at the Hospital das Clínicas (FMRP-USP) of Ribeirão Preto, S.P., and peripheral blood mononuclear cells (PBMC) were isolated, and used for the analysis of mitochondrial parameters, such as alterations in the membrane potential, mitochondrial ROS (Reactive Oxygen Species) level, mitochondrial mass, intracellular ROS assay and protein expression. A statistically significant difference was not observed for membrane potential, intracellular ROS and mitochondrial ROS. In the quantification of mitochondrial mass, a statistically significant difference ($p < 0.05$) was observed in the fluorescence intensity, indicating that patients with MCI may present mitochondrial dysfunction. The expression of TP53 tumor suppressor protein presented significant differences ($p < 0.05$) in patients with MCI, with an increase of approximately 187% in terms of relative expression. Regarding the SOD1 protein expression, there was a reduction of 18.5% in relative expression, but the differences were not statistically significant. The results obtained are similar to those obtained in a previous study with patients with AD, and may indicate that most probably, some alterations are already occurring before the development of the dementia. In addition, the parameters evaluated may be further studied as important markers to facilitate the diagnosis of MCI.

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Circular RNAs as potential biomarkers of gastric cancer

Laís Reis das Mercês[1*]; Leandro Magalhães[1]; Rafael Pompeu[1]; Gloria Tatiana Vinasco Sandoval[1]; Aline Maria Pereira Cruz Ramos[2]; Ândrea Ribeiro-dos-Santos[1,2]; Amanda F. Vidal[1]

[1]Laboratório de Genética Humana e Médica, Programa de Pós-Graduação em Genética e Biologia Molecular, Universidade Federal do Pará, Belém, Brazil; [2]Programa de Pós-Graduação em Oncologia e Ciências Médicas, Núcleo de Pesquisas em Oncologia, Universidade Federal do Pará, Belém, Brazil.

*laiscdreis@gmail.com

Keywords: Circular RNAs; Biomarker; Gastric Cancer

Circular RNAs (circRNAs) are noncoding endogenous RNA molecules, covalently joined by their 3' and 5' ends, and characterized as remarkably stable, abundant, exonuclease- resistant, and evolutionarily conserved transcripts. They are differentially expressed in several diseases, including gastric cancer, and one of its potential functions is to act as RNA binding proteins (RBPs) sponges. In a previous study, we found through next generation sequencing that the circRNAs hsa_circ_0000284, hsa_circ_0000211, hsa_circ_0000524, hsa_circ_0004771 and hsa_circ_0001136 were differentially expressed in gastric cancer tissue. In the present study, these circRNAs were selected to be validated by qRT-PCR in whole blood samples (gastric cancer patients and cancer- free individuals). Blood samples of gastric cancer patients and cancer-free volunteers were collected in PAXgene Blood RNA Tubes and total RNA was extracted using PAXgene Blood miRNA Kit. 50 ng of total RNA were reverse transcribed using SuperScript III First-Strand Synthesis and qRT-PCR of all circRNAs were realized using SsoAdvanced Universal SYBR® Green SuperMix with specific primers. All reactions were performed in triplicates, qPCR data was analyzed using the comparative Ct Method and ß-actin was used as an endogenous control. Student's T test was used to compare the expression of each circRNA between groups and p-values < 0.05 were considered to be statistically significant. We observed that the circRNAs hsa_circ_0000284, hsa_circ_0000211, hsa_circ_0004771 and hsa_circ_0001136 are overexpressed in gastric cancer patients when compared to cancer-free individuals (P = 5.96e-07, P = 4.97e-05, P = 0.0003 and P = 8.5e-07, respectively). When we performed a ROC curve analysis the differentially expressed circRNAs had a high sensitivity and specificity (AUC > 0.7), suggesting them as minimally invasive potential risk biomarkers for gastric cancer. Additionally, we identified that these circular RNAs have multiple binding sites, confirmed by CLIP-seq, for RNA binding proteins with important roles in the cell, such as spliceosome, RNA transport and mRNA surveillance. These findings show that the circRNAs have relevant roles in the regulatory epigenetic network and are minimally invasive potential biomarkers of gastric cancer.

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Circular RNA's global expression in triple-negative breast cancer and their potential as risk biomarkers

Leandro Magalhães[1*], André M. Ribeiro-dos-Santos[1,2], Amanda F. Vidal[1], Kivvi Duarte De Melo Nakamura[3], Rafael Brianese[3], Dirce Maria Carraro[3], Ândrea Ribeiro-dos-Santos[1,2]

[1]Laboratório de Genética Humana e Médica, Programa de Pós-Graduação em Genética e Biologia Molecular, Universidade Federal do Pará, Belém, Brazil; [2]Programa de Pós-Graduação em Oncologia e Ciências Médicas, Núcleo de Pesquisas em Oncologia, Universidade Federal do Pará, Belém, Brazil; [3]Genomic and Molecular Biology Group. CIPE, A.C. Camargo Cancer Center, São Paulo, Brazil.

leandromag@me.com

Keywords: CircRNAs; Triple-negative Breast Cancer; Biomarkers

Circular RNAs (circRNAs) are a recently rediscovered class of non-coding RNAs, characterized by its 3' and 5' ends covalently joined. Recently, changes in the characteristic circRNA expression profile of a tissue have been associated with the development of several complex diseases, including triple-negative breast cancer (TNBC). Although there are some studies regarding circRNAs and TNBC, the circRNAs' global expression profile hasn't been identified in this molecular subtype of breast cancer. In this context, the aims of this study were to characterize the circRNAs global expression profile of TNBC and identify those that are deregulated in this type of breast cancer. RNAseq data from 37 cases of TNBC (25 with paired tumor and adjacent non- tumor) were obtained from a previous study, in which the data was generated using TruSeq Stranded Total RNA kit and sequenced in an Illumina NextSeq500. The resulting reads were trimmed using fastp and aligned to the hg19 reference human genome using STAR. Mapped reads were then aligned to CircBase to identify noted circRNAs and counted using three different circRNA identification tools: CircExplorer2, CIRI and DCC. Only circRNAs that were identified by all three softwares were considered for further analyses. Differential expression analyses were performed using DESeq2 and circRNAs that had an absolute log2FoldChange ≥ 1.5 and an adjusted p-value < 0.05 were considered to be deregulated in TNBC. We found 4256 circRNAs counted by all three softwares, both in TNBC and in non-tumor adjacent tissue, of which 16 were differentially expressed (DE) in TNBC. All DE circRNAs were downregulated, being hsa_circ_0072309 having the lowest expression with a log2FoldChange of -2.26. Of all 16 DE circRNAs, "chr12:97886238-97954825" was not identified in CircBase database, suggesting that this is a new circRNA involved in TNBC development. Utilizing high throughput sequencing, it was possible to characterize the circRNA profile of TNBC and identify 16 DE circRNAs, including a new one, that act in the disease development and may be potential risk biomarkers of TNBC.

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GENETIC RESOURCES OF THE SPECIES *Brycon insignis* (CHARACIFORMES): CHARACTERIZATION AND STRATEGIES FOR CONSERVATIONIST MANAGEMENT OF THE SPECIES

Letícia Rafaela de Morais[1]; Danilo Caneppele[1,2]; Caio Augusto Perazza[1,3]; Guilherme Souza[4], Alexandre Wagner Silva Hilsdorf[1*]

[1]University of Mogi das Cruzes, Unit of Biotechnology, UMC, Mogi das Cruzes, SP; [2]Energy Company of São Paulo, CESP, Paraíbuna, SP; [3]Brazilian Fish Industry-Royal Fish, Jundiaí, SP; [4]Piabanha Project, Itaocara, RJ.

*wagner@umc.br

Key-words: Piabanha, microsatellite, restocking program.

Brycon insignis, popularly known as piabanha, was reported to occur abundantly in the Paraíba do Sul River and tributaries, as well as in the Itabapoana and São João Rivers. However, currently, piabanha wild populations are only found in some stretches these water bodies due to steep reduction of populations because of environmental disturbances. Against this background, this study aimed of using microsatellite loci developed for *Brycon opalinus* to assess intra e inter- population genetic variability of 264 individuals from seven wild and one captive populations, which have been maintained in two *ex-situ* germplasm banks. Our results demonstrated that cross amplification of microsatellite markers was efficient to amplify microsatellite loci of *B. insignis*, allowing the evaluation of its population genetic structure. The populations demonstrated significant levels of structuring (DEST = 0.15, p-value < 0.01) and low average genetic diversity (Ho = 0.48). Higher levels of inbreeding were verified in the captive population. The genetic relatedness between individuals kept as broodstock was estimated to assist the production of fingerlings from dam and sire breeders of *B. insignis* with less genetic kinship. The identification of the best pairs breeders based on their genetic relatedness is crucial to minimize inbreeding among the progeny to improve the success of restocking programs. Microsatellite markers have been key component in the genetic assessment of genetic variability, providing information about population structure and establishing breeding schemes for rational restocking programs. To recover jeopardized finfish populations is of paramount importance for the long-term survival of the species as a whole. That is the actual situation of the piabanha, a species once abundant, currently on the brink of extinction.

Funding Agencies: FAPESP, FAEP, CEIVAP, CNPq

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COMPLETE MITOCHONDRIAL GENOME OF *Astyanax altiparanae* (CHARACIFORMES, CHARACIDAE)

Matheus Lewi C. B. de Campos[1,2*]; Rubens Pasa[2]; Fabiano Bezerra Menegídio[3]; John Seymour (Pat) Heslop-Harrison[4]; Trude Schwarzacher[4]; Karine Frehner Kavalco[2]

[1]Pós-Graduação em Zoologia, Universidade Federal de Minas Gerais, Belo Horizonte, Minas Gerais, Brazil. [2]Laboratório de Genética Ecológica e Evolutiva, Universidade Federal de Viçosa campus Rio Paranaíba, Rio Paranaíba, Minas Gerais, Brazil. [3]Núcleo Integrado de Biotecnologia, Universidade de Mogi das Cruzes, São Paulo, Brazil. [4]Department of Genetics and Genome Biology, University of Leicester, United Kingdom.

*matheus.bio@outlook.com

Key-words: Genomics; mitDNA; WGS.

The genus *Astyanax* comprises about 170 species of fish popularly known as tetras, lambaris or piabas. It is one of the most diverse genera of the Neotropical's region being the second richest in species of the Characidae family. With such diversity, a number of studies on morphological, cytogenetic and molecular approaches can be found about the group. Nevertheless, genomic approaches are well exploited only about *Astyanax mexicanus*, a species with surface and troglomorphic phenotypes, which is a model in evolutionary studies. For brazilian species, some of them with huge chromosomal diversity, genomic studies are still scarce. In this way, so far only three mitogenomes were described for the genus (*A. mexicanus, A. paranae* and *A. giton*), thus, we aim to describe a fourth, the mitogenome of *Astyanax altiparanae*, a species from Upper Parana river basin. The *A. altiparanae* used in this study was collected in Ribeirão Pirapitinga (Paranaíba river basin) at Ibiá – MG. Total genomic DNA was extracted from liver and heart samples as per the instructions of the Invitrogen's DNA extraction and purification kit. After quality checking, the Whole Genome Sequencing was performed by Illumina technology. The raw reads were trimmed to remove the adaptors and low-quality sequences and then mapped against the complete mitochondrion genome of *A. paranae* available on GenBank (NC_031380.1), using Bowtie2 software. The consensus sequence was annotated on GeSeq. The total mitochondrial genome obtained from *A. altiparanae* contains 16,707bp of length and it's composed of 13 coding sequence genes (CDS) corresponding to 68.42% of the genome, 25 RNA transporters (tRNA) and 2 ribosomal RNA (rRNA) genes, as well as a terminal control region (D-loop) with 844bp. The final organization of the coding genes in *A. altiparanae* is very similar to the other *Astyanax* available in the literature. Differences exist in the position, length, and number of some genes. The *A. altiparanae* D-loop, for example, has 331bp more than *A. mexicanus* and is still smaller than that of *A. giton* and *A. paranae*. Just like *A. mexicanus*, *A. altiparanae* has two more tRNAs than *A. giton* and *A. paranae*. Thus, we concluded that the order of *A. altiparanae* mitogenome genes follows the typical conformation of the vertebrates, with small changes when compared to other fishes of this genus, as is expected for a very conserved genome.

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EFFECTS OF DIFFERENT LEVELS OF SULFUR AND COBALT IN THE DIET DURING THE PRE- AND PERICONCEPTIONAL PERIODS ON THE DNA METHYLATION PROFILE OF THE PROGENY IN CATTLE

Luna N Vargas[1,2*]; Allice R F Nochi[3]; Anelise S Mendonça[4]; Roberto C Togawa[5]; Marcos M C Costa[5]; Paloma S Castro[1,2]; Alexandre R Caetano[5]; Maurício M Franco[1,2,5]

[1]Programa de Pós-Graduação em Genética e Bioquímica, Instituto de Genética e Bioquímica, Universidade Federal de Uberlândia, Uberlândia - Minas Gerais, Brasil. [2]Laboratório de Reprodução Animal, Embrapa Recursos Genéticos e Biotecnologia, Brasília - Distrito Federal, Brasil. [3]Agência de Defesa Agropecuária do Paraná, Curitiba - Paraná, Brasil. [4]Instituto Federal do Triângulo Mineiro, Campus Avançado Uberaba Parque Tecnológico, Uberaba – Minas Gerais, Brasil. [5]Embrapa Recursos Genéticos e Biotecnologia, Brasília, Distrito Federal, Brasil.

*lunavargas@hotmail.com

Key-words: Epigenetic; DNA methylation; Nutrition

The epigenome can be modulated by environmental factors during gametogenesis and early embryogenesis, when a wide epigenetic reprogramming occurs. Low levels of specific compounds of the one-carbon cycle, which provides methyl groups for the establishment of histone and DNA methylation, in diet of oocyte donors may change the epigenetic reprogramming of embryos, leading to an aberrant transgenerational epigenetic profile. The aim of this study was to evaluate the effects of low levels of sulfur and cobalt (LowS/Co) in the diet of the oocyte donor Nellore heifers during the pre- and periconceptional periods on the DNA methylation of the progeny. Whole-genome bisulfite sequencing (WGBS) was performed in preimplantation embryos and some differentially methylated regions (DMR) that were identified in the embryos were used to be tested in blood cells of the male progeny in adulthood. We found 2,336 DMRs between LowS/Co and control groups in preimplantation embryos. Among those, five DMRs of genes involved in the epigenetic machinery was choice to be evaluated in the F1 progeny. DNA methylation profile of the lysine demethylase 2A (KDM2A), lysine demethylase 5A (KDM5A), lysine methyltransferase 2D (KMT2D), DNA methyltransferase 1 (DNMT1) and DNA methyltransferase 3 beta (DNMT3B) genes were evaluated. Genomic DNA was isolated (DNeasy Blood & Tissue kit), treated with sodium bisulfite (EZ DNA Methylation- Lightning™ Kit) and specific intragenic regions were amplified by PCR. Amplicons were purified from agarose gel (Wizard SV Genomic DNA Purification System), cloned (TOPO™ TA Cloning™ Kit PCR 2.1) and sequenced by dideoxi method. Only sequences with a minimum of 95% of homology and 97% of bisulfite conversion were used. The DNA methylation percentage in embryos was 10% and 100% for KDM2A, 51.5% and 15% for KDM5A, 96.6% and 46.6% for KMT2D, 10% and 77% for DNMT1 and 46.6% and 90% for DNMT3B in the control and LowS/Co groups, respectively ($p<0.05$). In the progeny in adulthood, differences were found just for DNMT3B ($p=0.0405$). The methylation percentage was 96.2% and 94.4% for KDM2A, 96.1% and 97.1% for KDM5A, 97.4% and 97.7% for KMT2D, 91.6% and 93.2% for DNMT1 and 85.3% and 88.7% for DNMT3B in the control and LowS/Co groups, respectively. These results demonstrates that the low levels of sulfur and cobalt in the diet of oocyte donors were able to alter the DNA methylation patterns in preimplantation embryos and in the blood of the progeny in adulthood for DNMT3B gene. Based on our results, we speculate that the LowS/Co diet of the oocyte donors affected the DNA methylation profile and/or the epigenetic machinery of the oocytes during gametogenesis and embryogenesis in DNMT3B gene. However, DNA methylation of the other genes were correctly reestablished during the waves of reprogramming that occurs in mammalian embryogenesis.

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ETHICAL AND GENETIC ISSUES: WHAT DO CURRICULAR DOCUMENTS INDICATE FOR BASIC EDUCATION?

Maria Helena Machado[1]; Rosane Moreira Silva de Meirelles[1,2]

[1]Programa *stricto sensu* em Ensino em Biociências e Saúde – IOC – Instituto Oswaldo Cruz – Fiocruz - RJ. [2]Departamento de Ensino de Ciências e Biologia - DECB-UERJ - Universidade do Estado do Rio de Janeiro.

helenamestrado.09@gmail.com; rosanemeirelles@gmail.com

Keyword: Ethics; Genetics; High School

The teaching of Genetics has been presented in the main Basic Education curricular documents comprising content and discussion guidelines regarding issues related to biotechnology, gene manipulation and bioethics. Considering the current relevance of Genetics, the starting point of this study was the analysis of official documents taking into consideration the Ethics theme, in order to understand the conceptions defended in the Basic Education curriculum guide documents. The present study presents the results of a doctoral research currently in progress, investigating the possibilities and challenges regarding the approach of ethical questions for the teaching of Genetics in Basic Education. The National Curricular Parameters for High School (PCNEM and PCN+) and the National Common Base (BNCC) were analyzed. A mapping was carried out to assess which moments the documents refer to, mention, indicate or approach concerning the ethics theme in the teaching of Genetics, as a subject to be studied and discussed in Basic Education. Initially, a floating reading was carried out, which consists in searching the selected documents descriptors pertinent to the ethical questions addressed in the teaching of Genetics. Based on these findings, a specific analysis of the curricular documents was performed. With regard to the PCNEM, the difference between what is proposed in the PCNEM and the PCN+ is clear. The amount of information and concern about ethical issues is greater in the PCN+. Terms related to ethics are more frequently applied in the Chemistry area of knowledge in the PCNEM, while allusive words related to ethics are mostly found in the field of Biology in the PCN+. In this sense, both the PCNEM and PCN+ emphasize the importance of linking the teaching of Genetics to ethics issues, although the PCN+ further emphasizes the theme. Regarding the BNCC, few textual fragments containing discussions related to ethical issues and Genetics were found, suggesting a lesser degree of importance of the subject in the document that intends to be the guiding document of the current curriculum. With these findings, we hope to understand the differences in curricular approaches, proposing answers to the issue of central research on ethical discussion possibilities and challenges in the teaching of Genetics in Basic Education.

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ASSOCIATION OF *MEF2C* RELATED PATHWAYS TO VOLUMES IN BRAIN REGIONS IMPLICATED IN ADHD

Maria Eduarda de Araujo Tavares[1,2]; Renata Basso Cupertino[1,2]; Bruna Santos da Silva[1,2]; Eduardo Schneider Vitola[2,3]; Vitor Thumé Breda[2,3]; Carlos Alberto Iglesias Salgado[2,3]; Felipe Almeida Picon[2,3]; Cibele Edom Bandeira[1,2]; Eugenio Horacio Grevet[2,3]; Claiton Henrique Dotto Bau[1,2,3]

[1]Department of Genetics, Institute of Biosciences, UFRGS, Porto Alegre, RS, Brazil. [2]ADHD Outpatient Program – Adult Division, Hospital de Clínicas de Porto Alegre, Porto Alegre, RS, Brazil. [3]Department of Psychiatry, School of Medicine, UFRGS, Porto Alegre, RS, Brazil

metavaresbio@gmail.com

Keywords: ADHD, *MEF2C*-gene-set, brain volumes

The *MEF2C* (Myocyte Enhancer Factor 2C) gene has been associated through Genome Wide Association Studies (GWAS) with a far-reaching range of psychiatric and neurodegenerative disorders, such as Attention Deficit/Hyperactivity Disorder (ADHD), Schizophrenia, Substance Use Disorder, Major Depressive Disorder, and Alzheimer disease. It has also been associated with the volume of several brain regions, including hippocampus, frontal lobe and lateral ventricles, as well as with intracranial volume (ICV). Gene-set analysis is a promising tool to further clarify the role of the *MEF2C* pathways in ADHD and related brain regions. Our goal was to evaluate if volumes in brain regions previously implicated with ADHD (putamen, hippocampus, thalamus, amygdala, accumbens and pallidum, as well as overall ICV were associated with enriched *MEF2C* gene-sets. Magnetic Resonance Imaging structural data were acquired for 100 ADHD cases and 60 controls, diagnosed following the DSM-5 criteria. All samples were genotyped within Infinium PsychArray-24 BeadChip platform. Gene-set analyses were performed in MAGMA v1.07b. ADHD diagnosis, ICV, sex, age and the 10 first principal components were included as covariates. GO enrichment of *MEF2C* provided 217 gene-sets within the Biological Process category, of which 111 overlapped with the gene-sets previously associated with ADHD through GWAS. Six overlapping sets comprised *MEF2C* among their constitutive genes and were significantly associated with ADHD: regulation of protein binding (GO:0043393), regulation of myeloid cell differentiation (GO:0045637), regulation of ossification (GO:0030278), bone development (GO:0060348), mesenchyme development (GO:0060485), and skeletal system morphogenesis (GO:0048705). Gene-set analysis resulted in associations between the regulation of ossification set and bilateral hippocampus (p_{cor}=0.008511), bilateral amygdala (p_{cor}= 0.007503) and ICV (p_{cor}=0.01) and between the regulation of protein binding set and bilateral hippocampus (p_{cor}= 0.009625). In addition, the *MEF2C* gene within the sets was not individually associated with the volume outcomes. The present results suggest a possible involvement of genes related to the ossification pathway with volumes of amygdala, hippocampus and ICV, and are in line with previous studies associating these regions with ADHD and *MEF2C*. Even though this gene was not significantly associated with the volumes *per se*, its possible effect cannot be ruled out, since *MEF2C* is an enhancer and it has been widely described as a transcription factor acting in neural development. In this sense, it could exert an indirect effect through other genes or gene-interactions, and possibly be relevant to ADHD etiology as part of a genomic net effect.

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RESEARCH OF GENETIC VARIANTS IN THE MTHFR AND FACTOR V GENES IN PATIENTS WITH SICKLE CELL ANEMIA OF THE STATE OF PARÁ

Matheus Caetano Epifane de Assunção[1]*; Yure Jefferson da Cruz do Nascimento[1]; Wandrey Roberto dos Santos Brito[1]; Aylla Nubia Lima Martins da Silva[1]; Greice de Lemos Cardoso Costa[1]; João Farias Guerreiro[1].

Laboratório de Genética Humana e Médica, Instituto de Ciências Biológicas, Universidade Federal do Pará; Belém, Pará, Brasil.

*matt.caet37@gmail.com

Keywords: Sickle Cell Anemia; MTHFR; Leiden Factor V

Sickle Cell Anemia (SCA) is characterized as a chronic hemolytic disease caused by a mutation in the κ globin gene, resulting in a hemoglobin variant, HbS. When under conditions of hypoxia, polymerization of HbS and consequently erythrocyte falcization occurs. The falcization results in unequal erythrocyte properties, such as rigid non- deformable character and high expression of adhesive molecules, factors that reduce its useful life and favor its deposition in the vascular endothelium, generating blood stasis and vaso-occlusive events. Such events are responsible for triggering ischemic processes and tissue death, especially in organs where blood flow is less intense, such as in the spleen and bone marrow. Sickle cell anemia is a pathology that exposes extensive clinical heterogeneity. This variation can be associated to several factors, among them, The polymorphism rs1801133 in the gene Methylenetetrahydrofolate reductase (MTHFR), as well as the G1691A mutation of Leiden's Factor V, molecules that were studied in association with vasoconstriction events. The present study aimed to investigate the MTHFR polymorphism and Leiden Factor V mutation as genetic modulators associated with hematological indices in individuals with sickle cell anemia. One hundred and thirty patients attended at the HEMOPA Foundation were genotyped and the hematological data of HbF, HbA2, Hb, Hematocrit, VCM and HCM were obtained at medical records. The results were organized in spreadsheets through the Excel program, and the averages of each hematological index associated to different genotypes were then calculated. It was not possible to observe a statistically significant association between hematological indices and the presence of Leiden Factor V mutation and MTHFR polymorphism. However, it was possible to observe a discrete increase in the hemoglobin levels (10.25 g%), as well as in the hematocrit (31.19%) of wild homozygous individuals for Factor V and mutant homozygotes for the MTHFR polymorphism, when compared to the other genotypes. Possibly, in isolation, there is no significant association of polymorphisms in these genes and the extensive clinical and hematological variability of patients with sickle cell anemia, and therefore, several and exhaustive investigations must be carried out in order to propose a panel of genetic variants that can direct the clinical prognosis of people with sickle cell anemia.

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EFFECT OF AIRE GENE ON THE CHEMOTACTIC PROPERTIES OF MEDULLARY THYMIC EPITHELIAL CELLS ON THYMOCYTES

Mayara C. Vieira Machado[1], Max Jordan Duarte[1], Pedro P. Tanaka[1], Ana C. Monteleone Cassiano[2], Geraldo A. Passos[1,3]

[1]Molecular Immunogenetics Group, Departament of Genetics, Ribeirão Preto Medical School, USP, Ribeirão Preto, SP [2] Departament of Basic and Applied Immunology, Ribeirão Preto Medical School, USP, Ribeirão Preto, SP [3]Discipline of Genetics and Molecular Biology, Departament of Morphology, School of Dentistry of Ribeirão Preto, USP, Ribeirão Preto, SP.

*mayaracvmachado@usp.br

Keywords: *Aire* gene; mTEC cells; Crispr-Cas9.

The autoimmune regulator gene (Aire) (human chromosome 21q22.3 and murine 10qC1 Mus musculus), is a controller of central immune tolerance and the emergence of autoimmune diseases. The Aire sequence shows about 80% similarity between human and mice and this gene regulates the transcription of peripheral tissue autoantigen genes (PTAs) in medullary thymic epithelial cells (mTECs) and this phenomenon was called promiscuous gene expression (PGE). The meaning of PGE is immunological, that is, the self-representation in the thymus that is the basis for the induction of the immunological tolerance. For the tolerance of PTAs occur, thymocytes (T-cell precursors) migrates within the thymus sequentially and in this order, cortex and then medulla, establishing respectively adhesion with cortical epithelial thymic cells (cTECs) and then with mTEC cells (mTEC-thymocyte). These interactions are referred to as thymic crosstalk. Migration and adhesion events are important for the positive selection (mediated by cTECs) and negative selection (mediated by mTECs) of thymocytes. Migration of thymocytes within the thymus is controlled by the CXCL12 (secreted by the cTECs), CCL19 and CCL21 chemokines (secreted by the mTECs). In our laboratory, we are investigating the role of Aire gene in mTEC cells through the strategy of loss of function (LOF) mediated by deleterious mutations induced by the Crispr-Cas9 system. In this project, we investigated whether LOF of Aire in mTECs could be associated to imbalance in thymocyte chemotaxis. Migration assays (transwell assay) using conditioned medium from cultures of these two cell types demonstrated that complete inhibition of Aire reduced the ability of mTECs to promote thymocyte migration. Analyzes of the large-scale gene expression profiles by microarrays revealed that Aire KO mTECs caused modulation (up- or down- regulation) of more than 1,000 genes including those encoding FN1, EFNA1, TNC migration molecules and CCL2 chemokine that were differentially expressed between WT and Aire KO mTECs. These results contribute to a better understanding of the role of Aire in the control of thymocyte migration toward mTECs, which is an essential process for the negative selection and consequently the induction of immunological tolerance. (Funded by CNPQ, CAPES and FAPESP 2018/06460-7 and 17/10780-4. We thank Cesar A. Speck-Hernandez for his help and discussions).

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GENOME SEQUENCING AND *DE NOVO* ASSEMBLY OF THE FISH PACU (*PIARACTUS MESOPOTAMICUS*)

Milena Vieira de Freitas[1*]; Vito Antonio Mastrochirico-Filho[1]; Diogo Teruo Hashimoto[1]

[1]Centro de Aquicultura da UNESP – CAUNESP, Jaboticabal.

*milenavfreitas@gmail.com

Keywords: Neotropical fish; Genomic; Serrasalmidae

Pacu (*Piaractus mesopotamicus*) is a Neotropical fish widely used in the South America aquaculture. This fish corresponds to one of the most produced species in the Brazilian aquaculture, especially due to its easy acceptance in the national and international market. However, the limited genetic and genomic information of this species limits its production. Therefore, the main purpose of this study was to perform the genome sequencing and *de novo* assembly of this species, which is an important step for assist the genetic improvement of pacu. Sequencing of the pacu genome by NGS was performed from a male individual, maintained by the Laboratório de Genética em Aquicultura e Conservação (LaGeAC), Caunesp, Jaboticabal, SP. Illumina technology was used (Truseq 350 bp) to sequence 45 Gb, with approximate coverage of 30x, considering the estimated size of the pacu genome of 1.5 Gb. After the quality analysis, the sequences were trimmed to remove adaptors sequences, and correction of possible sequencing errors, using the program Trimommatic (leadin=28, trailing=28, sliding window 4:15, mielin=75). Further analysis of K-mers and identification of error levels and contamination were performed. The normalization and correction of the reads were performed by the BBnorm program, using a mean depth of 100x and a minimum coverage of 5x, reads with an apparent depth of less than 5x were considered errors and discarded. The *de novo* assembly for contigs formation was performed using the SPAdes program, and evaluation of the assembly observed by the QUAST and BUSCO tools using the database for Actinopterygii. After trimming, 115,603,419 paired-end reads were generated, with 16,785,826,680 bp. After normalization/correction, we obtained 103,893,497 paired-end reads, with 15,200,335.58 bp. The assembly generated a total 1.11 Gb in length, and 312,844 contigs. The largest contig was composed by 123,015 bp. In total, 226,267 contigs presented more than 1,000 bp. The N50 was estimated in 6,826 bp and GC percentage was 39.37%. All evaluated results followed a normal distribution. When comparing the results obtained with the Actinopterygii database (BUSCO), we observed a total of 2,401 complete genes (52.4%) (2,331 single-copy and 70 duplicated), 1,037 fragmented genes (22.6%), and 1,146 missing genes (25%). The data presented in this study correspond to the first assembly of the pacu genome, being this information of relevance for the development of new technologies for the genetic improvement of this species.

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MITOGENOME OF *Coprophanaeus ensifer* (COLEOPTERA) AND PHYLOGENETIC RELATIONSHIPS OF THE SCARABAEIDAE FAMILY

Giuliene Rocha de Medeiros[1]; Catarine Melo[1]; Igor Costa Amorim[1]; Gabriel da Luz Wallau[2]; Rita de Cássia de Moura[1]*

[1]Laboratório de Biodiversidade e Genética de Insetos - Universidade de Pernambuco, Recife, PE, Brasil. [2]Departamento de Entomologia, Instituto Aggeu Magalhães – FIOCRUZ, Recife, PE, Brasil;

*ritamoura.upe@gmail.com

Key-words: Mitochondrial DNA; Phanaeini tribe; Phylogeny.

The genus *Coprophanaeus* d'Olsoufieff, 1924 belongs to the Phanaeini tribe and has about 40 described species, which are grouped into three subgenera. Among its subgenera, *Megaphanaeus* has only four valid species and stands out for presenting evidence of taxonomic uncertainty. This uncertainty was proposed based on molecular analyzes (using the genes: COI, COII, 16S and 28S) that grouped the *C. lancifer* species within the *C. ensifer* clade, suggesting that these species are not monophyletic. This uncertainty can be solved by using more robust genetic markers, including sequences from the mitochondrial genome (mitogenome). Thus, the objective of this work was to characterize the mitogenome of *Coprophanaeus ensifer* aiming to establish its position in the Scarabaeidae family as well as to contribute in the future to the resolution of taxonomic uncertainties of the subgenus *Megaphanaeus*. The DNA sequencing of an individual from the Pernambuco population was performed on the Illumina NGS platform. The sequences obtained to the mitogenome was assembled and characterized in the Mitobim software and the MITOS WebServer, respectively. In the phylogenetic analysis, the protein sequences of the mitochondrial DNA of 36 species belonging to 6 tribes of Scarabaeidae were used, which are deposited at the National Center for Biotechnology Information (NCBI). The phylogenetic tree was reconstructed from the maximum likelihood method on the PhyML server using MtZoa as an evolutionary model. The results demonstrated that the mitochondrial DNA of *Coprophanaeus ensifer* has 18369 bp, and it is therefore considered a large molecule since the animals usually have mitogenomes ranging from 15 to 18 Kb. Also, this mitogenome has genes for two ribosomal RNA (rRNA), 13 protein-encoding genes, 22 transporter RNAs (tRNA) and A- T richness (87%). The numbers and organization of these genes are similar to most species of Scarabaeoidea, except for some species that differ in the order of the trnN and trnR tRNA genes. Furthermore, intergenic spacers and gene overlap were observed, and the largest of them were located between the nad3-trnA and trnL1-rrnl genes, respectively. This is a common feature among Scarabaeidae species. These results suggest that the mitogenome of *Coprophanaeus ensifer* is structurally conserved. In turn, phylogenetic reconstruction indicates monophyly of the tribes analyzed, except for Coprini and Onthophagini, which are paraphyletic. As per *C. ensifer*, this species grouped in the same clade as *Dichotomius schiffleri* (Coprini tribe). The results suggest the Coprini tribe is not monophyletic as suggested in the literature by morphological characters. In addition, the results obtained may contribute in future to resolution of taxonomic uncertainties of the subgenus *Megaphanaeus* and work on population genetics and conservation.

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TRANSCRIPTOME ANALYSIS OF A CLEPTOPARASITE BEE AND THEIR HOST

Paulo Cseri Ricardo[1*]; Natália de Souza Araujo[2]; Maria Cristina Arias[1]

[1]Departamento de Genética e Biologia Evolutiva, Universidade de São Paulo – Instituto de Biociências, São Paulo, São Paulo, Brazil. [2]Université de Liège - GIGA Institute, Liège, Belgium.

*cseri.bio@gmail.com

Keywords: cleptoparasitism; *Coelioxoides*; *Tetrapedia*

Cleptoparasitism is a strategy frequently observed among hymenopteran species. Despite its great ecological relevance in the dynamics of different species, little is known about the molecular bases involved in the evolution and maintenance of this behavior. In this context, this study aimed at the prospection of genes whose biological functions have a potential association with cleptoparasitism. Therefore, we compared transcriptome data from two neotropical solitary bees: *Coelioxoides waltheriae*, an obligate cleptoparasite, and their specific host *Tetrapedia diversipes*. For both species, samples of female founders were collected (*C. waltheriae*: 6 samples; *T. diversipes*: 9). The RNA was extracted from each sample using the RNeasy Mini Kit (Qiagen). The quantification and quality measurements were performed on an Agilent 2100 Bioanalyzer (Agilent Technologies). Samples were pooled to normalize individual differences, generating three replicates for each species (pools of two or three individuals, for *C. waltheriae* and *T. diversipes*, respectively). Paired end reads of 100 bp were sequenced using the Illumina® HiSeq 2000 platform at LaCTAD (Unicamp, Brazil) and Macrogen (South Korea) facilities. The sequencing quality was visualized in FastQC 0.11.7, and the removal of low-quality reads was performed using Trimmomatic 0.38. The software Trinity 2.6.6 was used for digital normalization and assembly. Transcriptome assembly quality was evaluated by Qualimap 2.2.1, QUAST 4.6.3 and BUSCO 3.0.0. Gene annotation was performed in Annocript 1.1.3 software. Transcripts annotated as potential contaminants (acari, archaea, bacteria, fungi, protists, algae, plants and viruses) were removed. Differential gene expression analyses between both species were conducted only for orthologous genes predicted by the OMA 2.3.0. These analyses were performed using the programs Bowtie2.3.4.1, RSEM 1.3.0 and the DESeq2 package through scripts included on Trinity 2.6.6. Gene Ontology (GO) term enrichment analysis wasperformed using the one-tailed Fisher's exact test carry out on Blast2GO 5.2.5. RNA-Seq resulted in 336,182,388 reads for *C. waltheriae* and 212,338,448 for *T. diversipes*. After cleaning and digital normalization steps, for each species the number of reads decreased to 11,618,498 and 10,992,792, respectively. The annotation process, after filtering the possible contaminants, identified 39,470 transcripts for Coelioxoides and 43,901 for *T. diversipes*. Orthology analysis identified only 1,544 orthologous, of which 136 were determined as differentially expressed transcripts by DESeq2. From these, 19 were upregulated and 23 downregulated in the cleptoparasitic species, and no enriched term was identified among them in GO term enrichment analysis. Possibly this is a consequence of the small number of orthologous identified by the approach used. Further analyzes using a more permissive strategy for orthologous identification will be carried out in order to increase the set of transcripts and enable a more comprehensive investigation.

Funding Agency: CAPES; FAPESP.

NEUROPROTECTIVE EFFECT OF ACETYLCHOLINESTERASE INHIBITORS IN RESPONSE TO OXIDATIVE STRESS IN NEURONAL SH-SY5Y CELL LINE

Flores TSC[1]; Moreira NCS[2]; Chierrito TPC[3]; Carvalho I[3]; Sakamoto-Hojo ET[1,2]

[1]Faculty of Philosophy, Sciences and Letters at Ribeirão Preto, University ofSão Paulo–USP; Ribeirão Preto, SP, Brazil. [2]Department of Genetics, Ribeirão Preto Medical School University of São Paulo–USP; Ribeirão Preto, SP, Brazil. [3]School of Pharmaceutical Sciences of Ribeirão Preto, University of São Paulo- USP; Ribeirão Preto, SP, Brazil.

thaflores11@usp.br

Keywords: Alzheimer's disease; neurodifferentiation; inhibitors of AChE

Alzheimer's Disease (AD) is the most common dementia in the world. The most common symptom of AD is episodic and progressive memory loss in individuals older than 65 years; the aggregation of β-amyloid peptides (Aβ) and hyperphosphorylation of tau proteinare characteristics of the disease.; the decrease in the concentration of acetylcholine in the brain of AD patients is considered the main cause of cognitive decline, and inhibitors of acetylcholinesterase enzyme (AChEI) have been used in AD therapy. However, these drugs present several side effects for the patients, reinfocing the need to develop new effective molecules for the treatment of AD. The objective of this project is to evaluate the neuroprotective capacity of new hybrid compounds synthetized from donepezil-tacrine; they are acetylcholinesterase inhibitors, called TA8Amino and TAHB3, and were tested in SH-SY5Y cells, which are able to differentiate in mature neurons; under oxidative stress condition induced by H2O2, cytotoxicity assays were performed at 24 and 120h time-points. Next, the differentiation and quantification of neuronal cells was performed. After confirming the neuronal differentiation, neuroprotection assays were performed. The compounds did not present cytotoxic effects and did not change cell viability at the concentrations tested, TA8Amino: 0.0035 to 0.112 μM; TA88B: 0.088 to 2.84 μM. Neuronal differentiation was analyzed by using retinoic acid,being confirmed by an increased expression of β-III-Tubulin (a marker of neuronal differentiation). Neuroprotection assay was performed in differentiated cells, and we observed that only TA8amino (0.014 μM) was able to protect neurons against damage induced by H2O2. Further assays will be performed, mainly for TA8amino, which demonstrates a potential for AD treatment.

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DISSEMINATION OF THE CONSERVATION CONCEPT OF THE ATLANTIC FOREST AND ITS NATIVE FAUNA THROUGHT GAME-BASED LEARNING

Pedro Paulo de Oliveira Nogueira[1]; **Lígia Souza Lima Silveira da Mota**[2]

[1]Bolsista CNPq/PIBIC. Universidade Estadual Paulista - Faculdade de Medicina Veterinária e Zootecnia, Botucatu, São Paulo. [2]Departamento de Genética, Universidade Estadual Paulista - Instituto de Biociências

*pdroliveira21@gmail.com

Key-word: conservation; learning; game.

The conservation, recovery and sustainable use of the Atlantic Forest depend on the integration of multiple efforts and a strategic vision that makes it possible to overcome the obstacles that have made it one of the most destroyed and still threatened forests on the planet. The importance of passing habits and attitudes of environmental conservation for the next generations becomes ever greater. From an early age, children should be aware of the need to balance human life with the environment, and for this to happen, we must be aware of new learning methods that can help us to spread and popularize this knowledge. Environmental education activities can be seen as an educational strategy focused on conservation of the species and its environment. Such activities have great potential to generate changes of habits, acting as instruments of awareness and social direction. In order to improve, bring better results and arouse greater student interest, game-based learning (GBL) brings games as a playful way of learning. This project aims to provide subsidies for a pedagogical strategy that will aid in the educational process of elementary school students regarding conservation biology, allowing discussions about social and environmental responsibilities and the development of citizenship actions. In your composition have a four-player board where, during your course of game, are found houses that take cards with different themes such as: animal tracks, your means of locomotion, feeding, scientific name, habitat, and other biomes. As results we hope to make knowledge more accessible and demonstrate the importance of forest conservation and how they are directly linked to our lives.

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POST TRANSCRIPTIONAL CONTROL OF THE AUTOIMMUNE REGULATOR (AIRE) GENE BY THE miR-155.

Pedro P. Tanaka[1], Ernna H. Oliveira[1], Max Jordan Duarte[1], Geraldo A. Passos[1,2]

[1]Group of molecular immunogenetics, Department of Genetics, FMRP, USP. 2 Department of Oral and Basic Biology, FORP, USP.

Pedro.tanaka@usp.br, passos@usp.br

Keywords: Aire; miR-155; mTEC

The thymus is a primary lymphoid organ where the establishment of central immune tolerance occurs through the elimination of self-reactive T lymphocyte clones. Precursors of T lymphocytes (termed thymocytes) enter the thymus, where they undergo two processes, positive selection (which occurs in the thymus cortex and is mediated by cortical thymic epithelial cells or cTECs) and negative selection (which occurs in the medulla and is mediated by medullary thymic epithelial cells or mTECs). During negative selection, the mTEC cells express a wide variety of tissue-restricted antigens (TRA) and present them to thymocytes. Those clones that recognize some of these autoantigens with high affinity are deleted by apoptosis and this ends up avoiding peripheral autoimmune aggressiveness. The property of mTECs to express a diverse range of autoantigens was termed promiscuous gene expression (PGE), which encompasses virtually all the body's tissue antigens. The PGE ensures the representation of the self- constituents in the thymus and consequently setting up of central immunological tolerance. The main PGE controller is the Autoimmune Regulator (Aire) gene, which acts as an unconventional transcription factor and promotes the expression of thousands of TRAS mRNAs and non-coding RNAs in mTECs. The Aire gene is a transcriptional and post-transcriptional controller in mTEC cells. However, little is known about how Aire is controlled. One of the current questions, which was explored in this work, is whether Aire could be controlled by microRNAs. To try to respond to this, we performed an *in silico* analysis of the microRNAs that predicted hybridization with the 3'UTR region of the mouse (*Mus musculus*) Aire mRNA. The miR-155 predicts thermodynamically stable hybridization between the nucleotides 185 and 202 of that region. Our work focused on the functional evaluation of the post-transcriptional control of miR-155 on Aire mRNA and its consequences on mTEC cells *in vitro*. Transfection of mTEC 3.10 cell line with miR-155 (mimic) resulted in reduction in Aire mRNA 24 hours after the transfection and a reduction up to 37 % in AIRE protein levels within the same period. We performed large-scale transcriptional expression using microarray hybridizations with total RNA of transfected cells, and observed the modulation of a set of mRNAs, including those encoding TRAs, mTEC-thymocyte adhesion proteins, apoptosis, and alternative splicing of mRNAs and cell migration cytokines. Molecular observations were validated experimentally when we demonstrated that miR-155 reduced about 34 % the chemotactic properties of mTEC cells on thymocytes.These results showed for the first time that miR-155 modulates Aire and a set of mRNAs in mTEC cells, affecting chemotaxis, one of its properties most important for maturation of T lymphocytes.

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POPULATION STRUCTURE OF THE ATLANTIC SPOTTED DOLPHIN (*Stenella frontalis*)

Luísa Schlude Marins[1,2]; Carolina Pereira Dias[2]; Eduardo Secchi[3]; Tatiana Lemos Bisi[2]; Alexandre de Freitas Azevedo[2]; José Lailson Brito Junior[2]; Haydée Andrade Cunha[2*]

[1]Instituto de Biologia, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil. [2]Laboratório de Mamíferos Aquáticos e Bioindicadores, Faculdade de Oceanografia, Universidade do Estado do Rio de Janeiro, Rio de Janeiro, Brazil.[3] Laboratório de Ecologia e Conservação da Megafauna Marinha (EcoMega), Instituto de Oceanografia, Fundação Universitária do Rio Grande, Rio Grande, Rio Grande do Sul, Brazil

*haydeecunha@yahoo.com.br

Key-Words: phylogeography; *Stenella frontalis.*

The Atlantic spotted dolphin (*Stenella frontalis*) is one of the most commonly sighted cetaceans off southeastern and southern Brazil. Along the coast of South America, the species shows a preference for continental shelf waters and a reported hiatus in its distribution (6º - 21ºS) could indicate the existence of at least two populations in Brazilian waters. The present study aims to widen our understanding of the genetic structure of the species as a whole by increasing sampling from a possibly geographically isolated population in southern Brazil. Therefore, skin samples from 76 Atlantic spotted dolphins from Brazil were obtained either from stranded animals or through remote biopsy. A fragment of the mitochondrial control region was amplified and sequenced. Sequences were aligned using MEGA v.10.0.5 and two datasets were built. The first dataset included 74 sequences of 540bp from five different localities in south and southeastern Brazil. The second dataset contained a total of 728 sequences of 347bp, adding nearly all publically available sequences from different areas of the species range (Western North Atlantic, Gulf of Mexico, Azores, Madeira and the Bahamas). In the first dataset, 17 different haplotypes were found using DNAsp v.6.12.03. Diversity indexes for this dataset were high (h=0,.851 and π=0.0081). Pairwise F_{ST} and Φ_{ST} values among localities were calculated using Arlequin v.3.5.2.2. There was no evidence of differentiation between localities in south and southeastern Brazil (p> 0.05), suggesting a single population. A total of 115 haplotypes were identified in the second dataset, and estimated h and π were 0.9436 and 0.01539, respectively. A Median Joining Network was built using Network v.5.0.1.1 in order to summarize the haplotypes frequencies, geographical distribution and relationship. The best AMOVA scenario (Φ_{CT}= 0,11276 and p= 0.0) indicated no differentiation between the oceanic individuals from Azores and Madeira, but significant levels of structuring were found between all coastal populations, except within the Gulf of Mexico. Pairwise F_{ST} and Φ_{ST} analysis revealed significant differentiation between all localities, except between Azores and Madeira. The south/south-eastern Brazilian population had the lowest fixation indices when compared to Azores and Madeira. These results support the hypothesis of recent gene flow between oceanic individuals and those inhabiting Brazilian continental shelf waters, and reinforce the notion that the species' biogeography is shaped by factors other than geographic distance alone. Oceanic individuals show greater mobility and may contribute to the genetic homogeneity of the species as a whole.

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ANCESTRY OF THE X CHROMOSOME IN ADMIXED POPULATIONS – AN EXAMPLE WITH QUILOMBOLAS POPULATIONS IN VALE DO RIBEIRA (SP)

Carlos H. Passos[1]; Kelly Nunes[1]; Regina Célia Mingroni Netto[1], Diogo Meyer[1*]

[1]Departamento de Genética e Biologia Evolutiva, Universidade de São Paulo - Instituto de Biociências, São Paulo, Brazil (IB/USP)

*diogo@ib.usp.br

Keywords: Ancestry; X Chromosome; Population Genetics

Since the 16th century, the immigration of Europeans, followed by the arrival of Africans for slave labor in America, had important implications to the genetic constitution of the Brazilian population. The meeting of Europeans, Africans and Native Americans resulted in an admixture profile that varies between the regions of Brazil and types of social organization. Quilombo communities were founded by small groups of escaped, abandoned or freed slaves which founded semi-isolated rural communities. In the state of São Paulo most Quilombos are in the Vale do Ribeira region. Despite predominantly African cultural ties, studies based on autosomal markers indicate a high admixture degree. The admixture dynamics can be investigated by several approaches. For example, we can infer the mating pattern between these people (was there assortative mating or random mating through these years?) or we can search for signals of natural selection (if some ancestry is overrepresented in a specific genomic region). The present work focuses on recovering this information, using the X chromosome. We analyzed 650 individuals from 12 Quilombo communities of Vale do Ribeira, genotyped with high density SNP array (the Affymetrix Axiom Human Origins array). We identified the global admixture pattern in autosomes and the X chromosome using ADMIXTURE and tested for differences in ancestry proportion between them using the Chromosomal Ancestry Differences (CAnD) routine. We used Hardy-Weinberg tests (Chi-squared and Exact tests) on the markers of the X chromosome, in order to detect if there is disequilibrium in some genomic region, which can be an indicative of breakdown of the assumptions for this model, such as assortative mating and natural selection. We found that the X chromosome has, on average, 52.01%, 27.21% and 20.78% of African, European and Native American ancestry respectively in contrast to 47.69%, 37.56% and 14,75% estimated for the autosomal ones. The CAnD test revealed significant differences between autosomal and X chromosome for the European (p-value 0.0172) and Native American (p-value 0.0046) components. We identified 74 markers with evidence of significant deviation from Hardy-Weinberg (p-value < 0.01). This study reveals a greater African and Native American contribution on the X chromosome than on autosomes, indicating a pattern of marriages mainly between men of European origin with African and Native women. We also identified some regions along the X chromosome that may be candidates for natural selection or assortative mating.

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ANALYSIS OF HEREDITARY CANCER-PREDISPOSING SYNDROME BY USING MULTIPLE-GENE SEQUENCING PANEL

Rafaella Sousa Ferraz[1], Amanda Ferreira Vidal[1], Antonette Souto El Husny[1,2,3] Tatiana Vinasco-Sandoval[1], Milene Raiol-Moraes[1], Paulo Pimentel Assumpção[2], William Barra[2], Leonardo Miranda de Brito[1], Ricardo Assunção Vialle[1], André M. Ribeiro dos Santos[1,2], Sidney Santos[1,2], Ândrea Ribeiro-dos-Santos[1,2]

[1]Human and Medical Genetics Laboratory, Federal University of Pará – Belém, Brazil. [2]Oncology Research Center, Federal University of Pará – Belém, Brazil. [3]Bettina Ferro de Souza University Hospital, Federal University of Pará – Belém, Brazil.

rafaellaferraz.16@hotmail.com

Key-words: Hereditary cancer; panel testing; pathogenic variant

Next generation sequencing (NGS) has been a very useful tool in clinical practice, mainly due to its efficiency and cost-effective approach. NGS has been widely used in genetic diagnosis of several inherited diseases and its use in clinical oncology may enhance the discovery of new susceptibility genes and enable individualized care of cancer patients. In order to investigate germline variants in Brazilian patients presenting clinical criteria for hereditary cancer syndromes or familial history, we built a NGS custom pan-cancer panel containing 16 high and moderate penetrance genes previously associated to hereditary cancer syndromes (*APC, BRCA1, BRCA2, CDH1, CDKN2A, CHEK2, MSH2, MSH6, MUTYH, PTEN, RB1, RET, TP53, VHL, XPA* and *XPC*). ClinVar database was used to determine the clinical significance of all reported variants. We analyzed blood samples of 71 patients, being 60 cancer patients - breast cancer (68,3%), gastric cancer (16,7%), and other types of cancer (15%) - and 11 unaffected individuals with family history of cancer, mainly familial adenomatous polyposis (81,8%). Thirteen pathogenic/likely pathogenic variants were identified in *APC, BRCA1, CDH1, MSH2, MSH6* and *MUTYH* in 21 individuals. The functional consequences of the pathogenic variants identified were primarily frameshift effects (47,6%), followed by missense (19%), stop gained (14,3%), splice region variant (14,3%) and splice donor variant (4,8%). The most prevalent pathogenic variant was c.1187G>A (p.Gly396Asp) in *MUTYH*, which was detected in 4 probands. This variant has been reported in *MUTYH*-associated polyposis, increasing colorectal cancer risk. A total of nine (9) variants of uncertain significance (VUS) were identified in eight (8) genes in 34 participants. According to the Mutation Taster, five (5) of these VUS have disease-causing potential. Overall, analysis of all these genes in NGS-panel allowed the identification not only of pathogenic variants related to hereditary cancer syndromes but also of some VUS that need further clinical and molecular investigations. The results obtained in this study had a great impact for the patients and their relatives since it allowed genetic counselling and personalized management decisions.

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DNA BARCODE IN THE IDENTIFICATION OF SPECIES OF RAYS COMMERCIALLY EXPLOITED IN SÃO PAULO STATE

Raul Barrera Camacho Oliveira[1]; Cahique Moraes Daneluz[1]; Fernanda Dotti do Prado[1]; Carlos Egberto Rodrigues Júnior[2]; Fábio Porto-Foresti[*]

[1]Universidade Estadual Paulista (Bauru), Departamento de Ciências Biológicas. [2]Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis (IBAMA).

raulbarrera_3@hotmail.com

Palavras-chave: Chondrichthyes; illegal trade; DNA barcoding

Stingrays are cartilaginous fish that present the body flattened dorso-ventrally, plus six or seven pairs of gill slits and developed pectoral fins fused to the head. In Brazil, 29 species of rays are in danger of extinction. Due to decharacterization and the grouping of all species of rays in a single NCM (Mercosur Common Nomenclature), the species of commercialized rays are often difficult to identify. The DNA Barcode is a species- specific molecular identification system based on the mitochondrial DNA sequence Cytochrome Oxidase Subunit I (COI) with about 650 base pairs. This system has been used successfully to identify samples that are marketed, such as sharks and other groups threatened with extinction. Thus, the present work aims to generate data about the species of streak marketed in the state of São Paulo and to investigate if there is an illegal commercialization of endangered species, besides validating the technique as a routine tool to control the trade in stingrays. For this, 39 specimens of individuals marketed in the cities of Bauru, São José do Rio Preto, Campinas and São Paulo were sampled and analyzed. Genomic DNA was extracted from the muscles of the individuals and preserved for further studies. Species level identification was performed with amplification of the COI genes by the Polymerase Chain Reaction (PCR). Subsequently, the PCR products were purified and sequenced, where the results were aligned in the Geneious 4.8.5 program, resulting in consensus sequences. The sequences were submitted to the database of the BOLD platform, where they were compared to the 100 most similar sequences, obtaining identification at the species level. After analysis, 6 species were identified, of which 13 individuals are of the species *Paratrygon ajereba*, 10 *Potamotrygon motoro* and 10 *Hypanus dipterurus*, all classified as insufficient data (DD) according to the IUCN Red List of Threatened species, in addition to 3 *Gymnura altavela* and 2 *Atlantoraja platana*, classified as vulnerable (VU) and 1 *Atlantoraja castelnaui*, categorized as endangered. In this way, we can see that there is at least three species at risk of extinction being marketed in the state, and three other species are not officially in a threat status, since animals that lack information are classified in category DD about conservation status based on population status and distribution.

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HJURP OVEREXPRESSION PROMOTES OPENING OF CHROMATIN STRUCTURE AND INCREASES THE EXPRESSION OF DNA REPAIR GENES

Rodrigo de Almeida[1]; Rodolfo Bortolozo Serafim[2]; Geovana Navegante[1]; Valeria Valente[1*]

[1]Department of Clinical Analysis, School of Pharmaceutical Sciences of São Paulo State University (UNESP). [2] Department of Genetics, Ribeirão Preto Medical School, University of São Paulo (USP).

*valenteval@gmail.com

Key-words: DNA repair; glioblastoma; HJURP.

The *Holliday Junction Recognizing Protein* (HJURP), a novel protein involved in DNA repair, is highly overexpressed in glioblastoma (GBM) and other cancers, and the elevated levels are usually correlated with poor prognoses. We have previously demonstrated that the U87MG cell line overexpressing HJURP shows increased proliferation and reduces Heterochromatin Protein 1 (HP1) foci, suggesting an overall opening of the chromatin structure that possibly allows the expression of repressed genes. The aim of this work was to analyze whether the overexpression of HJURP promotes increased expression of other genes. Using online available tools (cBioPortal), we searched for genes co-regulated with HJURP in GBM samples of the TCGA (The Cancer Genome Atlas) database, and generated a list of 100 genes showing Spearman's correlation with HJURP levels of at least 0.8. Using the KEEG tool we defined as the most enriched pathways: cell cycle control, progesterone- mediated oocyte maturation, oocyte meiosis, p53 signaling pathway, DNA replication, homologous recombination, cellular senescence, FOXO signaling pathway and pyrimidine metabolism. The expression of six DNA repair genes strongly correlated with HJURP, namely EXO1, NEIL3, RAD54L, BRCA2, XRCC2 and PIMREG, was analyzed by quantitative PCR in different astrocytoma cell lines (U186, UW467, U87MG and U138MG) genetically modified for HJURP overexpression. As controls we used cell lines transformed with the empty vector. We found increased levels of all analyzed genes in cells overexpressing HJURP, which corroborates the hypothesis that when HJURP is highly expressed, the chromatin is more relaxed and permissive to the expression of selected genes. New experiments are being carried out to better understand this unknow activity of HJURP.

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A novel plant DEAD-box RNA helicase associated with transcription factors

Aziani, R[1]; Pinoti, VF[1,2]; Strini, EJ[1,2]; Ferreira, PB[1,2]; Lubini, G[1,2]; Thomé, V[1,2]; Cruz, JO[1,2]; Quiapim, AC[1]; Goldman, GH[3]; DePaoli, HC[4]; Goldman, MHS[1]

[1]FFCLRP/University of São Paulo (USP), Ribeirão Preto, SP– Brazil; [2]PPG – Genética, FMRP/University of São Paulo (USP), Ribeirão Preto, SP– Brazil; [3]FCFRP/University of São Paulo (USP), Ribeirão Preto, SP– Brazil; [4]Biological Systems & Engineering Division, Lawrence Berkeley National Laboratory, Berkeley, CA - United States

rd.aziani@gmail.com

Key words: protein interactions, NtDDX41, Y2H screening

Previously, we characterized the *SCI1 (Stigma/style Cell-cycle Inhibitor 1)* gene, which controls cell proliferation in the pistil of *Nicotiana tabacum* and *Arabidopsis thaliana*. Recent experiments of *in situ* hybridization and analyses of transgenic plants demonstrated that *SCI1* is expressed not only at the pistil, but at the whole floral meristem, since its specification. As the molecular mechanism(s) through which SCI1 controls cell proliferation is still unknown, we performed the screening of a yeast two-hybrid (Y2H) *N. tabacum* stigma/style cDNA library, using SCI1 as bait. Among the interactions partners identified, we revealed the homolog of human DDX41, a DEAD-box RNA helicase that acts as tumor suppressor. This plant protein has not been studied yet and, for this reason, we conducted a new Y2H screening using NtDDX41 as bait. In the screening, the homolog of the plant epigenetic regulator HISTONE MONOUBIQUITINATION1 (HUB1) was found. Further, the homologs of the plant transcription factors (TFs) ASIL2, TCP7 and TCP15, FAR1-RELATED SEQUENCE 5 (FRS5) and ZINC-FINGER HOMEODOMAIN 22 (ZFHD22) were also uncovered in the screening. The interaction between NtDDX41 and NtZFH22 was further investigated and it was confirmed by Bimolecular Fluorescence Complementation (BiFC) experiments. Y2H experiments have demonstrated that NtZFHD22 also interacts with SCI1. Additionally, BiFC experiments confirmed the interaction between SCI1 and NtZFHD22, which occurs at the nucleus. Taken together, our results establish the association of NtDDX41 with transcriptional regulators, point toward an overlap between NtDDX41 and SCI1 interactomes and indicate that SCI1 and NtDDX41 may function in the transcription process.

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IN SILICO ANALYSIS OF THE TRANSCRIPTION FACTORS XYR1 AND CRE1 IN *TRICHODERMA* SPP.

Rafaela R. Rosolen[1*]; Jaire A. Ferreira Filho[1]; Déborah A. Almeida[1]; Anete P. Souza[1,2].

[1]Center for Molecular Biology and Genetic Engineering (CBMEG), University of Campinas (UNICAMP), Campinas, SP, Brazil. [2]Departament of Plant Biology, Biology Institute. University of Campinas (UNICAMP), Campinas, SP, Brazil.

rafaelarosolen@gmail.com

Keywords: *Trichoderma* spp.; biomass degradation; gene regulation.

Trichoderma reesei is one of the most efficient producers of cellulolytic enzymes involved in biomass deconstruction being widely used in the biotechnology industry, mainly in the production of biofuels. However, *Trichoderma harzianum* has also demonstrated great potential as a producer of enzymes that act in the conversion of lignocellulosic substrates to soluble sugars. One of the key topics in this process is the regulation of the expression of genes related to the production of these enzymes, which has as main positive regulator the transcription factor XYR1, while the repression is mediated by CRE1. Here we used four approaches to a better understanding of the role and the differences of these regulators in *T. harzianum* (IOC-3844 and CBMAI-0179), *T. reesei* CBMAI-0711 and *T. atroviride* CBMAI-0020, which include: phylogenetic analysis, genomic comparison, co-regulation networks analysis and identification of specific binding sites for XYR1 and CRE1 in target genes. For the phylogenetic analysis, the trees were constructed from the alignment of the Ascomycetes protein sequences by the Maximum Likelihood statistical method. SimpleSynteny software was used for comparison of genes next to *cre1* and *xyr1* among different species of *Trichoderma* spp. For the search of co- regulation clusters, genes of interest were filtered in networks assembled from co- regulated genes under cellulose and glucose growth conditions for the strains of study. For the *in silico* identification of the regulatory elements, 1.5 kb upstream for each gene selected were extracted from *T. reesei* RUTC-30, *T. harzianum* T6776 and *T. atroviride* IMI206040 scaffolds using the software Bedttols. With the *in silico* analyzes, it was possible to notice that these regulators are strongly present in the filamentous Ascomycetes fungi, although, as reported by the literature, XYR1 can present different functions according to the life style of the species analyzed while CRE1 is the only conserved throughout the fungal kingdom, suggesting a preserved mechanism for the catabolic repression of fungi. It has also been demonstrated that regions of the genome around *xyr1* and *cre1* are remarkably conserved in the species considered, however, *T. reesei* presents a greater difference in relation to them, which can be explained by the loss of genes and genomic modifications carried out in lineages of this fungus to increase its performance during enzymatic saccharification. Also been demonstrated that *xyr1* of *T. harzianum* IOC-3844 is co-regulated with genes encoding the MFS transporters, which can carry different small molecule inducers from the extracellular environment into the fungus influencing the expression of CAZyme-encoding genes. We also showed that the motifs are diffused in the 5'-upstream region of genes regulated by XYR1 and CRE1. The data generated in this work can be used as basis for further studies of the regulation involved in the degradation of biomass in *T. harzianum*.

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Validation of the amh-y gene for molecular sex identification in Nile Tilapia breeders from CAUNESP

Rubens Ricardo de Oliveira Neto; John F. Gomez[1]; Raquel B. Ariede[1]; Carolina Heloisa de Souza Borges[1]; Diogo Teruo Hashimoto[1]

[1]Centro de Aquicultura, Universidade Estadual Paulista, Jaboticabal, Brazil.

rubensricardo.mv@gmail.com

Keywords: molecular marker, aquaculture, sex control, *Oreochromis niloticus*

The TGF-β signaling pathway has already shown an important mechanism for determining gonadal sex in Nile Tilapia (*Oreochromis niloticus*). Recent studies have suggested the hypothesis of a duplicated copy of the amh gene on the Y chromosome of males in Nile Tilapia. Therefore, this gene be considered a strong candidate as master gene in the sex determination of this species. Nowadays, all the production of Nile Tilapia is based on sex-reverted male individuals treated with 17α-methyltestosterone, because males grows faster than females. However, the treatment with this hormone is not 100% effective, and it can result in residues to the effluents. Therefore, males with the genotype YY are desirable to mating with normal XX females, which will result in 100% of the progeny XY. However, to obtain YY males is needed initially the mating between XY male and XY female, which can be identified by using a molecular marker, since Nile Tilapia does not present sex chromosomes with apparent dimorphism. The present study aimed to validate *amh-y* in tilapia breeders. Blood samples were collected from seven males and eleven females from the breeding facility of the Aquaculture Center of UNESP (CAUNESP, Jaboticabal, Brazil). DNA was extracted and PCR was performed with previously designed primers (Amh-y-F2 and Amh-Y-R2). Individuals genetic sex was observed through agarose gel 1%. Results showed amplification of the duplicated copy of the amh gene on the Y chromosome only in six males (fragment of approximately 600 bp). One male was considered with the genotype XX, since we did not detect the diagnostic band. This atypical male can be result of a sex reverted individual. All the females were identified with the genotype of XX. The results of the present study show that the gene *amh-y* can be used as molecular marker for sex identification and chromosome manipulation to obtain YY males in Nile Tilapia breeders from CAUNESP.

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EVOLUTION AND SELECTION OF TOXINS IN THE PHYLUM NEMERTEA

Gabriel Gonzalez Sonoda[1]*, Sónia Cristina da Silva Andrade[1]

[1]Laboratório de Diversidade Genômica, Departamento de Genética e Biologia Evolutiva do Instituto de Biociências da Universidade de São Paulo

*gabriel.sonoda@usp.br

Keywords: Toxinas; Molecular evolution; Nemertea

Nemertea is a phylum composed of vermiform animals, mostly marine and bentonic. Animals from this phylum secrete toxins, which they use for both defense and predation. Until now, little is known about the diversity, action and evolution of these toxins, but bioinformatic studies revealed many transcripts of putative toxins in transcriptomes from different Nemertea species. The objective of the present study is to identify and describe the evolution of toxin genes of Brazilian nemertea by using bioinformatic and biomolecular tools. Using BLAST, several putative toxin sequences were found in transcriptome of different species of nemertea. Of these sequences, the cytotoxin A-III and the plancitoxin-1 sequences were chosen for further analysis. Four species have been chosen for this study: *Lineus bonaerensis, Nemertopsis bivittata, Ototyphlonemertes erneba* and *O. lactea*. Specimens were collected at the North littoral of the state of São Paulo and South littoral of the State of Rio de Janeiro. The available reads of transcriptomes from *Lineus sanguineus,* closely related to *L. bonaerensis*, were used to assemble new transcriptomes from specimens collected in different localities. These new transcriptomes were not only used for designing primers which would anneal to conserved regions of these transcripts, but were also used to make selection tests in these transcripts. The plancitoxin-1 transcripts were found to be under positive selection (average omega for models M1 and M2 are, respectively, 0.516 and 1.203, $p < 0.0001$) matching the pattern of other toxins used for predation. We found no significant evidence of selection acting on cytotoxin A-III (average omega for models M1 and M2 are, respectively, 0.396 and 0.688, $p=0.302$). As for the *Ototyphlonemertes* and the *Nemertopsis* species, the lack of transcriptome from closely related species asks for the assembly of *de novo* transcriptomes from total RNA extraction. Toxin transcripts found in these transcriptomes will be used for designing primers to amplify these regions through PCR, which will be assayed for selection analyses.

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MOLECULAR ANALYSIS AND MICROSATELLITE DEVELOPMENT FOR THE STUDY OF NATIVE OYSTERS IN BRAZIL

Jakeline Rangel Monteiro[1]; Alexandre Wagner Silva Hilsdorf[1]; Márcia Santos Nunes Galvão[2]

[1]Laboratório de Genética de Organismos Aquáticos e Aquicultura, Núcleo Integrado de Biotecnologia, Universidade de Mogi das Cruzes, Mogi das Cruzes, São Paulo, Brazil [2]Unidade de Pesquisa e Desenvolvimento de Pirassununga, Centro de Pesquisa de Aquicultura, Instituto de Pesca, Pirassununga, São Paulo, Brazil

fef_jake@hotmail.com

Key-words: oyster; molecular analysis; microsatellite

Oysters represent an important global economic resource. *Crassostrea brasiliana* (= *C. gasar*) and *C rhyzophorae* are native species of the Brazilian coast, representing economic fishing resources. Molecular markers are need for the differentiation of these species due that they show high phenotypic plasticity. The present work aimed of identifying native and exotic species via molecular markers. In addition, to develop microsatellite markers by next generation sequencing for genetic population assessment of native oysters. Oysters were collected from mangrove roots and rocks in three estuaries in the southeast of Brazil (Paraty, Bertioga and Cananeia). Sequences of 16S ribosomal gene and PCR-RFLP techniques were used to identify the species. *C. brasiliana* DNA was used to develop microsatellite markers by next generation sequencing (NGS). From 249 samples collected in the three estuaries, 70 were *C. brasiliana*, 55 were *C. rhyzophorae* and 124 were an exotic species *Saccostrea* sp. The three species were present in the sampling sites except for *C. brasiliana* that was not found in Paraty. In Bertioga, this species was found only on the river bottom. PCR-RFLP using AluI enzyme was effective to discriminate *C. brasiliana* from *C. rhyzophorae*, whereas *Saccostrea* sp. was easily identified by shell morphological characteristics. Microsatellite markers were isolated and selected from a genomic library obtained by NGS. A set of 30 primer pairs was selected and the conditions for PCR were established for each of them. The results of the present study show a decrease of the native species *C. brasiliana* in places where it was abundant in the past. On the other hand, there were a considerable number of *Saccostrea* sp. specimens in all collection sites. Considering that the first report of *Saccostrea* sp. in Bertioga occurred in 2014 and in Cananeia in 2017, we realize a substantial expansion of this species along the coast that can seriously compromise the natural stocks of native oysters. The development of microsatellite markers for genetic population assessment of native oyster is of paramount importance to reveal the status of genetic diversity of this face the hazard of exotic oyster invasion we are currently witnessing.

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DUSP6 IMPACTS EXPRESSION PATTERN OF GLUCOSE METABOLISM-RELATED GENES IN PANCREATIC CANCER CELLS

Mariana Tannús Ruckert[1], Carlos Alberto Oliveira de Biagi Júnior[1], Vanessa da Silva Silveira[1]*

[1]Department of Genetics, Ribeirão Preto Medical School, University of São Paulo, Ribeirão Preto, São Paulo, Brazil

*vsilveira@fmrp.usp.br

Keywords: pancreatic cancer; dual-specificity phosphatases; glucose metabolism.

Pancreatic ductal adenocarcinoma (PDAC) is one of the most lethal tumors with an overall 5-year survival rate of only 7%, being the 7th cause of cancer-related deaths worldwide. Among the mutations associated with PDAC, 95% of the cases are driven by mutations in *KRAS* proto-oncogene, which cannot be directly targeted. KRAS is responsible for activation of several signaling pathways, such as MAPK/ERK, which has been reported as the main responsible for glycolysis activation on PDAC cells. Constitutive activation of MAPK/ERK leads to several downstream effects resulting in uncontrolled proliferation, which requires the cell to reprogram its metabolism to sustain the anabolic demand. Regulation of MAPK pathways is orchestrated by a negative feedback network mediated by the balance between protein tyrosine kinases (PTKs) and protein tyrosine phosphatases (PTPs). Among numerous phosphatases, dual-specificity phosphatases (DUSPs) constitute one of the major classes responsible for downregulation of MAPKs. DUSP6 has been described for controlling ERK1/2 activity, and its expression is highly regulated in PDAC. As previous studies have demonstrated that metabolic reprogramming in PDAC might be mediated by ERK1/2, this study aimed to elucidate the complex mechanisms behind it, to help us identify new therapeutic targets for this highly lethal tumor. For this matter, we induced constitutive overexpression of DUSP6 in MIA PaCa-2 using lentiviral transduction. Then we assessed ERK1/2 phosphorylation levels using Western blotting and observed a 63% reduction in the context of DUSP6 overexpression. After validating the model, we performed a PCR array analysis with a set of 92 glucose metabolism-related genes. We used HTqPCR package in R to analyze data. Statistical analysis was performed using t- test and results were considered significant when $P<0.05$. Interestingly, we identified eight differentially expressed genes from which five are directly involved in the TCA cycle. Among them there is *PDK4*, which showed a negative correlation with *DUSP6* in PDAC patient samples in *in silico* analysis. We also observed that the lower expression of *PDK4* is related to a poorer prognosis in these cases. In a clonogenic assay, we observed that DUSP6 overexpression decreases survival of MIA PaCa-2 cells. Other recent studies have provided concrete evidence that breaking down metabolicaddiction in PDAC is an innovative and promising field to improve therapeutic approaches in this type of tumor. Therefore, the results we obtained so far bring new insights into metabolic reprogramming dependence, and it might reveal new target molecules that could ease the challenge of treating PDAC.

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AN OVERVIEW OF THE *HLA-B* CODING AND PROTEIN VARIABILITY IN A ADMIXED SAMPLE FROM BRAZIL

Nayane dos Santos Brito Silva[1*], Andreia da Silva Souza[2], Thálitta H. A. Lima[2], Heloisa A. Andrade[2], Marília A Passos[1], Camila Ferreira Bannwart Castro[3]; Jaqueline Wang[4], Marília O. Scliar[4], Guilherme L. Yamamoto[4], Yeda A. O. Duarte[5], Mayana Zatz[4], Michel S. Naslavsky[4], Maria Rita Passos-Bueno[4], Diogo Meyer[5], Celso T. Mendes-Junior[6], Erick C. Castelli[1]

[1]Universidade Estadual Paulista (UNESP), Faculdade de Medicina de Botucatu, Brasil. [2]Universidade Estadual Paulista (UNESP), Instituto de Biociências de Botucatu, Brasil. [3]Centro Universitário Sudoeste Paulista – UniFSP [4]Centro de Pesquisas sobre o Genoma Humano e Células Tronco, Instituto de Biociências, Universidade de São Paulo (USP), Brasil. [5]Faculdade de Saúde Pública, Escola de Enfermagem, Universidade de São Paulo (USP), Brasil. [6]Departamento de Química, Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto, Universidade de São Paulo, Ribeirão Preto, SP, Brasil.

*nayanebritto@yahoo.com.

Keywords: HLA-B, NGS, polymorphisms

The Human Leukocyte Antigen B (HLA-B) gene is possibly the most variable gene in the human genome, with more than 6 thousand different sequences reported in the Immuno Polymorphism Database (IMGT/HLA). This gene encodes a key molecule for antigen presentation to T CD8+ lymphocytes and NK cell modulation. Because of sequence similarity with other HLA genes and its polymorphic nature, to evaluate HLA- B variability using second-generation sequencing (NGS) is quite challenging. Moreover, most of the known HLA-B sequences are characterized for only exons 2 and 3, thus genetic variability in other segments might be underestimated. Here we present a bioinformatics pipeline to characterize the complete HLA-B exon variability using NGS. We evaluate HLA-B genetic variability in 920 individuals surveyed in the State of São Paulo, Brazil, 492 using whole-genome sequencing (WGS) and 428 using targeted sequencing. We identified 113 variable sites in exons, 99% of them with a minor allele frequency (MAF) higher than 1%. As expected, exons 2 and 3 were the most variable ones, but the segment encoding the leader peptide presented 11 variable sites (63.7% non-synonymous). Controversely, other HLA-B segments (alpha-3, transmembrane, and cytoplasmic domains) presented few non-synonymous variants. The alpha-3 domain interacts with CD8 in the T lymphocyte, and possibly with some NK receptors. The variable sites configure 117 coding haplotypes encoding 111 different HLA-B proteins. The most frequent ones were B*35 (10%), B*44 (9%) and B*51 (8%), and 99.7% of HLA-B coding sequences were already reported (at least for some exons) in the aforementioned database. However, here we describe the complete exon sequences of each different HLA-B coding allele detected in our sample. Frequencies for HLA-B supertypes and proteins are compatible with the ones reported for other samples from the São Paulo State. In conclusion, here we present a bioinformatics pipeline suitable to evaluate the complete HLA-B exon variability from targeted sequencing and WGS data. Although highly variable considering the leader peptide and the alpha-1 and alpha-2 domains, other HLA-B segments are conserved. These segments might be under strong selective pressure against variation.

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CYTOGENETIC STUDIES IN BRAZILIZAN NATIVE HERB *Hyptis radicans* (Laminaceae)

Maria Clara Stocco[1*], **Ricardo Augusto Lombello**[2]

[1]Bacharelado em Ciência e Tecnologia, Universidade Federal do ABC. [2]Universidade Federal do ABC – São Bernardo do Campo – SP.

*maria.stocco@aluno.ufabc.edu.br

Palavras-chave: cytogenetics; CMA/DAPI; pollen viability

The widespread tropical genus *Hyptis* Jaqc. (Lamiaceae) presents more than 350 species, some of them with verified medicinal properties. Despite the pharmacological importance of the genus there are few cytogenetic studies published for *Hyptis* species. The literature presents chromosome number reports for only 10 species of *Hyptis* and presents the basic number x = 7 or 8 for the genus. In the present study meiotic analysis, pollen viability, classical cytological assays and CMA/DAPI fluorochrome banding were carried out in *Hyptis radicans* (Pohl) Harley & J.F.B.Pastore, a Brazilian native species collected at Paranapiacaba Biological Reserve, Santo André, São Paulo, with anti-HIV activity for its extracts. The meiotic process was regular, with 88.1% of meiotic index, percentage of regular tetrads observed, although some abnormalities were registered, such as triads and polyads. The viable pollen index observed was 87.3% indicating high potential male fertility for the species. The chromosome numbers were n = 16 and 2n = 32. Both counts are unpublished. The chromosomes observed were small and similarly sized, with lengths ranging from 1.77µm to 3.04µm, and the karyotype was symmetric. No DAPI positive bands were observed. Four CMA positive bands, two distal and two in intercalary positions, were registered for *H. radicans* chromosomes. The haploid and diploid numbers observed agree and reinforce the basic chromosome number x = 8 earlier proposed for the genus. The chromosome counts presented here and registered in the literature allow us to infer the important role of polyploidy for the karyotype evolution in *Hyptis*. The wide distribution of *Hyptis* species is probably related to this important derivation process due to the adaptive potential conferred by multiple genomes.

GENETICS APPLIED TO THE STUDY OF REPRODUCTIVE BIOLOGY ASTYANAX ALTIPARANAE

Yasmin Garcia[1]; Caio F. da Silva[1]; George Shigueki Yasui[2]; Fabio Porto-Foresti[1]

[1]Universidade Estadual Paulista (Bauru). [2]Centro Nacional de pesquisa e conservação da biodiversidade aquática continental- CEPTA.

*yasmingarcia_yg@hotmail.com

Palavras-chave: paternity; markers microssallite; aquaculture

Brazil is considered the country with the most diverse ichthyofauna in the world. In the Neotropical region there is a great richness of species, in prominence of the orders Characiformes and Siluriformes. Astyanax altiparanae, popularly known as yellowtail lambari, is one of the Characiformes species widely produced in Brazil, due to its easy handling, fast reproduction and ecological importance. It is fundamental to develop studies about the reproduction of this species, in order to study the amount of individuals that contribute genetically at each reproduction, avoiding the incorrect management of lambaris. Microsatellite molecular markers are frequently applied tools for this kind of studies, resulting in efficient studys, such as paternity and kinship. The objective of the study was to understand the reproductive biology of the species, performing two breedings in different sex ratios by applying the markers that were previously described for the genus, and analyzing how many individuals generated offspring. By the end of the study it will be possible to make future predictions regarding the reproduction of the species, obtaining greater efficiency. For the development of the study, were analyzed thirty larvae of the first reproduction in the proportion of five males to one female (C1) and thirty embryos of the second reproduction, in the proportion of four males to two females (C2) , both performed by the seminatural method at the National Research Center and Conservation of Continental Aquatic Biodiversity (CEPTA), ICMBio, Pirassununga, SP. After that the offspring and the parentals were taken to the Fish Genetics Laboratory, at UNESP of Bauru, to extract the genomic DNA. The integrity and amount of extracted DNA were observed by 1% agarose gel electrophoresis. Seven microsatellite loci were amplified through the Polymerase Chain Reaction (PCR). Therefore, the loci were arranged together for sequencing and the results of the amplifications were genotyped. The paternity test was done through the COLONY 2.0.6.2® program. The paternity estimation of the offspring analyzed in the present study allowed to conclude that only three of the five males of the C1 contributed genetically to the reproduction, while in C2 two of the four males and one of the two females generated offspring. Although only a few males and one female reproduced, the justification for the lack of participation regarding the other individuals in generating gametes is little known, but sperm motility or concentration may have caused some influence in the results. However, studies on the reproductive biology of this fish should be more applied and can lead to an improvement in reproductive efficiency in fish farming by enabling a correct number of individuals to contribute during reproduction, since Astyanax is an extremely diverse genus and used in aquaculture.

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INTEGRATIVE CONJUGATIVE ELEMENTS OF SXT/R391 FAMILY IN *PROTEUS MIRABILIS* AND ITS CORRELATION WITH RESISTANCE, MUTAGENESIS AND CONJUGATION

Sato, J.L.[1], Fonseca, M.R.B.[1] and Galhardo, R.S.[1]

[1]Department of Microbiology, Institute of Biomedical Science - University of São Paulo, São Paulo, SP, Brazil

juliana.sato@usp.br

Keywords: *Proteus mirabilis*; SOS response; Mutagenesis; Resistance; Conjugative transposon

Proteus mirabilis is a bacterium that causes catheter-associated urinary tract infections (CAUTIs) that has its clinical importance associated with prolonged catheterizations. Due to its beta-lactam-sensitive nature, the emergence of resistance to this class of antibiotics led to the identification of the integrative conjugative elements (ICEs) of the SXT / R391 family, which carried such resistance genes. ICEs have genes that regulate the transfer mechanism itself, as well as several other conserved genes of unknown or apparently non- essential functions for the transfer process. Among these conserved genes is the *rumAB* operon, a homolog of E. coli *umuDC* that encodes a DNA polymerase from the Y family, characterized by the high error rate. The regulation of *rumAB* expression is under control of the SOS response, the same regulation controlling the conjugative functions of ICE. This response is induced when DNA damage is caused, for example, by exposure to antibiotics. By inducing the transionic polymerases, the SOS response leads to the transient increase in the mutation rate. Mutations in the chromosome, in turn, may lead to the emergence of resistance to certain antimicrobials such as fosfomycin and ciprofloxacin. In the genome of P. mirabilis, there are no homologues of these translesion polymerases, except in those lines harboring the ICE SXT / R391, which has *rumAB* with it. Therefore, in this project, we identified bla_{CMY-2} in one of our isolates, and the sequencing data shows that the structure of this ICE may be identical to the ICE*Pmi*Jpn1, described in Japan. We will perform some functional analysis of *rumAB* translesion polymerase, analyzing their role in mutagenesis processes and in the maintenance and conjugation of ICEs.

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THE XIA-GIBBS SYNDROME CAUSED BY A NOVEL VARIANT IN THE AHDC1 GENE

Carvalho LML[1*]; Costa SS[1], Goloni-Bertollo EM[2]; Galbiatti-Dias ALS[2], Pavarino EC[2]; Krepischi A[1], Koiffmann CP[1]; Rosenberg C[1]

[1]Instituto de Biociências da Universidade de São Paulo. [2]Faculdade de Medicina de São José do Rio Preto.

*lauralara@usp.br

Keywords: Xia-Gibbs syndrome; *AHDC1* gene; Whole Exome Sequencing

INTRODUCTION: The proband of this study, a 3-year-old male, presents hyperphagia, early obesity, developmental delay, intellectual disability, speech delay, macrocephaly, low nasal bridge and agenesis of the corpus callosum. His parents (non- consanguineous) and sister are healthy, with no other similar cases in the family. **OBJECTIVE:** To identify genetic causes underlying the clinical manifestations of the proband. **METHODS**: Previously, G-banding karyotype, Prader Willi region methylation test and MLPA (P036, P070, P245 and GOD) were performed, all of them with negative results. We then performed a whole exome sequencing (WES) on DNA from the parents-proband trio (HiSeq Platform - Read length: 2 x 150 Paired End; Library type: SureSelect V6). Exome data were analyzed using the VarSeq software (Golden Helix), with the reference genome GRCh37. The following annotation and filtering strategies were used: read depths ≥ 10, genotype qualities ≥ 17, population frequency ≤ 0.01 (1KG Phase3, gnomAD, NHLBI, ExAc and ABraOM); missense and loss-of- function variants were selected, and functional interpretation was performed based on ClinVar 2018, Ensembl, and dbNSFP 3.0. Additionally, we also used the ACMG (American College of Medical Genetics) classification of variants. Sanger sequencing was used to confirm the existence of candidate variants identified by exoma analysis. **RESULTS**: We identified by WES a heterozygous novel nonsense variant in the *AHDC1* gene (1:27877633 G>A), which was confirmed by Sanger sequencing. According to the ACMG classification, the variant was classified as pathogenic. **CONCLUSION**: More than 25 nonsense variants in *AHDC1* gene have been described as causing the Xia-Gibbs syndrome, which has clinical features compatible with those presented by our proband (intellectual disability, speech delay, dysmorphic facial features and hypoplasia of the corpus callosum). As we did not find in the literature references to hyperphagia and obesity in this syndrome, we contacted a patient parents' organization (Xia-Gibbs Society) and Dr. Richard Gibbs (Baylor College of Medicine), both confirming that some patients have obsession with food and satiety dysfunctions. Therefore, we report a novel *AHDC1* gene variant that cause Xia- Gibbs syndrome. Xia-Gibbs diagnosis should be considered amongst patients with syndromic obesity.

Compliance with ethical standards: This study was approved by the ethics commitee (Comitê de Ética em Pesquisa – Seres Humanos) of IB-USP (CAAE80921117500005464).

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MISMATCH URACIL DNA GLYCOSYLASE FROM *CORYNEBACTERIUM PSEUDOTUBERCULOSIS* RECOGNIZES AND REMOVES URACIL FROM DNA *IN VITRO* AND IS PRESERVED IN PATHOGENIC SPECIES OF CORYNEBACTERIUM GENUS

[1]Cássio Siqueira Souza Cassiano; [1]Thalia Queiroz Ladeira; [2]Bruno Carvalho Resende; [2]Diego Lisboa Rios; [2]Carlos Renato Machado; [2]Vasco Ariston de Carvalho Azevedo; [1]Luciana Lara dos Santos; [3]Carlos Valenzuela; [3]Gonzalo German Cabrera Vallejos; [1]Debora de Oliveira Lopes.

[1]Universidade Federal de São João del-Rei - *Campus* Centro Oeste Dona Lindu. [2]Universidade Federal de Minas Gerais. [3]Facultad de Medicina, Universidad de Chile.

cassiossc@gmail.com

Key-words: DNA Repair; *Corynebacterium pseudotuberculosis*; Mismatch uracil DNA glycosylase.

Corynebacterium pseudotuberculosis is the etiological agent of caseous lymphadenitis, a chronic infection disease affecting different species of mammals, causing significant economic loss all over the world. To improve the knowledge about this bacteria it becomes necessary to access its genetic composition, and verify genes related to pathogenicity, and virulence. As it's known, parasites always find a hostile environment inside the host, and have to adapt to modification in pH, temperature or salt concentration. As a result that, their DNA can be damaged by several agents inducing many kinds of DNA lesions. Without DNA repair, accumulated lesions can drastically modify the genome committing its stability. Fortunately, cells have evolved a sophisticated DNA damage system to repair these lesions maintaining genomic stability. Uracil in DNA may result from spontaneous deamination of cytosine or incorporation of dUMP during DNA synthesis, and an important DNA glycosylase, Mismatch Uracil DNA glycosylase (Mug), recognizes and removes this base from the DNA. The purpose of this study was to characterize CpMug protein from *C. pseudotuberculosis,* it's optimal activity condition and determine its involvement with DNA repair. We compared 15 Corynebacterium genomes, and *mug* gene is preserved in 6 pathogenic species, the 3D Structure of the CpMug was conducted through comparative modeling using 9 homologous structures from PDB. The CpMug protein was produced, and incubated with 60 pb 5' fluorescein-labeled oligonucleotide containing an uracil at the 40th position in one of the strands. The CpMug activity was evaluated in an automatic sequencer by release of a 40-mer fragment. This assay showed CpMug protein was capable to recognize and excise uracil from the oligonucleotide, showing evidence of Glycosylases/AP lyase activity *in vitro*. The activity of CpMug was tested in different conditions of temperature, pH, reaction time, presence or absence of NaCl, or magnesium. CpMug showed better activity in pH 8, temperature of 40 °C, after 18 hours of reaction, without magnesium addition. A second trial was conducted using labeled phosphate in 5' strand oligonucleotides carrying others DNA lesions such 8-oxoguanine, tetrahydrofuran, thymine glycol and uracil. These oligonucleotides were incubated with CpMug, and this enzyme was functional only in Uracil-containing oligo, showing that CpMug can discriminate uracil from others DNA lesions, and it has a high specificity for this lesion. Taken together, the results suggest CpMug protein is an important target to study pathogenic species showing evidence of functional activity in Uracil mismatch repair, preventing GC → TA transversions and protecting DNA to damage. Therewith, to evaluate the participation of CpMug pathogenicity of *C. pseudotuberculosis* and their role in DNA Repair, we are performing knockout cells through the CRISPR-Cas technique to conduct *in vivo* functional assays in genotoxic conditions.

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GENOTYPING OF INDEL MARKERS IN A BRAZILIAN URBAN POPULATION SAMPLE

Juliet Figueiredo Gonçalves de Souza[1*]; Maria Luísa de Barros Rodrigues[1]; Cláudia Emília Vieira Wiezel[1]; Ayling Martins Ng[1]; Aguinaldo Luiz Simões[1]

[1]Faculdade de Medicina de Ribeirão Preto – USP

*juliet.goncalves@usp.br.

Keywords: Indel, Polymorphisms and PCR multiplex.

Indels are polymorphisms characterized by the insertion or deletion of one or more nucleotides. Indel polymorphisms can be used as alternative for STR's in human identification, criminal or paternity exams due its abundance and wide distribution in the genome. It can have significantly different allele frequency between geographically distinct populations, hence acting as ancestry indicator markers. Indels alleles can be genotyped in small amplicons, increasing degraded DNA analysis success rate. Indel markers are very useful in Population Genetics studies, since it allows drawing of insertion and deletion allele-specific primers. For a locus, an individual could be considered either homozygous, due presence of insertion allele (in / in) or absence (del/del), or heterozygous (in / del). PCR Multiplex reactions decrease analysis time and specific primers to deletion or insertion allow detection and different DNA lineage dosage in the same solution. It can be used to non-invasive fetal diagnosis or chimerism analysis in patients submitted to hematopoietic cell transplantation. In this work, conventional PCR, PCR-multiplex and polyacrylamide gel electrophoresis laboratory conditions are described for five Indel type locus of Brazilian urban population with flaking and alleles-specific primers, under the hypothesis that its allele frequency and other forensic parameters are informative. Forensic and populational parameters of two locus (rs67718373 and rs34656661) were analyzed: PIC (0,3405 and 0,3481); Discrimination probability (0,5863 and 0,5955); insertion aleric frequency (0,3400 and 0,6800); Heterozygosis observed (0,3333 and 0,4400); expected Heterozygosis (0,4370 and 0,4503) and Probability of Exclusion (0,1703 and 0,1740), hence both locus (rs67718373 and rs34656661) are informative for the studied population and can be used in DNA mixturesanalysis.

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MOLECULAR ANALYSIS OF EIF5A-LIKE 1 (EIF5A-L1) GENE IN HUMAN CELLS CULTURE

Nathalie Fortes Pestana[1,2]; Leticia Meneguello[3]; Fábio C. P. Navarro[3]; Isadora C. B. Pavan[1,4]; Ana Paula Morelli[1]; Mariana R. Tavares[1]; Luiz Guilherme S. da Silva[1]; Augusto Ducati Luchessi[3]; Fernando Moreira Simabuco[1].

[1]Laboratório Multidisciplinar em Alimentos e Saúde, Faculdade de Ciências Aplicadas, Universidade Estadual de Campinas (FCA-UNICAMP), Limeira, São Paulo, Brazil. [2]Centro Universitário Hermínio Ometto (UNIARARAS), Araras - SP.[3]Laboratório de Biotecnologia (BIOTEC), Faculdade de Ciências Aplicadas, Universidade Estadual de Campinas, Limeira, São Paulo, Brazil. [4]Laboratório de Mecanismos de Sinalização, Faculdade de Ciências Farmacêuticas, Universidade Estadual de Campinas (FCF- UNICAMP), Campinas, São Paulo, Brazil.

nathalie.f.pestana@gmail.com.

Key words: eIF5AL1; translation; cellular metabolism.

Protein translation is an essential process for metabolism and homeostasis that occurs by highly conserved mechanisms between organisms. This process is dependent of specific translation factors, being eIF5A1 (eucaryotic translation initiation factor 5A 1) necessary for all stages of protein translation. This is the only cellular protein that undergoes to a modification called hypusination, essential for its functions. Recently, it was discovered by bioinformatic tools an amino acid sequence with high identity to the canonical eIF5A, that possible encodes for a protein denominate eIF5A Like 1 (eIF5AL1). There are no evidences of eIF5AL1 gene expression detection in indexed literature and it is not clear if this protein is really expressed and hypusinated. In this context, molecular assays are necessary for characterization and understanding the role of this protein in eukaryotic cells. This study aims to evaluate if *EIF5AL1* is expressed in human cells and if it is post translated modified by hypusination, verifing its possible functions in cellular metabolism. Firstly, analysis of eIF5AL1 sequence and evolutionary origin were made by bioinformatic tools. Also, genomic DNA was obtained from HEK293 cells for gene detection and sequencing. Besides, RNA was extracted from HEK-293, THP1 and Jurkat cells and treated with DNAse I, for cDNA construction and PCR. A restriction assay was performed with *Acc*I enzyme to differentiate the eiF5AL1 sequence from the canonical gene. Moreover, a plasmid containing eIF5AL1 gene in fusion with HA peptide was constructed for expression in HEK-293. Protein extracts were analyzed by Western Blotting with specific antibodies for eIF5A1 and HA detection. Bioinformatic analysis indicated that eIF5AL1 originates from eIF5A1 in a common ancestor between gorillas, chimpanzees and humans, probably by a retrotransposition event. Sequencing of DNA fragment confirmed the detection of *EIF5AL1 in vitro*. Moreover, eIF5AL1 RNA was found in THP1 cells but not in HEK 293 and Jurkat, indicating that expression pattern is dependent on the cell line. Peptide HA in fusion with eIF5AL1 was detected in protein extracts obtained from transfected cells, and eIF5A1 specific antibody was able to detected eIF5AL1, possible due the high similarity between both proteins. However, the efficiency of detection was lower for eIF5AL1. All together, these data shown that *EIF5AL1* gene is present in human cells genome and RNA detection indicates that protein could be expressed, but only in specific cell lines. Heterologous eIF5AL1 expression evidences that cells can translated its mRNA, but more assays must be performed for understanding efficiency of expression. As perspectives, hypusination and effects of eIF5AL1 expression will be analyzed for understanding the possible functions in cellular metabolism.

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ASSOCIATION LINE-1 – rDNA 45S IN MANATEES (*Trichechus manatus manatus* and *Trichechus inunguis*)

Flávia dos Santos Tavares, Manoella Gemaque Cavalcante, Cleusa Yoshiko Nagamachi, Julio Cesar Pierckzarka, Renata Coelho Rodrigues Noronha*

Centro de Estudos Avançados da Biodiversidade, Federal University of Pará, Belém – Pará, Brazil.

*renatarcrn@gmail.com

Key-words: repetitive DNA; LINE-1; *Trichechus*

Transposable elements (TEs) are much present in eukaryotes DNA and was found associated with multigene families. Retrotransposon non-LTR LINE-1 (*Long Interspersed Nuclear Element – 1*) has a large distribution in eutherian mammals comprising around 20% of that genomes. Ribosomal genes (rDNA) 45S (18S, 5.8S and 28S), among multigene families, its responsible for nucleous organizer region (NOR). Thus, the purpose this study was mapping and analyzing rDNA 45S and LINE-1 in *Trichechus manatus manatus* – TMM (marine manatee) and *Trichechus inunguis* – TIN (Amazonian manatee) karyotypes. Biological samples was collected in Centro Nacional de Pesquisa e Conservação de Mamíferos Aquáticos (CMA) (Itamaracá, Pernambuco – Brazil) and in the captivity of the Faculdade Integrada do Tapajós (Santarém, Pará – Brazil). It was made lymphocyte culture getting metaphasic chromosomes, and Fluorescence *in situ* Hybridization (FISH) for physical mapping of repetitive sequences. Our results confirmed that 2n=48 in TMM and 2n=56 in TIN; rDNA 45S, localized in short arm of pair 20, it was found preserved form to both species. LINE-1 retroelement presented distribution highly dispersed in all karyotypes of the investigated species, besides accumulation in pericentromeric regions. That accumulation of the retrotransposon LINE-1 was observed in more abundance in heterochromatic regions and also being associated to the rDNA 45S in both species. However, about intensive distribution of the retrotransposon LINE-1, including euchromatic regions, it can be indicative of possible interference of the retroelement for genomic dynamics of the studied species. It is worth mentioning that retrotransposon action can cause recombination events, and that can produce genomic breaks and rearrangements, homologous non-allelic recombination, and/or interrupting coding sequences of endogenous genes that modify its expression. In conclusion, our data open prospect to investigate the interference of the retrotransposon LINE-1 in *T. m. manatus* and *T. inunguis* genomes.

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ASSOCIATION LINE-1 – rDNA 45S IN MANATEES (*Trichechus manatus manatus* and *Trichechus inunguis*)

Flávia dos Santos Tavares, Manoella Gemaque Cavalcante, Cleusa Yoshiko Nagamachi, Julio Cesar Pierckzarka, Renata Coelho Rodrigues Noronha*

Centro de Estudos Avançados da Biodiversidade, Federal University of Pará, Belém – Pará, Brazil

*renatarcrn@gmail.com

Key-words: repetitive DNA; LINE-1; *Trichechus*.

Transposable elements (TEs) are much present in eukaryotes DNA and was found associated with multigene families. Retrotransposon non-LTR LINE-1 (*Long Interspersed Nuclear Element – 1*) has a large distribution in eutherian mammals comprising around 20% of that genomes. Ribosomal genes (rDNA) 45S (18S, 5.8S and 28S), among multigene families, its responsible for nucleous organizer region (NOR). Thus, the purpose this study was mapping and analyzing rDNA 45S and LINE-1 in *Trichechus manatus manatus* – TMM (marine manatee) and *Trichechus inunguis* – TIN (Amazonian manatee) karyotypes. Biological samples was collected in Centro Nacional de Pesquisa e Conservação de Mamíferos Aquáticos (CMA) (Itamaracá, Pernambuco – Brazil) and in the captivity of the Faculdade Integrada do Tapajós (Santarém, Pará – Brazil). It was made lymphocyte culture getting metaphasic chromosomes, and Fluorescence *in situ* Hybridization (FISH) for physical mapping of repetitive sequences. Our results confirmed that 2n=48 in TMM and 2n=56 in TIN; rDNA 45S, localized in short arm of pair 20, it was found preserved form to both species. LINE-1 retroelement presented distribution highly dispersed in all karyotypes of the investigated species, besides accumulation in pericentromeric regions. That accumulation of the retrotransposon LINE-1 was observed in more abundance in heterochromatic regions and also being associated to the rDNA 45S in both species. However, about intensive distribution of the retrotransposon LINE-1, including euchromatic regions, it can be indicative of possible interference of the retroelement for genomic dynamics of the studied species. It is worth mentioning that retrotransposon action can cause recombination events, and that can produce genomic breaks and rearrangements, homologous non-allelic recombination, and/or interrupting coding sequences of endogenous genes that modify its expression. In conclusion, our data open prospect to investigate the interference of the retrotransposon LINE-1 in *T. m. manatus* and *T. inunguis* genomes.

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IDENTIFICATION OF VARIANTS INVOLVED WITH UMBILICAL HERNIA IN PIGS

Igor Ricardo Savoldi[1]*; Adriana Mércia Guaratini Ibelli[2]; Mauricio Egídio Cantão[2]; Jane de Oliveira Peixoto[2]; Marcos Antônio Zanella Morés[2]; Jader Silva Lopes[3], Mônica Corrêa Ledur[1,2]

[1]Programa de Pós-Graduação em Zootecnia, Universidade do Estado de Santa Catarina, Chapecó, SC, Brasil. [2]Embrapa Suínos e Aves, Concórdia, SC, Brasil. [3]BRF S.A, Curitiba, PR, Brasil.

*igorsaavoldii@gmail.com

Keywords: Whole Exomic Sequencing, SNPs, swine.

Umbilical Hernia (UH) is a condition that affects pig production by reducing welfare and performance. There are several potential causes that can lead to the umbilical hernia development, such as bacterial infections, management conditions and genetic factors. UH is characterized by the passage of part of the intestine through the umbilical canal forming the hernia sac, being related to disorders associated with collagen metabolism, muscular structure, and connective tissue repair. Since the genetic components involved with UH are poorly understood, this study aimed to identify polymorphisms associated with UH in pigs through the whole exomic sequencing, trying to identify genes involved with this condition. Briefly, samples from 5 healthy (without a family history of UH) and 5 UH-affected pigs were collected for DNA extraction. Exomic libraries were prepared using the swine SeqCap EZ Library SR v. 1.0 kit (NimbleGen/Roche) and sequencing was performed using Illumina HiSeq 2500 (2x100bp). The sequences were submitted to quality control using Trimmomatic tool and mapped against the reference pig genome (Sscrofa11.1) using BWA-MEM software. The polymorphism identification was performed using the Genome Analysis Toolkit (GATK) and the Variant Effect Predictor (VEP) from Ensembl was used to annotate and determinate the effect of the variants. A total of 459,728 polymorphisms were identified in the analyzed samples. From those, 209 variants differed between healthy and UH-affected pigs and were grouped as: 31% in introns, 20% downstream of genes, 14% synonymous, 11% in region 3' UTR, 7% upstream gene, 7% missense, 3% in splice region, 3% in 5' UTR and 2% in intergenic regions. According to the results from the VEP tool, 204 polymorphisms were located in 70 genes. Some of them, such as *ITGAM, CNR2* and *FUCA1,* are involved with immune innate system, inflammatory responses, adhesive interaction of monocytes, macrophages and granulocytes, and have not been previously associated to UH. Other genes, such as *MYH13, MYH3, MYH4, MYH8* and *MYO19* have already been related to muscle metabolism. Biological processes associated to muscle development have already been described to be involved in the occurrence of hernias and some of the myosin gene variants identified in our study were different between normal and UH-affected groups. Therefore, the variants found in this study, especially those related to muscle, are strong candidates to be involved in the development of umbilical hernia in pigs and should be further investigated to confirm their role in this condition.

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CHARACTERIZATION OF SENSE AND ANTISENSE TRANSCRIPTION THROUGHOUT THE X-INACTIVE SPECIFIC TRANSCRIPT (XIST) LOCUS IN THE PLACENTA OF CATTLE

Paloma Soares de Castro[1,2]; Luna Nascimento Vargas[1,2]; Anelise dos Santos Mendonça[3]; Álvaro Fabrício Lopes Rios[4]; Maurício Machaim Franco[1,2]

[1]Instituto de Genética e Bioquímica, Universidade Federal de Uberlândia, Uberlândia- Minas Gerais, Brasil. [2]Laboratório de Reprodução Animal, Embrapa Recursos Genéticos e Biotecnologia, Brasília- Distrito Federal, Brasil. [3]Instituto Federal do Triângulo Mineiro, Campus Avançado Uberaba Parque Tecnológico, Uberaba- Minas Gerais, Brasil. [4]Laboratório de Biotecnologia, Centro de Biociências e Biotecnologia, Universidade Estadual do Norte Fluminense, Campos dos Goytacazes- Rio de Janeiro,

Brasil paloma_cp@hotmail.com

Key-words: X chromosome inactivation; XIST; Antisense transcription.

X chromosome inactivation (XCI) is a well-understood event in mouse. XCI occurs as a way of compensating the imbalance between the homo and heterogametic sexes with respect to the gene expression. In mouse it is established that the lncRNA X-inactive specific transcript (XIST) is essential to the initiation of the XCI process, performing chromosomal silencing. The most well-known antisense transcript in the mouse XIST locus is the TSIX, a negative modulator to XIST. However, in cattle these events are not yet totally established. The aim of this study was to characterize the patterns of strand- specific transcription along the XIST locus in the bovine foetal placenta. Total RNA was isolated from a cotyledon sample using the TRIzol™ Plus RNA Purification Kit (Invitrogen). To detect strand-specific transcription, we synthesized cDNA using forward and reverse primers specific to exon 1, exon 4 and exon 6 of XIST gene using the GoScript™ Reverse Transcription System (Promega). As positive control, cDNA synthesized using Oligo(dT) primer was used. As negative control, it was performed a cDNA using Oligo(dT) primer in the absence of the reverse transcriptase enzyme. Strand-Specific Reverse Transcription Polymerase Chain Reaction (SS-RT-PCR) analysis were run in a 7500 Fast RealTime PCR System (Applied Biosystems) using the SYBR Green PCR Master Mix (Applied Biosystems) and the same primer pairs that were used to synthesize cDNA. GAPDH gene was used as an endogenous control. Each cDNA sample (n= 8) was amplified and analysed in duplicate and interpreted qualitatively, as presence or absence of expression. The specificity of each amplicon was confirmed by the size on agarose gel (exon 1 - 172bp, exon 4 - 96bp and exon 6 - 159bp) and by the melting curve analysis. Together, our results showed that there are sense and antisense transcription throughout the XIST locus in bovine placenta. Based on the sequences deposited in GenBank, in this study we probably detected the XIST X2 isoform (XR_001495596.2) because we used a primer pair annealing in exon 1 that is specific for this isoform. However, amplicons from the exon 4 and exon 6 that were detected does not allow us identified which specific isoform we detected, because those exons are common for the three known isoforms of bovine XIST. As the molecular events related to the XCI is very scarce in cattle, our results are important to improve our understanding about XCI in this specie. However, future studies should still be done to better characterize the events involved in the XCI in cattle. Finally, our results show the relevance to take into account the possibility of antisense expression when it is proposed to perform gene expression studies, especially in non-coding RNA loci, where transcription from the two DNA strands is frequent.

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THE GENOME OF *Tetrapedia diversipes*: INSIGHTS ABOUT DIAPAUSE EVOLUTION

Priscila Karla Ferreira dos Santos[1]*; Maria Cristina Arias[1]

[1]Departamento de Genética e Biologia Evolutiva, Universidade de São Paulo, São Paulo, Brazil.

*pkfsantos@usp.br

Keywords: solitary bee, hybrid assembly, Illumina, Pacbio, transcriptome, ICA

Tetrapedia diversipes is a solitary oil-collecting bee distributed along the Neotropical region. This species is the most abundant in studies using trap-nests and has been an interesting model organism for genetics, physiology, ecology, behavior and evolutionary studies. An interesting feature is the ability of this species to pass through diapause during the winter. In São Paulo city *T. diversipes* is bivoltine and only part of population will express this alternative way of life. Previous studies reported the global pattern of gene expression of diapause for this species and to proceed on the understanding of the molecular mechanisms underlying this phenotype we aimed to generate the complete genome for the species. Sequencing was performed using second and third generation platforms (Illumina HiSeq200 and Pacbio, respectively). The Illumina data were cleaned by quality and size using Trimmomatic and Pacbio were corrected using Canu. The hybrid assembly was performed using MaSurCA. The high quality genome comprises 332,342,503 bp distributed in 2,494 scaffolds. The gene set was identified using the Maker2 pipeline and the functional annotation using Blast and Interproscan. The annotation identified 15,028 gene set for *T. diversipes*, similar number compared to other bee genomes. The software BUSCO identified 95% of the expected single-copy orthologous of Hymenoptera. We re-analyzed our previous transcriptome data obtained from diapause and non diapause larva now assembling the transcripts using the *T. diversipes* genome as reference. The number of differentially expressed genes (DGE) decreased from 2,274 to 914. Additionally, independent component analysis (ICA) was performed using MineICA to identify the group of genes with similar pattern of expression that most explain the diapause. The ICA analysis resulted in seven components that most explain the DGE, the genes of the components are mainly related to digestion, lipid metabolism and cuticle. We are now investigating expansion and contraction of gene families in *T. diversipes* genome and in others to get some clues about these phenomena and diapause in bees

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LOOKING FOR POLYGENIC ADAPTATION BEHIND THE DISTRIBUTIONS

Carolina Silva-Carvalho[1]*, Isabela Alvim[1], Marla Mendes[1], Victor Borda[2], Giordano B Soares-Souza[1], Heinner Guio[3], Eduardo Tarazona-Santos[1]

[1]Federal University of Minas Gerais – UFMG – Brazil. [2]Laboratory of Bioinformatics- National Laboratory of Scientific Computation- Petrópolis, RJ - Brazil. [3]Instituto Nacional de Salud – Peru

*carolina160195@hotmail.com

Keywords: natural selection; Population Branch Statistic; polygenic adaptation.

In humans, one of the main well-known adaptive events comprises the ability to deal with the lack of oxygen in high altitude. In view of this fact, Andean population has been used for decades as a model to understand the evolutive response and physiological consequences of high altitude. Most studies focused on monogenic selection, however, some theoretical approaches conclude that most natural selection acts as polygenic selection. The detection of this kind of natural selection has become a challenge, since the variants have a subtle shift in their frequencies. Most polygenic adaptation tests are based on GWAS, except the method developed by Gouy *et al.* (2017). However, it have a gene-based approach, which loses variability of SNPs. To use the information from the SNPs and find pathways that could have been under selective pressure, we calculated the difference between their distributions of population-based population statistics (PBS) (precalculated between Andean and Amazonian) using Kolmogorov- Smirnov (ks). We tested only pathways with more than one gene with the maximum value greater than the 95th percentile of the dataset. We find a p-value of 3.82×10^{-6} for the ks test for the pathway of thyroid hormone generation. All the 11 genes related to this pathway, according to Gene Ontology, are represented in our data. One compound of this pathway is the gene DUOX2, pointed as candidate to natural selection on Andean populations by Jacovas *et al.* (2018) and for our PBS results. We are still ~~are~~ developing a method to infer pathways involved in polygenic adaptation based on differentiation parameters distribution. We have a long way to have a robust statistical method, but, our partial results indicate that we can contribute to actual scenario of polygenic selection tests.

Study of the transcriptome of *Trypanosoma cruzi*

Bottaro, T.[1]; Ürményi, T.P.[1]

[1]Instituto de Biofísica Carlos Chagas Filho, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil

thayanebottaro@biof.ufrj.br

Keywords: Trypanossoma cruzi, heat shock, oxidative stress, transcriptome, next generation sequencing, thermal stress.

Trypanosoma cruzi, the causative agent of Chagas disease, is a parasitic protozoan of the family Trypanosomatidae, order Kinetoplastida, which exhibits a complex life cycle characterized by several cellular forms, the ability to infect the insect vector and then mammalian hosts. Since *T.cruzi* naturally undergoes temperature variation and oxidative stress during its life cycle, when it transitions between the insect vector and the mammalian host, it is believed that the parasite synthesizes many proteins to prepare and maintenance the infection. However, more extensive analysis of involved differential gene expression is currently unknown. High throughput next generation sequencing (NGS) provides large scale analysis of gene expression by RNA-seq methodology. The aim of this work is to analyze the transcriptome of heat shock and oxidative stress on a large scale to identify transcripts involved in the response to stress in *T. cruzi.* Exponentially growing epimastigotes from clone CL Brener and Y were growth in LIT medium for 24 hours and incubated at different temperatures (29ºC, 37ºC and 40ºC) or in diffenrent concentrations of CO_2 (absence and presence - 100µM) for 3 or 2 hours. Total RNA was extracted using Trizol® protocol (Invitrogen), enriched for poly(A)+ RNA using Dynabeads mRNA (Life Technologies) and enzimatically fragmented. cDNA was generated and the library for NGS was prepared and sequenced in Ion PGM (Life Technologies) or in Ion Proton (Life Thecnologies). Reads obtained were analyzed using CLC Genomics Workbench software (CLC Bio). In a reference-based strategy, reads were mapped to the publicly available genomic sequence of *T. cruzi* using the CLC Genomics Workbench. Preliminary differential gene expression analysis of transcriptomes shows stress, chaperone, proteolysis and translation genes upregulated at 37ºC, while a large number of categories of genes, including energy metabolism, cytoskeleton and splicing, are downregulated at 40ºC. Results about oxidative stress are still being analyzed. Molecular mechanisms involved in thermal stress and oxidative stress of *T. cruzi* is unknown. The study of these mechanisms will help the comprehension of cellular diferentiation, metabolism, protein synthesis and degradation of this organism.

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IN VIVO GENE EDITING IMPROVES CARDIOVASCULAR ALTERATIONS IN MUCOPOLYSACCHARIDOSIS I MICE

Esteban Alberto Gonzalez[1,4]; Roselena Silvestri Schuh[1,2]; Angela Maria Vicente Tavares[1,3]; Luisa Natalia Pimentel Vera[1,4]; Francyne Kubaski[4]; Edina Poletto[1,4]; Roberto Giugliani[1,4]; Ursula Matte[1,4]; Helder Ferreira Teixeira[2]; Guilherme Baldo[1,3,4]

[1]Centro de Terapia Gênica do Hospital de Clinicas de Porto Alegre, Porto Alegre, Brazil. [2]Programa de Pós-Graduação em Ciências Farmacêuticas da Universidade Federal do Rio Grande do Sul (UFRGS), Brazil. [3]Programa de Pós-Graduação em Fisiologia da UFRGS, Porto Alegre, Brazil. [4]Programa de Pós-Graduação em Genética e Biologia Molecular da UFRGS, Porto Alegre, Brazil.

*egonzalez@hcpa.edu.br

Key-words: Mucopolysaccharidoses I; CRISPR/Cas9; Cardiovascular alterations

Mucopolysaccharidosis I (MPS-I) is a lysosomal disorder caused by mutations in the alpha-L-iduronidase gene (*IDUA*), which is involved in the catabolism of the glycosaminoglycans (GAG) heparan (HS) and dermatan sulfate (DS). Accumulation of GAGs interferes with the normal functioning of cells, tissues, and organs, leading to multiple abnormalities. Cardiovascular alterations are commons in MPS-I patients, are the main cause of death, and include heart dilatation, growth and dysfunction, valve thickening and aortic dilatation. Current treatments have limited efficacy on this disease thus novel therapies such as gene therapy are needed. Previously we reported that neonatal hydrodynamic injection of liposomal CRISPR/Cas9 complexes that insert the murine *IDUA* in the mouse genome resulted in long-lasting IDUA activity in MPS I mice, and secreted IDUA could be taken-up from blood by multiple organs. In this study, we evaluated the effect of this therapy on the alterations found in the cardiovascular tissue of MPS-I mice. Newborn MPS-I mice were treated with a single hydrodynamic injection of liposomes containing plasmids with the CRISPR/Cas9 system in the superficial temporal vein (n=8). Normal and untreated MPS-I mice were used as control groups (n=14 each). Echocardiographic analyses were performed at 6-months-old, before euthanasia, to determine the left ventricular (LV) dimensions, heart function and aortic diameter. Additionally, aortic diameter was measured *in situ* using a digital caliper. Cardiac and aortic sections were stained with H-E & Alcian-Blue to visualize GAG storage and valve thickness; or VVG for elastin breaks. Heart GAG levels were measured by tandem mass spectrometry. Statistics was performed using ANOVA and Tukey post hoc. Treated mice showed normalization of LV chamber diameter and cardiac mass. Cardiac function was also improved, although heart valves were still abnormal showing hyperplasia and lysosomal storage. Furthermore, MPS I treated mice had a mild improvement in the aortic diameter and a reduction in elastin breaks. Total GAG levels were reduced after treatment; while DS levels were not different from untreated mice, HS were reduced in heart samples. We conclude that neonatal gene therapy can improve some aspects of cardiovascular pathologies in MPS I mice. However, a better delivery of our gene product to this tissue, usually considered “difficult-to-treat”, will be necessary to achieve more profound effects.

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OPTIMIZATION OF GENE EDITING PROTOCOLS USING CRISPR/Cas9 TECHNOLOGY IN *Leishmania infantum*

Tatiane Souto[1]; Gabriela A. Burle Caldas[2]; Viviane G. Silva[2]; Santuza M. R. Teixeira[2], Ana Paula Salles Moura Fernandes[1]

[1]Laboratory of Molecular Genetics – Pharmacy Faculty- The Federal University of Minas Gerais-UFMG. [2]Laboratory of Genomics and Molecular Genetics of Trypanosomatids- Department of Biochemistry and Immunology-UFMG

tatianecristinasouto@gmail.com

Keywords: *CRISPR-Cas9; Leishmania; miltefosine transporter*

CRISPR/Cas9 (clustered regularly interspaced short palindromic repeats Cas9 associated) technology has been shown to be a powerful tool in genome editing, by knockout (KO) or knockin. However, in *Leishmania*, studies using CRISPR/Cas9 are still scarce, although it is a strategy to, among other advances, validate new targets for drugs and vaccines. The breakdown of the double DNA strand (DSB) generated by Cas9 can be repaired by microhomology mediated end joining (MMEJ) or by homologous recombination (HR), but in *Leishmania* the involvement of these mechanisms for DNA repair is still unclear. In this study, as a proof of concept, we tested protocols using episomal (pLdCN) or integrative plasmid (pRM006) that allow the expression of Cas9 of Streptococcus pyogenes (SpCas9), in order to editing the gene coding for the *L. infantum* miltefosine transporter (MT). The Cas9 expressing parasites were then transfected with two different *in vitro* transcriptional single-guide RNAs (sgRNAs) specific for the MT gene and with a donor sequence containing three stop codons and the restriction sequence of the *Xho*I enzyme, flanked by 25 nucleotides of the MT gene. As a result, MT resistant parasites were generated, although in the presence of a donor sequence, the parasites recovered from the selection more quickly. Sequence analysis of the transfected parasites in the absence of a donor sequence revealed the presence of similar deletion in the selected clones near the Cas9 cleavage site. Analysis of the MT gene sequence in the presence of a donor sequence confirmed HR insertion of the *Xho*I site and stop coding. These results confirm the release of the MT gene independently of the constitutive or non-constitutive expression of Cas9 in *L. infantum* and suggest that HR repair is more efficient than by MMEJ in *Leishmania*. In addition, they indicate that the CRISPR/Cas9 platform has been successfully implemented, allowing its use in future studies of gene function and development of vaccines and new terapeutical targets in *Leishmania.*

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TRACKING MAJOR HISTOCOMPATIBILITY COMPLEX MAPPING AND GENOTYPING ERRORS WHEN USING CONVENTIONAL READ ALIGNERS

Andréia da Silva Souza[1*]; Thálitta Hetamaro Ayala Lima[1]; Heloísa de Souza Andrade[1]; Emiliana Weiss[1]; Nayane dos Santos Brito Silva[2]; Marília Rodrigues Silva Passos[2]; Camila Ferreira Bannwart Castro[3]; Jaqueline Wang[4]; Marília O. Scliar[4]; Guilherme L. Yamamoto[4]; Yeda A. O. Duarte[5]; Mayana Zatz[4]; Michel S. Naslavsky[4]; Maria Rita Passos-Bueno[4]; Diogo Meyer[5]; Celso T. Mendes-Junior[6]; Erick C. Castelli[2]

[1] Universidade Estadual Paulista (UNESP), Instituto de Biociências de Botucatu, Brasil. [2] Universidade Estadual Paulista (UNESP), Faculdade de Medicina de Botucatu,Brasil. [3] Centro Universitário Sudoeste Paulista – UniFSP,Brasil. [4] Centro de Pesquisas sobre o Genoma Humano e Células Tronco, Instituto de Biociências, Universidade de São Paulo (USP), Brasil. [5] Faculdade de Saúde Pública, Escola de Enfermagem, Universidade de São Paulo (USP), Brasil. [6] Departamento de Química, Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto, Universidade de São Paulo, Ribeirão Preto, SP, Brasil.

Palavras-chave: HLA; sequence mapping; WGS. The authors declare noconflict of interest.

The human Major Histocompatibility Complex (MHC) comprises many genes related to the antigen presentation process and the modulation of T and NK cell activity. These genes are highly polymorphic and present high sequence similarity. The HLA-A, HLA-B, HLA-C, and HLA-DRB1 genes within the MHC complex are the most variable genes in the human genome, and this variability influence clinical outcomes in infections, autoimmune diseases, tumors, and allografts. Efforts have been made to develop reliable mapping and genotyping approaches when evaluating HLA in second- generation sequencing (NGS) data. The HLA polymorphic nature hinders the mapping of reads presenting many mismatches compared to the reference. Here we demonstrate the importance of applying strategies alignment optimization in the MHC region to avoid genotyping errors. We used whole-genome sequencing (WGS) of 50 samples to track HLA mapping and genotyping errors when sequences are mapped with conventional aligners. To optimize read mapping in the MHC region, we used an updated version of the hla-mapper software that supports WGS and HLA class II genes. We have mapped reads using the Isaac aligner, further extracting all reads mapped to the MHC segment and the unmapped ones as FASTQ files. FASTQ files were processed using hla-mapper, and we have tracked (a) reads that changed their target genes (b) unmapped reads mapped after optimization. Briefly, the major issues were the cross-mapping between HLA-B and HLA-C (reaching 3.91%), HLA-A reads mapping to HLA-H (reaching 4.51%), and the high proportion of HLA-A, HLA-B, HLA-C, and HLA-DRB1 reads with zero mapping quality (MQ=0) or not mapped at all in the non-optimized files, reaching 83% for HLA-DRB1 and 46% for HLA-C. Further, weused the GATK Haplotypecaller to genotype the samples before and after optimization, comparing the genotypes. In order to avoid the comparison between low-quality genotypes, we have processed both VCF files with vcfx checkpl to introduce missing alleles in genotypes with low likelihood. This comparison revealed around 5.0%, 2.6%, 3.2%, and 22.25% of genotype divergence between both files, for HLA-A, HLA-B, HLA- C, and HLA-DRB1, respectively. These genotype differences translate into the detection of a higher number of heterozygous sites for all these genes after optimization, and also the lower rate of heterozygous sites in the HLA-H gene. The number of heterozygous sites at HLA-DRB1 increased 3 times after optimization because most of the HLA-DRB1 reads presented MQ=0 or were not mapped at all in the original alignment. Taken together, these findings demonstrate the importance of the hla-mapper software and mapping optimization in the MHC segment in order to achieve a reliable genotyping in this very polymorphic and repetitive genome segment. Similar results were observed with HLA class I genes by usingBWA.

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Clinical, cytogenetics and molecular characterization of mosaicism for a small supernumerary marker chromosome derived from chromosome 8 in a girl with developmental delay, dysmorphia and malformations, but without corpus callosum agenesis

Carlos Roberto Fonseca[1]; Ingrid Bendas Feres Lima[1]; Sara Fabíola da Silva Oliveira[1]; Anna Luisa Serrão[1]; Patrícia Santana Correa[1]; Elenice Bastos[1]; Juliana Mazzeu[2]; Juan Clinton Llerena Jr[1]; Lucia de Fátima Marques de Moraes[1]

[1]Departamento de Genética Dr. José Carlos Cabral de Almeida/ Instituto Fernandes Figueira/FIOCRUZ - Laboratório de Citogenética/Citogenômica, Rio de Janeiro, Brazil. [2]Faculdade de Medicina, Universidade de Brasília, Brasília, Brazil

*lucia.moraes@iff.fiocruz.br

Key-words: supernumerary marker chromosomes, partial trisomy of 8, Chromosome analysis by Microarray

The presence of supernumerary marker chromosomes are cytogenetic findings in patients with global developmental delay, dysmorphia and / or malformations. This extra material is responsible for partial trisomies of certain chromosomal regions, with distinct or nonspecific clinical pictures. Phenotypic variability is mainly associated with the genes present in the additional region and the origin of the chromosome. The characterization of this material is fundamental for the determination of the prognosis and genetic counselling of patients and their families. The present study aimed to identify a small supernumerary marker chromosome, detected by conventional GTG karyotype in a patient referred for Reference Center for Rare Diseases (National Fernandes Figueira Institute - IFF / FIOCRUZ). For the identification of the marker chromosome, a Microarray (CMA) analysis was performed. A mosaic gain of 40,215 Kb involving 8p11.21 to 8q21.11 regions of chromosome 8 [arr [hg19] 8p11.21 to 8q21.11 (8: 39.580.893 - 79.796.337)x 3) was detected. Thus, a partial trisomy of 8 and the karyotype was defined as mos 46, XX [9] / 47, XX, + der (8) (8p11.21 - 8q21.11). Clinical examination of the patient revealed a speech delay, intellectual deficit, hyperacusia, left motor-ocular paralysis, gross ears with thick lobes, pectus excavatum, long thorax, deep furrows in feet, ligament laxity, hydronephrosis, atrial septal defect; however, without agenesis of the corpus callosum (ACC). Agenesis of the corpus callosum (ACC) is a frequent malformation in cases of complete trisomy 8; however, ACC was not observed in our patient, suggesting that genes located in other regions of chromosome 8 different from the regions 8p11.21 to 8q21 may correlate to this malformation. Our study emphasizes the need to combine different cytogenetic and molecular techniques for the identification of small supernumerary marker chromosomes. It reinforces CMA as an important tool for the diagnosis and genetic counseling of affected patients and their families, assisting in the genetic mapping of each region involved for future karyotype-genotype-phenotype correlation. It also contributes to the delineation of the partial trisomy 8 phenotype.

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THE ROLE OF NEK6 PROTEIN IN PROGRESSION OF CASTRATION-RESISTANT PROSTATE CANCER (CRPC) USING THE CRISPR/CAS9 GENE EDITING SYSTEM

Isadora Carolina Betim Pavan[1]; Luidy Kazuo Issayama[1]; Fernando Riback Silva[1]; Ana Paula Morelli[2], Nathalie Fortes Pestana Pereira[2], Luiz Guilherme Salvino da Silva[2], Fernando Moreira Simabuco[2]; Jörg Kobarg[1*]

[1]Laboratório de Mecanismos de Sinalização, Faculdade de Ciências Farmacêuticas, Universidade Estadual de Campinas (FCF-UNICAMP), Campinas, São Paulo, Brasil. [2]Laboratório Multidisciplinar em Alimentos e Saúde (LabMAS), Faculdade de Ciências Aplicadas, Universidade Estadual de Campinas (FCA-UNICAMP), Limeira, São Paulo, Brasil.

*jorgkoba@unicamp.br

Key-words: NEK6, Castration-resistant prostate cancer, CRISPR/Cas9

Prostate cancer (PCa) is the most prevalent malignant neoplasm in man, with increasing incidence due to the aging of the population. Advanced prostate cancer is characterized by metastasis in different organs, such as bones and lymph nodes. Surgical or chemical castrations are the indicated treatments for metastatic PCa. However, it is very common that cancer cells continue to grow after castration, which allows the progression of the disease. This condition is called castration-resistant prostate cancer (CRPC) and is the leading cause of death for prostate cancer. Constitutive activation of some specific kinases has been implicated in mediating castration resistance. NEK6 protein, member of the NIMA-related serine/threonine kinase family, plays an important role in mitotic cell cycle progression. Some studies have shown that NEK6 participates in tumorigenesis, however it is unclear in which mechanisms and signaling pathways NEK6 is involved. Recently, a study showed that NEK6 is overexpressed in samples derived from advanced prostate cancer patients. A double hydride study conducted previously by our group showed that NEK6 interacts with cellular survival proteins, such as proteins related to NF-kappaB signaling pathway. Thus, we hypothesized that NEK6 can mediate castration resistance in prostate cancer by regulating cell survival signaling pathways. The present study generated a NEK6 knockout (NEK6 $^{-/-}$) for DU-145 cell line, an in vitro model of metastatic castration-resistant prostate cancer cells, using the CRISPR/Cas9 gene editing system. We used the PX459 V2.0 plasmid available from the Zhang's Laboratory for cloning of a specific guide sequence (gRNA) between *Bbs*I restriction sites. Here, we report that NEK6 deletion significantly decreased viability, proliferation and migration of DU-145 cells. We also demonstrated that NEK6 knockout altered Bcl- 2 proteins family expression, such as Bcl-2 and Bim, which act as regulators of the intrinsic apoptotic pathway. In addition, we found that NEK6 $^{-/-}$ DU-145 cells presented reduced p62 expression, an important NF-kappaB mediator in tumorigenesis, and also reduced JNK protein phosphorylation (p-JNK). Both p62 and JNK are proteins related to cell survival. Docetaxel treatment of NEK6 $^{-/-}$ DU-145 cells led to a lower viability compared to the docetaxel-treated control cells. These data suggest that NEK6 is involved in the progression of castration-resistant prostate cancer through modulation of apoptotic and survival pathways. The next steps will be the generation of constitutive active and kinase dead forms of NEK6 in DU-145 cells using the HDR mediated CRISPR/Cas9 system. Besides, we aim to identify new phosphorylation targets of NEK6 by phosphoproteomic to finally better understand the role of NEK6 in the progression of CRPC.

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BIOINFORMATIC ANALYSIS AND LITERATURE REVIEW REVEAL microRNAs AS POTENTIAL BIOMARKERS FOR GESTATIONAL DIABETES MELLITUS

Izabela M. C. A. Conceição[1]; Lúcio R. Queiroz[2]; Marcelo R. Luizon[1*]

[1]Departament of Genetics, Ecology and Evolution, Institute of Biological Sciences, Federal University of Minas Gerais. [2]Departament of Biochemistry and Immunology, Institute of Biological Sciences, Federal University of Minas Gerais.

iza.mamede@gmail.com

Palavras-chave: Gestational Diabetes Mellitus, Literature Review, microRNA

Gestational Diabetes Mellitus (GDM) is described as a condition in which woman have any degree of glucose intolerance during pregnancy without the previous presence of those symptoms. GMD affects 16.2% of all pregnancies worldwide what equals 19 million live births per year, and woman with GDM are at higher risk for maternal hypertensive disorders. Although there are no known molecular biomarkers for GDM, several single nucleotide polymorphisms and microRNAs (miRNAs) were proposed as possible biomarkers for GMD. While miRNAs have been proposed as alternators of disease pathogenesis in cancer and diabetes, their use as clinical predictors remains a field in expansion. However, miRNAs are multivalent posttranscriptional regulators, and one miRNA is able to target a large group of genes. Therefore, it is not straightforward to prove their functionality in an independent miRNAs-gene analysis. In this study, we used an integrative approach to relate miRNAs to gene pathways known to be involved in GDM physiopathology, to come up with a simplified biomarker finding approach in complex diseases that could be translated to clinical use. We compiled the main miRNAs found to be related to GDM in the literature using the search terms ([GDM] OR [GESTATIONAL DIABETES MELLITUS]) AND [miRNA] on Pubmed Scopus and Web of Science databases from 2011 to 2019. The known gene pathways related to GDM were compiled by using the terms [GDM] AND [GENE] or ([ENDOTHELIAL DYSFUNCTION] OR [INSULIN RESISTANCE]) AND [GENE], in order to include genes related to GDM physiopathology. Single case analysis articles were excluded. A bioinformatics analysis was made comparing miRDB target prediction data from those previously compiled microRNAs. We found a total of 56 precursors and extracted 71 mature microRNAs, which were imputed to miRDB. We retrieved 12576 target genes, which were filtered by miRDB 90% score resulting in 4245 genes. From the 111 genes related to GDM, 32 (28.8%) were predicted to be targeted by 49 of the filtered miRNA. From the 49 miRNA, we selected those that target most of the 111 genes related to GDM: mir-95-5p, mir-16-5p, mir-195-5p, mir-30d-3p and mir-92a-3p. These miRNAs are related to ACE-RAGE signaling pathway in diabetic complications: angiotensin stimulates pro-inflammatory cytokines as NF-κB and IL-17, linked to diabetes induced nephropathy. All of those miRNAs were also present on the FuncMir collection, a tab in miRDB for those miRNAs predicted to play active functional roles, as precursors or mature miRNA. Using bioinformatic tools and literature reviews, we identified five miRNAs that may be useful to develop potential biomarkers for GDM. Although our findings require further functional studies for a potential clinical application, we were able to establish a possible correlation between the miRNA found and a common pathway related to diabetic complications.

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CHARACTERIZATION OF PROMOTERS ELEMENTS RESPONSIVE TO THE QUORUM SENSING (QS) IN *ZYMOMONAS MOBILIS*

Juliana de Fátima dos Santos Silva[1], Valquíria Campos Alencar[2], Renata Ozelami Vilas Boas[2], Fabiano Menegidio[2], David Aciole Barbosa[2], Daniela Leite Jabes[2], Regina Costa de Oliveira[2], Luiz Roberto Nunes[1]

[1]Centro de Ciências Naturais e Humanas, Universidade Federal do ABC, Santo André, São Paulo, Brazil [2]Núcleo Integrado de Biotecnologia, Universidade de Mogi das Cruzes, Mogi das Cruzes, São Paulo, Brazil

luiz.nunes@ufabc.edu.br

Key-words: *Zymomonas mobilis*; quorum sensing; bacterial promoters

Zymomonas mobilis is a gram-negative facultative anaerobic bacterium, which is considered a microorganism of major potential for industrial applications, since it presents several important attributes that favor its use in alternative bioconversion processes. *Z. mobilis* has attracted the attention of the bioethanol industry for several years, especially for presenting some advantageous features regarding ethanol production, when compared to the yeast *Saccharomyces cerevisiae*. In addition, genetic manipulation techniques have been developed for this bacterium, which has been genetically modified and/or adapted to produce different high value-added products (as levan, sorbitol, lactate and 2.3-butanediol). Therefore, *Z. mobilis* possesses a number of desirable characteristics for the bioconversion industry and a better understanding of the mechanisms used to control gene expression in this bacterium can help in the development of new genetically manipulated strains, with high capacity of industrial production. This project aims to characterize the global transcriptional response of *Z. mobilis* to the process of Quorum Sensing (QS), a chemical communication process employed by bacteria to assess population density in their surrounding environment and synchronize behavior in a community scale. The activation of the QS response reduces cell growth, activating expression of genes involved with a series of alternative activities, such as biofilm formation, bioluminescence and production of pathogenicity factors, for example. Thus, QS-responsive promoters may be used to redirect cellular resources for the production of substances of interest in recombinant *Z. mobilis* strains. To characterize such promoters, RNA-seq libraries were constructed using RNA obtained from *Z. mobilis* cells at two phases of growth in minimal medium (MM): (i) cells growing at the exponential phase and (ii) cells in stationary phase. The libraries were sequenced using the Illumina NextSeq platform and the sequences obtained were processed by the Rockhopper software, using the reference genome of *Z. mobilis* ZM4. Comparisons involving these two transcriptomes demonstrated that the QS response induced expression of a total of 588 genes (q value ≤ 0.01), including 292 upregulated genes and 296 downregulated genes (32% of the bacterium genome). These studies led to the identification of chromosomal regions carrying QS-responsive promoter elements, which are currently being mapped and identified. Such inducible promoters may constitute an important tool for *Z. mobilis* manipulation, with possible application in a wide variety of industrial bioprocesses, allowing the expression of transgenes in this bacterium, under controlled and optimized conditions.

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GENETIC ASSOCIATION STUDY OF SINGLE NUCLEOTIDE POLYMORPHISMS IN NEUROTROPHIN GENES AND LEPROSY PHENOTYPES

Karina Talita de Oliveira Santana Jorge[1]; Rodrigo Anselmo Cazzaniga[2]; Mauro Martins Teixeira[1]; Maria Amélia Ribeiro de Jesus[2]; Frederico Marianetti Soriani[1*]

[1]Instituto de Ciências Biológicas da Universidade Federal de Minas Gerais. [2]Universidade Federal de Sergipe

*fredsori@gmail.com

Palavras-chave: leprosy; polymorphisms; neurotrophins

Neural damage is a frequent manifestation in leprosy and it is associated with disability degree. Neural impairment may occur in all clinical forms of leprosy being paucibacillary leprosy characterized by impairment of one or few nerves and multibacillary leprosy characterized by impairment of a higher number of nerves. After neural damage, regeneration can occur with the axonal endings seeking out by a new microenvironment for re-establishment, otherwise, it persists as irreversible damage. This process depends on neurotrophins which allow axonal endings to extend into the perineural space. Neurotrophin family includes BDNF (brain derived neurotrophic factor), NGF (nerve growth factor), NT3 (neurotrophin 3) and NT4 (neurotrophin 4). Due to the crucial role played by neurotrophins and its receptors in neural plasticity, polymorphisms in neurotrophins genes may be associated with neural impairment and disability degree in leprosy. In this context, we studied the genetic association of the following single nucleotide polymorphisms and different clinical manifestations in leprosy: rs6265 in *BDNF* (-196 G/A), rs11030099 in *BDNF* (-3613 A/C), rs6330 in *NGF* (-273 C/T), rs6332 in *NT3* (-502 A/G) and rs2072446 in *P75NTR* (-739 C/T). For this purpose, DNA was extracted from peripheral blood and the genotypes were determined by Real-time PCR using TaqMan® Assays. In this study, 173 leprosy patients were included divided into groups with each clinical form of leprosy, 66 non-consanguineous contacts, and 73 consanguineous contacts. After evaluating the adherence of each polymorphism to Hardy-Weinberg Equilibrium, the association of allelic and genotypic frequency with each phenotype was evaluated with Fisher's Exact Test. The following associations were evaluated: leprosy *per se*, leprosy clinical form (Ridley-Jopling and WHO classification), disability degree, leprosy reaction, neuritis, number of lesions and number of impaired nerves. The main finding of this study consists in an association of genotypes "AC" and "AA + AC" of rs11030099 in BDNF and the number of impaired nerves in the studied population, being these genotypes protective against nerve impairment due to *M. leprae* infection. This association may be related to post-transcriptional modulation of BDNF, once this polymorphism is located in a miRNA binding site. The possible functional role of this polymorphism still needs to be investigated.

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The dual role of mast cells in sporadic colorectal tumorigenesis: clinical and experimental evidence

Juliana Y Sakita[1], Luciana Yamamoto Almeida[2], Emerson de Souza Santos[3], Tathiane M. Malta[4], Mariângela O Brunaldi[5], Sergio Britto Garcia[5], Fernando Q Cunha[6], Guilherme Cesar Martelossi Cebinelli[6], Jeremy A Squire[1,7] Sergio A Uyemura[3], Vinicius Kannen[1,3,8]

[1]Department of Genetics, University of Sao Paulo, Ribeirao Preto, Brazil; [2]Department of Clinical Medicine, University of São Paulo, Ribeirão Preto, Brazil; [3]Department of Toxicology, Bromatology, and Clinical Analysis, University of São Paulo, Ribeirao Preto, Brazil; [4]Department of Neurosurgery, Henry Ford Hospital, Detroit, United States of America; [5]Department of Pathology, University of São Paulo, Ribeirão Preto, Brazil; [6]Department of Pharmacology, University of São Paulo, Ribeirão Preto, Brazil; [7]Department of Pathology and Molecular Medicine, Queen's University, Kingston, Canada; [8]Department of Biomedical and Molecular Sciences, Queen's University, Kingston, Canada.

Keywords: Intestines; Colon cancer; Immune system; Proliferation; Carcinogen

Mast cells (MCs) impact significantly on the development of colorectal cancer (CRC). However, the precise MC activity in CRC remains controversial. Here, we analyzed human samples and performed a comprehensive meta-analysis to show that MC numbers vary in CRC cases, a fact related not only to different cell proliferation rates but also to complex immunological reactions. In carcinogenically exposed mice, deficient MC numbers ($Kit^{W/sh}$ mouse model) increased the development of colorectal tumors. An RNAseq analysis showed this finding to be significantly associated with a T cell- dependent immune response. Before tumors were fully developed, we also found that MC deficiency increased the number of preneoplastic lesions and proliferative events. Exposing interleukin 33 receptor knockout (KO) mice to the same carcinogenic protocol revealed that increased MC density was related to a decrease in cell proliferation and β- catenin expression levels. Our experiments further showed that the major histocompatibility complex I decreased MC density while its type II increased their numbers. Indeed, MC deficient mice given half of the carcinogenic dosage showed reduced development of preneoplastic lesions. Bone marrow transplantation increased not only stromal MC density but also the expression of CRC biomarkers in $Kit^{W/sh}$ mice. Whether pharmacologically inhibiting MC activity throughout carcinogenic exposure promoted the development of preneoplastic lesions, applying the same treatment following carcinogen injections inhibited such events. Our findings suggest that the MC activity impact on the CRC development, although these cells can also be altered by several immunological reactions controlling the multi-stepped evolution of the disease.

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PHENOTYPIC CHARACTERIZATION OF ARABIDOPSIS INSERTION MUTANTS FOR THE MITOCHONDRIAL UNCOUPLING PROTEIN (UCP) GENES REVEALS AN IMPORTANT IMPACT ON PLANT FERTILITY

Mariana de Lara Campos Arcuri[1]; Alessandra Vasconcelos Nunes-Laitz[2]; Pedro Paulo Barreto[3]; Paulo Arruda[3]; Ivan de Godoy Maia[1]

[1]Departamento de Genética – Instituto de Biociências de Botucatu, Universidade Estadual Paulista "Júlio de Mesquita Filho" (UNESP) [2]Instituto Federal de Educação, Ciência e Tecnologia de Rondônia (IFRO), Campus Colorado-do-Oeste [3]Centro de Biologia Molecular e Engenharia Genética (CBMEG), Universidade Estadual de Campinas (UNICAMP)

mariharcuri@gmail.com

Key words: UCP; gene expression; plant fertility.

Mitochondrial Uncoupling Proteins (UCPs) are present in the inner mitochondrial membrane and catalyze the uncoupling between the electron transport chain and oxidative phosphorylation. These proteins are crucial for the maintenance of mitochondrial homeostasis and variations in their expression trigger various physiological changes. Studies using single T-DNA insertion mutants for the three UCP genes present in Arabidopsis (*AtUCP1-3*) revealed increased ATP levels in *atucp1* and *atucp2*, indicating clear alterations in energy metabolism. High levels of reactive oxygen species in the mitochondrial compartment were also observed in all mutants. Phenotypic characterization of these single mutants revealed *atucp2* plants displayed a decrease in the total number of leaves, a 3-day delay in flowering time, and a 5-fold increase in the number of sterile siliques. A 2-fold increase in the number of sterile siliques was equally observed in *atucp1*, which also exhibited a reduction in silique length, thus suggesting an effect in plant fertility. To further test this hypothesis, we investigated the relative expression of transcription factors implicated in gene regulation during female (*AtHEC1-3*) and male (*AtDYT* and *AtAMS*) reproductive organ/tissue development, respectively. As a result, these genes were strongly downregulated in *atucp1-3* flowers at different stages of development compared to WT Col-0. Moreover, transgenic tobacco plants harboring an *AtUCP1* or *AtUCP2* promoter: GUS fusion displayed reporter activity mainly in the stigma and anthers. Overall, these data give support for an important role of UCPs in flower metabolism and fertility.

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The *Nicotiana tabacum* CDKG;2 is involved in flowering, transcription, splicing and cell cycle control

Pedro B. Ferreira[1,2]; Greice Lubini[1,2]; Vanessa Thome[1,2]; Fabio S. Silva[2]; Juca A. B. San Martin[2]; Edward J. Strini[1,2]; Vitor F. Pinoti[1,2]; Andrea C. Quiapim[2]; Gustavo H. Goldman[3]; Maria Helena S. Goldman[2]

[1]PPG Genética, FMRP - Universidade de São Paulo; [2]Depto. Biologia, FFCLRP – Universidade de São Paulo; [3]Depto. Ciências Farmacêuticas, FCFRP – Universidade de São Paulo.

pedrobf@usp.br

Key words: cell proliferation, mitotic spindle, SCI1 interaction

NtCDKG;2, a Cyclin-Dependent Kinase (CDK) of *Nicotiana tabacum*, is an interaction partner of SCI1 (Stigma/style Cell-cycle Inhibitor 1), a cell proliferation regulator in the pistil of both tobacco andArabidopsis. Tobetter characterize NtCDKG;2, we screened a *N. tabacum* stigma/style cDNA library in the yeast two-hybrid (Y2H) system, to search for its interaction partners. We found partners whose homologues in Arabidopsis have functions in flowering, transcription control, RNA metabolism, and cell cycle. We found the proteins NtRPB7, NtRPB11, NtCYCLIN-L, NtLARP6a, NtRSZ21 and the spliceosome component NtU2A', which are involved in transcription and splicing. In flowering, there is potential involvement of NtFRIGIDA-LIKE 3 and NtBRG3, NtDNAJ3, NtMBD10, NtHB22, NtTCP13 and NtTCP15. Our most interesting findings are the proteins with roles in the regulation of the cell cycle, such as NtCDKF;1, NtF-BOX LIKE 17, NtPROTEIN PHOSPHATASE 2A (PP2A), NtCDC25-LIKE and NtUNICORN-LIKE. Sequence similarity analyses suggest that NtCDKG;2 is a homologue of human CDK11 (33% overall identity and 66% at the kinase domain). Human CDK11 is involved in transcription and splicing control, as well as in mitotic control by regulating centriole organization. However, until now there was no evidence of plant CDKG;2 involvement in cell cycle control. Our results provide the first evidence in this direction. Additionally, we found two Y2H interaction partners involved in the organization of the mitotic spindle: a κ-tubulin and a Ran-Binding Protein (RanBP1). In animals, alterations in RanBP1 levels caused defects in spindle polarization, delayed mitosis, inhibition of DNA replication, and chromatin decondensation at the telophase/interphase transition. Our results showed that NtRanBP1-RFP is cytoplasmic localized during interphase and moves to the nucleus at the beginning of cell division, when it is concentrated near the chromatin. Tobacco BY- 2 cells stably expressing GFP-NtCDKG; 2 have revealed its localization next to the mitotic spindle in metaphase and anaphase. The possibility of NtCDKG;2 participation in cell cycle as a mitotic spindle co-organizer is a novel function for plant CDKs and could explain how its interaction with SCI1 regulates cell proliferation in the pistil.

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ANALYSIS OF GENE EXPRESSION OF NADPH OXIDASE IN HIPSC-DERIVED CARDIOMYOCYTE FROM NORMOTENSIVE AND HYPERTENSIVE SUBJECTS

Sarafian, R.[1], Morato-Marques[2], M., Borsoi[2], J., Montezano, A.[2], Touyz, R.[2], Pereira, LV[1].

[1]University of São Paulo - Institute of Bioscience – Department of Genetics and Evolutionary Biology – São Paulo – Brazil [2]University of Glasgow – Institute of Cardiovascular and Medical Sciences – Glasgow - Scotland

*raquel.sarafian@gmail.com / lpereira@usp.br

Key-words: hiPSC; cardiomyocytes; NOXs

Hypertension is an important risk factor for various cardiovascular diseases, such as stroke, acute myocardial infarction and chronic kidney disease. It is a complex, multifactorial disease whose cellular mechanisms are not well understood. Recently several studies have focused on the role of oxidative stress regarding the development of hypertension. Increasing evidence over the last decades indicates an association between reactive oxygen species (ROS) and arterial hypertension. ROS are essential for cellular physiology, but in an unbalanced situation, an exacerbated production of ROS can damage cellular components and trigger pathological processes. Among the different ROS sources that are present in the heart, NADPH oxidases (NOXs) are particularly important because they are involved in many features of heart dysfunction. However, in human, even if redox regulation of some of the signalling proteins is well established, the role of NOXs in cardiac pathologies caused by hypertension and the relation to Nox-derived ROS and hypertension are misunderstood. In this scenario, human induced pluripotent stem cells (hiPSC), which are already recognized as a tool for modelling various diseases in vitro, can be a powerful tool for understanding cellular mechanisms in response to oxidative stress in hypertension – induced cardiomyocyte dysfunction. **Objectives:** in the present work, we propose to use hiPSC-derived cardiomyocytes from normotensive, responsive and resistant hypertensive patients as a source for the study of NOXs. **Methods:** We generated in a total of 9 cell lines of hiPSC from hypertensives and normotensives subjects; after this, we differentiation of these cells in cardiomyocytes for the quantitative PCR analysis of NOXs expression. **Results and Discussion:** Our results showed different expression profile between groups. Resistant hypertensive showed increased expression of NOX 1 and 2, which are described as increased in hypertension, and decrease of NOX 4, which has a protective role. **Conclusion:** The hypertensive group showed up is different from normotensive, which means that these cells can maintain the phenotype related to hypertension in vitro, even after the nuclear reprogramming. More interesting is the fact of existing two different groups of hypertensives (responsive to drugs and resistant to drugs), and these cells can keep it this difference, mainly because we see important changes related to NOXs expression between these groups after differentiation.

Fouding Agency: FAPESP, CNPq, BNDES

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THE FUNCTIONING OF THE TRANSCRIPTION FACTOR StuA OF THE DERMATOPHYTE *trichophyton rubrum*: INTERACTION WITH HOST MOLECULES

Larissa Daniela Ribeiro de Souza; Maíra Pompeu Martins; Tamires Aparecida Bitencourt; Pablo Rodrigo Sanches; Antônio Rossi; Nilce Maria Martinez-Rossi.

Department of Genetics, Ribeirão Preto Medical School, University of São Paulo, Ribeirão Preto, SP, Brazil.

larissaribeiro@usp.br

Keywords: *stuA*, transcription factor APSES, *Trichophyton rubrum*

Dermatophytes are filamentous fungi that use keratin as a source of nutrients during infection. Dermatophytoses are one of the most common fungal infections, with *Trichophyton rubrum* being the primary cause of dermatophytoses in human hosts. The dermatophytes secrete a variety of hydrolytic proteins, including several proteases, to produce nutrients from keratinized structures, which are important virulence factors in these organisms. Previous studies showed that *T. rubrum stuA* gene, a family member of the transcription factors APSES, is differentially expressed during the growth of this fungus in keratin. Several functions have been attributed to these transcription factors, such as regulation of cell development, virulence or pathogenicity, in several species of fungi. However, the role of this gene in *T. rubrum* remains poorly known. In this sense, we evaluated the expression profile of genes modulated by the *T. rubrum stuA* transcription factor (wild type X ΔStuA), during growth in minimal medium containing keratin and minimal medium containing glucose (MM) at culture times of 24, 48, and 96 hours using the RNA-Seq methodology. Our data show that StuA acts by inducing the expression of a more significant number of genes than repressing, regardless of carbon source and culture times, an effect observed as a function of *stuA* gene deletion. That is, the absence of this transcription factor decreased the number of genes induced in the different experimental conditions used. We highlight here the most modulated genes (up or down) that encode proteins components of the integral membrane, oxidation-reduction processes, and transferase activity. These results will help us to better understand the functionality and regulatory effects of the *T. rubrum stuA* gene during the host infection process.

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EFFECT OF DUAL-SPECIFICITY PROTEIN PHOSPHATASE DUSP11 MODULATION ON GEMCITABINE SENSITIVITY IN PANCREATIC DUCTAL ADENOCARCINOMA CELL LINES

Verena Silva Santos[1]; **Gabriela Maciel Vieira**[1]; **Pamela Viani de Andrade**[1]; **Vanessa da Silva Silveira**[1]

[1]Departamento de Genética, Universidade de São Paulo – Faculdade de Medicina de Ribeirão Preto, Ribeirão Preto, São Paulo, Brasil.

verenasilva@usp.br

Key-words: Pancreatic adenocarcinoma; gemcitabine; phosphatase.

The malignant and aggressiveness potential of pancreatic ductal adenocarcinoma cells (PDAC) are mainly promoted by the activation of the *KRAS* oncogene, which triggers signaling pathways essential for progression and chemoresistance, such as the MAPK pathways. MAPKs coordinate complex signaling networks and are precisely regulated by protein phosphatases. One of the major classes of phosphatases involved is represented by dual-specificity protein phosphatases (DUSPs) and their role in PDAC cells remains to be elucidated. Here we investigate the impact of DUSPs on chemoresistance and PDAC progression. The PDAC cell lines (MIA PaCa-2 and PANC-1) stably expressing the Cas9 endonuclease were submitted to CRISPR using an Edit- R library (GE Dharmacon) of crRNAs targeting all DUSP genes with 3 crRNAs per gene. Phenotypic analysis was conducted to address PDAC cells response to gemcitabine on a cytotoxic assay performed in a 4-day MTT experiment. Gene expression analysis of selected target has been addressed in cell lines and normal pancreatic tissue by quantitative RT-PCR. The knockout of the selected target gene was confirmed by Western blot and Sanger sequencing after single cell cloning of the MIA PaCa-2 cell line. To assess the impact of possible target modulation on gemcitabine sensitivity, *in vitro* assay as apoptosis, (by cell binding with Annexin V-APC), clonogenic assay, cell migration (Wound Healing) and cell invasion in matrigel were performed. Among the whole DUSP family members, CRISPR screening revealed some DUSPs involved in gemcitabine response modulation and pointed out to a possible target, *DUSP11*. *DUSP11* inhibition significantly increased gemcitabine responsiveness in both cell lines studied. Moreover, its inhibition combined to gemcitabine treatment reduced the cell proliferation rate by more than 30% in both cell lines compared to isolated gemcitabine treatment ($p \leq 0.04$). Analysis of the RT-qPCR gene expression revealed that *DUSP11* is upregulated in pancreatic adenocarcinoma cell lines in comparison to normal pancreatic tissue in mRNA analysis. *DUSP11* inhibition in MIA PaCa-2 cell line was able to *sensitize* cells to gemcitabine, increase cell death by apoptosis in about 30% (compared to treatment with gemcitabine in the wild type cell line) ($p=0.036$) and also impaired the colony forming capability ($p=0.037$). DUSP11 did not present any relevant effect on invasive and migratory capacity of PDAC cells. Taken together, these data suggest that protein phosphatase DUSP11 may play an important role in the process of PDAC tumor cell survival and gemcitabine response *in vitro*, which brings additional information to contribute to therapeutic target possibilities against this dismal disease.

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ANALYSIS OF HIF1A GENE EXPRESSION AND CHEMORESISTANCE OF ALDH+ CANCER STEM CELLS IN HYPOPHARYNX CELL LINE

Vilson Serafim Junior[1], Glaucia Maria Mendonça Fernandes[1], Ana Livia Silva Galbiatti-Dias[1], Maria Antonia dos Santos Bezerra[1], Caroline Izak Cuzziol[1]. Juliana Garcia de Oliveira-Cucolo, Rosa Sayoko Kawasaki-Oyama[1], José Victor Maniglia[2], Érika Cristina Pavarino[1], Eny Maria Goloni-Bertollo[1]

[1]Genetics and Molecular Biology Research Unit - UPGEM, FAMERP- São José do Rio Preto Medical School – Brazil. [2]Otorrinolaringology and Surgery Head and Neck Departament, FAMERP- São José do Rio Preto Medical School – Brazil.

juniorgrolla21@gmail.com and eny.goloni@famerp.br

Key-words: Cancer stem cells; Pharyngeal Cancer; HIF1κ gene

Pharyngeal cancer (PHC) occurs 5-10% of airways neoplasms and show a low survive that can be explained by presence of a subpopulation of cells named Cancer Stem Cell (CSC), that have self-renewal, migration, invasion and colony formation abilities, confering to the tumor proliferation and drug resistance. CSC can be identify using specific biomarkers such as ALDH1. Another factor related with drug resistance is the Hypoxia Inducible Factor (*HIF1α)* overexpression that can activate angiogenesis, to regulate apoptosis and cell proliferation. Paclitaxel alone or in combination with Cetuximab can be used in the treatment of PHC. The aim of this work were to identify and isolate CSC of Hypopharyngeal Cell Line (FADU) using ALDH1 biomarker; to evaluate the combinated effect of the Cetuximab and Paclitaxel treatment and to analyze *HIF1α* gene expression in both subpopulations. FADU cell line was cultivated using DMEM and sorted using ALDH1 biomarker by Fluorescent-activated cell sorting (FACSAria Fusion) technique in ALDH+ (CSC) and ALDH- (non-CSC). The colony forming assay was performed to confirm the stemness properties. After, the cells were treated using 0.06mg/ml of Cetuximab with 0.05mg/ml of Paclitaxel to 24, 48 and 72 hours and MTS assay was used to evaluate the cell proliferation. *HIF1α* gene expression was evaluated using Quantitative real time PCR (qPCR) by TaqMan® Assay (Thermo Fisher) and non-CSC were considered control. The amount of CSC and non-CSC obtained after cell sorting was 57.6% and 8.1%, respectively. The colony forming assay showed that CSCs present high colony forming hability when compared with non-CSC (mean = 19 and 13.3 colonies respectively), but these results had not statistical significance (p=0.0581). After treatment, MTS assay showed that with 24 hours the CSC proliferation was 51% and non-CSC was 53%. After 48 hours CSC proliferation was 56% and non-CSC was 43%. After 72 hours, CSC proliferation was 72% and non-CSC was 63% wich presented cell proliferation statistical significance (p=0.001). The *HIF1α* gene was slightly increased in CSC compared to non-CSC (RQ=1.026). The cell sorting using only ALDH1 was useful to sort two cell subpopulations. Howhever the number of CSC was greater than reviews studies. Therefore the association with others biomakers may be more efficient to identify CSC. Cetuximab with Paclitaxel treatment was most efficient in 48 and 72 hours. *HIF1α* gene expression was higher in CSC compared to non-CSC, howhever the results not shows statistical significance. Therefore, more studies in primary tumor are needed to confirm our results.

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Construction of a genetically modified lineage of *Zymomonas mobilis* for the production long chain alcohols

Vinícius Manganaro Farnézio[1]; Geovane Felippe Alves[1], Juliana Fatima dos Santos Silva[1], Valquíria Campos Alencar[2], Luiz Roberto Nunes[1*]

[1]Universidade Federal do ABC, SP, Brazil. [2]Universidade de Mogi das Cruzes, SP, Brasil.

*Luiz.nunes@ufabc.edu.br

Key-words: *Zymomonas mobilis*; long chain alcohol; biotechnology.

The gram-negative bacterium *Zymomonas mobilis* presents several characteristics that make it attractive to the industrial production of bioethanol. For example, *Z. mobilis* is a facultative anaerobic organism, which can be grown under both aerobic and anaerobic conditions, displays a higher glucose-to-ethanol conversion rate and an increased tolerance to ethanol, when compared to the yeast *Saccharomyces cerevisiae*. However, the possibility of using *Z. mobilis* to produce alternative biofuels is yet to be fully explored. This work describes experiments aiming at the development of a genetically modified *Z. mobilis* strain, capable of producing long chain alcohols (LCA). This strategy (originally developed in recombinant *E. coli*) involves incorporating only two new genes to the genome of *Z. mobilis*: the first gene (*kivd*, from *Lactococcus lactis*) encodes a broad-spectrum 2-ketoacid decarboxylase, capable of converting 2-ketoacids (produced as intermediates, during the biosynthesis of amino acids) into their respective aldehydes. The second gene (*adh2*, from *S. cerevisiae*) encodes a broad-spectrum alcohol dehydrogenase, which converts these aldehydes to their corresponding alcohols, including a series of LCAs, such as isobutanol, 1-butanol, 2-methyl-1-butanol, 3-methyl-1-butanol e 2-phenylethanol, for example. Thus, the above mentioned genes have been isolated by PCR and cloned in plasmid pBBR1MCS-2 (a broad-range plasmid that can replicate both in *E. coli* and *Z. mobilis*), along with *Z. mobilis*-specific promoters, ribosomal-binding sites (RPS) and 3´transcriptional termination sequences, using the *Gibson Assembly Method.* This recombinant plasmid was recovered from transformed *E. coli* and used to transform competent *Z. mobilis* cells through electroporation. Expression of these transgenes is currently being evaluated in this transformed *Z. mobilis* lineage, which will next be tested for its capacity to produce different types of LCAs, using Nuclear Magnetic Resonance (NMR).

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Identification of variants related to increased susceptibility to essential hypertension in large pedigrees from the African- derived *quilombo* populations in Vale do Ribeira (SP, Brazil)

Vinícius Magalhães Borges[1]; Lilian Kimura[1]; Regina Célia Mingroni-Netto[1]

[1]Centro de Pesquisas sobre o Genoma Humano e Células-tronco (CEGH-CEL), Departamento de Genética e Biologia Evolutiva, IBUSP, São Paulo - SP.

vinyborges@usp.br

Keywords: hypertension; *quilombo* populations; whole-exome sequencing

Essential Hypertension (EH) is the major risk factor for cardiovascular diseases (CVD) and it is the cause of 9.4 million deaths per year worldwide. The condition is characterized by high Systolic Blood Pressure (SBP) and/or high Diastolic Blood Pressure (DBP). In Brazil, reference values were established as SBP ≥ 140 and/or DBP ≥ 90 mmHg and the latest data indicated that 24.3% of the population has hypertension. EH has heritability rates for global population estimated between 15-60%. Specifically, values of 36.1% (SBP) and 42.92% (DBP) were estimated in the case of Brazilian partially isolated populations of African ancestry (*quilombo* populations). The objective of this study is identify candidate variants and genes to explain increased susceptibility to essential hypertension in the African-derived admixed remnants of *quilombos* from 'Vale do Ribeira' region (Southeastern Brazil). To accomplish that, we selected for study two large pedigrees: one from Abobral population (pedigree 1) with 47 affected individual and the second (pedigree 2) from Galvão and São Pedro populations with 40 affected individuals. We performed Whole-Exome Sequencing (WES) of samples from 17 affected individuals (10 from pedigree 1 and 7 from pedigree 2) and 7 unaffected ones, belonging to the two large pedigrees in which EH is segregating. Assuming the "common disease-common variant" hypothesis, the variants list was filtered according to two strategies: (a) considering common variants between all affected individuals in each pedigree, with MAF ≤ 0.20 and (b) considering genes in common containing variants (with MAF ≤ 0.20) between all the affected individuals in each pedigree. As a result, were found 14 variants in common in the first pedigree and 7 in the second pedigree. We found 213 genes containing variants in common in the first pedigree and 141 in the second pedigree. By crossing the findings and comparing to databases such as GWAS Catalogue, OMIM, Genecards, VarElect and NCBI, and also comparing to UCSC genome browser, it was possible to identify six genes which are the most likely to be related to the phenotype: *CPY11B2, SCNN1A*, *ADRA2C* and *WDR72* for Pedigree 1, and *ARHGEF17* and *ZCCHC2* for Pedigree 2. All genes are protein coding, two of them with roles in the aldosterone pathway (*CPY11B2* and *SCNN1A),* one with role in the presynaptic control of transmitter release from sympathetic nerves in the heart (*ADRA2C*), one with role in sodium and potassium levels (*WDR72*) and two related to blood pressure regulation (*ARHGEF17* and *ZCCHC2*).Our findings suggest a role of variants related to these genes in the elevated blood pressure observed in the *quilombo* populations.

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EVALUATING THE BIOLOGICAL EFFECTS OF THE *LEISHMANIA MAJOR*'S TELOMERASE TERT KNOCK OUT

Whisnayder M. Gentil[1], Maria Isabel N. Cano[1]

[1]Genetics Dept., Biosciences Institute, São Paulo State University (UNESP), Botucatu, SP, Brazil

*whisnayder@gmail.com

Keywords: *L. major;* telomerase; CRISPR/Cas9

Telomeres are important nucleoprotein structures that maintain genome homeostasis, by protecting the end of the chromosomes from DNA damage and fusions. In most eukaryotes, including *Leishmania* sp., telomere length is maintained by the action of telomerase, minimally composed by TERT (Telomerase Reverse Transcriptase) and an lncRNA, TER (Telomerase RNA). Among the *Leishmania* genus are the causative agents of leishmanisis, a neglected tropical disease to which there is no effective treatment and control. Our study aims to understand the real importance of telomerase in parasite survival. To reach this goal we induced the knock-out of the *L. major* telomerase TERT component using a CRISPR-Cas9 technology developed by Beneke et al., (2017). We were able to induce *L. major* TERT double knockout and to isolate TERT KO parasite clones using double drug selection. These clones had their genotypes confirmed by PCR, Southern-blot, and DNA sequencing. The absence of TERT expression was checked using RT-PCR and western blot comparing three TERT KO clones with wild type parasites. Growth curves of TERT KO parasites compared to wild type showed that the absence of TERT does not seems to alter cell viability and parasites proliferation capacity after short term cultivation. Also, as previously shown for *Trypanasoma brucei*, no visible morphological changes were detected in *L. major* TERT KO parasites after few population doublings. We are now checking the impact of telomerase loss in parasite telomere length maintenance, metacyclogenesis and infectivity capacity. We intend to repeat these experiments after long term parasite cultivation and check if in the absence of telomerase, parasites can use alternative modes to maintain telomere length in order to survive.

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ALTERATIONS IN GUT MICROBIOTA VERIFIED IN AN EXPERIMENTAL MURINE MODEL OF CANCER CACHEXIA

Yara N. L. F. de Maria[1]; Fabiano B. Menegidio[1]; David Aciole Barbosa[1]; Rafael dos S. Gonçalves; Lucas M. Carvalho[1]; Kaltinaitis, B. N. H. dos Santos[1]; Miguel L. Batista Jr.[1]; Regina Costa de Oliveira[1]; Luiz R. Nunes[2]; Daniela Leite Jabes[1*]

[1]Universidade de Mogi das Cruzes. [2]Universidade Federal do ABC.

*danielajabes@umc.br

Key-words: Microbiome; Cachexia; Cancer

Cachexia is a complex wasting syndrome, associated with a marked detrimental effect upon life quality and survival in patients with cancer and other chronic diseases. Cancer- induced cachexia (CC) is characterized by severe loss of fat and skeletal muscle mass, often accompanied by inflammation, and cannot be reversed by conventional nutritional support. The human gut is a robust ecosystem composed of a dynamic microbial community, which has important roles in the acquisition of energy from feedstock and in regulating host physiology through immune modulation. Alterations in the population of gut bacteria (dysbioses) have been shown to occur during the development of many prevalent disorders such as obesity, diabetes and cancer-induced cachexia. However, the studies involving cachectic animals have not been able to differentiate the dysbiotic effects induced by the tumor from those directly associated with de development of cachexia *per se*, probably due to the employment of highly aggressive tumors to promote CC. This work describes a thorough microbiome analysis performed with a group of mice that were submitted to CC with the use of Lewis Lung Carcinoma (LLC) tumor cells, which induce the development of moderately aggressive cancers, allowing the development of both cachectic and non-cachectic animals among the tumor-bearing mice population. Thus, two groups of male C57Bl/6 mice were injected with: (i) LLC cells and (ii) vehicle-saline, for controls. Body weight of these animals was assessed daily, and fecal pellets were collected at days 0 and 28 (when CC was identified in a series of LLC-inoculated animals). DNA was extracted from these stool samples and used for construction of amplicon libraries (encompassing the V4-V5 16S rRNA gene) for the characterization of the bacterial populations present in the gut of: (i) Control animals, at day 0 (C0); Cachectic animals, at day 29 (CQ); non-cachectic, tumor-bearing mice, at day 29 (TB) and healthy animals, inoculated with saline, at day 29 (SC). Libraries were sequenced in an Illumina MiSeq NGS sequencer, generating ~500,000 reads per library. Microbiome analyses, conducted with a 16S rRNA pipeline specifically developed by our group, effectively differentiated the four groups of animals mentioned above, allowing identification of a microbiome dysbiosis signature specifically associated with the development of cachexia (group CQ), which differs from the dysbiosis signature associated only with the presence of tumor (group TB).

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ANALYSIS OF THE EARLY DEVELOPMENTAL ACTIVATION OF *APIS MELLIFERA*

Franciene Rabiço Oliveira[1*]; Zilá Luz Paulino Simões[2]

[1]Faculdade de Medicina de Ribeirão Preto – Universidade de São Paulo – USP, Departamento de Genética, Ribeirão Preto, São Paulo, Brasil. [2]Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto – Universidade de São Paulo – USP, Departamento de Biologia, Ribeirão Preto, São Paulo, Brasil

*franciene.oliveira@usp.br

Keywords: Development; embryo*; Apis mellifera*

The onset of embryonic development is unique and has its own characteristics, where each component is programmed to act in a precisely defined place and time. In its initial phase, the embryo does not present zygotic transcription, being maintained by molecular components deposited by the mother during oogenesis. The transcriptional activation of the zygotic genome is coordinated with the degradation of the maternally deposited mRNAs resulting in a shift in the transcriptional profile of the embryo. Thus, during this maternal-to-zygotic transition (MZT), the zygote establishes control of its future development. The degradation of maternal products occurs mainly by the joint action of proteins Smaug and Zelda. In *Drosophila melanogaster*, zygotic transcription gradually increases from cycle 2 through cycle 13. In order to verify the initial zygote expression in *Apis mellifera* embryos was performed an analysis of 5 genes, selected from RNAseq libraries of embryos with 0, 2 and 6 hours. The post- and pre-splicing transcripts of the zelda, smaug, tropinin C type IIb and takeout genes were analyzed by qPCR in the first 6 hours of development. The results obtained point to a synthesis of new transcripts in the first hour of embryo development. To complement the findings and verify the mitotic cycles of that period, the nuclei of diploid embryos with 1 hour of development after the posture were marked with DAPI, in order to verify the mitotic cycles of that period. We observed that at 1 hour of development, the embryos have 3 cleavage nuclei, indicating that they have passed for at least 2 mitotic cycles and the division is possibly asynchronous. These findings strongly indicate that there may be zygotic gene expression in the first mitotic cycles of the *A. mellifera* embryo and will help to clarify the scenario that occurs at the beginning of embryogenesis and open the field to other studies in this area.

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X-LINKED MYOTUBULAR MYOPATHY: GENETIC DIAGNOSIS AND HIGH FREQUENCY OF MANIFESTING CARRIERS IN THE RECESSIVE FORM

Lucas Santos e Souza[1]; Camila de Freitas Almeida[1]; Guilherme Lopes Yamamoto[1]; Leonardo Galleni Leão da Silva[1]; Isabela Pessa Anequini[1]; Silvana Amanda do Carmo[1]; Rita de Cassia Mingroni Pavanello[1]; Juliana Gurgel-Giannetti[2]; Paulo Alberto Otto[3]; Edmar Zanoteli[4]; Mariz Vainzof[1]

[1]Centro de Pesquisa sobre o Genoma Humano e Células-Tronco, Universidade de São Paulo, São Paulo, Brazil. [2]Departamento de Pediatria, Faculdade de Medicina, Universidade Federal de Minas Gerais, Minais Gerais, Brazil. [3]Departamento de Genética e Biologia Evolutiva, Instituto de Biociências, Universidade de São Paulo, Brazil. [4]Departamento de Neurologia, Faculdade de Medicina, Universidade de São Paulo, São Paulo, Brazil.

lucassz@usp.br

Key-words: Myotubular myopathy; manifesting carriers; next-generation sequencing

Myotubular myopathy is a rare genetic disease which affects skeletal and respiratory muscles and it is caused by mutations in the *MTM1* gene. The phenotype is classified as a recessive X-linked inheritance and manifests in living born males with an estimated incidence of 1/50,000. Myotubular myopathy is characteristic and very severe, including hypotonia and generalized muscle weakness since birth. Most patients die in the first year of life due to respiratory failure. However, many cases with a more benign phenotype have recently been identified through molecular analysis. Women carrying the mutations are usually asymptomatic, but many cases of symptomatic heterozygous females have been reported, as compared with the lower frequency of manifesting carriers in other X-linked recessive diseases. Patients with structural congenital myopathies have been studied for more than twenty years at The Human Genome and Stem Cell Research Center (HUG-CELL) in Sao Paulo. Here, we have performed the molecular diagnosis of Brazilian families with myotubular myopathy, previously diagnosed by clinical and muscle biopsy findings, identified the female carriers of the families, and clinically evaluated their phenotype. Mutations in the *MTM1* gene were identified in patients from twelve different families, using a custom Next Generation Sequencing panel for neuromuscular disorders. Predicted pathogenic variants were found in all our male patients, some of which show a milder phenotype and are still alive at the age of three years or older. Four out of eleven identified mutations were novel. In two families, we identified 4/8 and 2/4 female carriers presenting some degree of clinical manifestation. Adding our manifesting carriers' cases to others presented in the international literature, we estimated the penetrance rate of the disease in about 30% in females, which is compatible with a pattern of incomplete penetrance and could explain the higher frequency of manifesting women. Skewed X-chromosome inactivation analysis pointed out to random X-chromosome inactivation. A whole exome study was done to try to identify possible modifier genes that could explain the observed clinical variability. We could identify a haplotype of variants, composed by genes from the KIR cluster located in the 19 chromosome. As it was present in the normal women, it became considered as a protective haplotype. The analysis of additional 95 unrelated exomes showed that the protective haplotype may have a frequency of about 30% in the Brazilian population, being compatible with a more frequent variable acting as a possible modifier. Further studies are currently taking place to strengthen the KIR haplotype modifying effect hypothesis.

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Production of L-asparaginase using genetically modified microorganism

Juliano Sales Mendes[1*]; Gabriela Barbosa de Paiva[1]; Ana Paula Mora Tavares[2]; Danielle Biscaro Pedrolli[1]; Valéria de Carvalho Santos Ebinuma[1]

[1]Universidade Estadual Paulista "Júlio de Mesquita Filho" (UNESP), Faculdade de Ciências Farmacêuticas de Araraquara, São Paulo, Brazil. [2]CICECO, Department of Chemistry, University of Aveiro, 3810-193, Aveiro, Portugal.

*juliano16@gmail.com

Key-words: L-asparaginase; *Aliivibrio fischeri*; *Bacillus subtilis*

L-asparaginase (E.C.3.5.1.1) (ASNase) is the enzyme that catalyzes the hydrolysis reaction of the amino acid side chain of the amino acid L-asparagine, releasing aspartic acid and ammonia, being widely used as anti-neoplastic agent. This enzyme can be produced by a number of microorganisms, however, there is a need for new producing sources with higher yields and lower adverse effects. Thus, the objective of this project is to evaluate the production of L-asparaginase by recombinant microorganism through new process and product development solutions, namely in the optimization of the production process and in the use of different microorganisms with greater production capacity. Initially, screening of ASNase production will be done with different heterologous microbial expression systems on a rotary shaker. In this stage, we performed a cloning of L-asparaginase from *Aliivibrio fischeri* into specific plasmid to expression in *Bacillus subtilis*. Afterwards, a rotating shaker study will be performed using different sources and concentrations of nitrogen, glucose concentration (carbon source), initial pH and culture temperature. The development of new L-asparaginase production lines will lead to a more effective, more effective therapeutic with reduced side effects. In the area of health, this project has a strong component of new biotechnologies and advanced solutions for the production of biopharmaceuticals to be used as therapeutic agents in various types of cancer, specifically in leukemia.

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IDENTIFICATION OF MICROSSATELLITE MARKERS CORRELATED TO ANTIOXIDANT ACTIVITY IN *Lactuca sativa L.*

Bárbara Gonçalves Bastos Silva[1*], Cíntia Raquel de Freitas[1], Joana D'Arc Mendes Vieira[2], Alexandre Henrique de Oliveira Mendes[1], Matheus de Souza Gomes[1], Marcos de Souza Gomes[1], Terezinha Aparecida Teixeira[1], Luiz Antônio Augusto Gomes[1]

[1]Instituto de Biotecnologia, Universidade Federal de Uberlândia, Campus de Patos de Minas, Patos de Minas, Minas Gerais, Brazil. [2]Departamento de Agricultura, Universidade Federal de Lavras, Lavras, Minas Gerais, Brasil

*barb@ufu.br

Keywords: Lettuce; antioxidant activity; microssatellite markers

Lettuce (*Lactuca sativa* L.) can be considered the main leafy vegetable consumed in Brazil. It is present in the daily menu of the Brazilian, preferably in the form of salad and its consumption presents great nutritional importance. Greenery is an abundant source of nutrients. It is rich in minerals such as calcium and iron; vitamins, such as A, B1, B2 and C, in addition to other phytochemical compounds. Among the phytochemicals present in lettuce, we highlight carotenoids (provitamin A) and anthocyanins. These substances have antioxidant action and act to prevent aging and control the oxidative stress related to the action of free radicals. The present work had the objective of studying the genetic divergence between 12 lettuce cultivars (Gabriela, Luiza, Model, Optima, Raider Plus, Red Star, Salad Bowl, Silvana, Sophia, Thaís, Verônica and Darkland) in 10 pairs of microsatellite primers and to associate these results to the evaluation of the antioxidant activity of each cultivar performed by the DPPH • and ABTS + • free radical capture methods, reduction of the Phosphomolybdenum complex and quantification of the phenolic compounds by means of the *Folin-Ciocalteau* reagent. The methodology consisted of evaluating the phenotypic characteristics of the cultivars, by verifying the color of each cultivar and quantifying the antioxidant activity; and genotyping of samples from 10 pairs of SSR primers. The antioxidant activity values obtained by all methods were correlated with the staining of the cultivars through Pearson's Correlation and Mantel's Test. In order to evaluate the dissimilarity between the cultivars, a distance matrix of Gower was generated. Microsatellite loci were compared with the levels of antioxidant activity obtained for each cultivar using the ABTS + •, DPPH •, phosphomolybdenum complex reduction and total phenol content; as well as the coloring of the plants. The results showed that the cultivar Red Star (dark purple coloring) showed higher% AA in most of the tests performed. The loci KSL-37, KSL-137, KSL-245 showed a positive and statistically significant correlation with the "dark purple" cultivars, Red Star and Gabriela. SML-022 was positively related to the results obtained by the phosphomolybdenum complex reduction test. Thus, these markers have been shown to be good indicators of antioxidant activity, and may be used in future studies that seek to associate this characteristic with lettuce cultivars.

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IT IS NEVER TOO LATE FOR A CHANGE – DNA METHYLATION IS NOT A DRIVER OF BEHAVIORAL (RE)PROGRAMMING IN HONEYBEE WORKERS

Carlos Antônio Mendes Cardoso Júnior[1,2], Boris Yagound[2], Mário Cervoni[1], Lucas Tavares[1], Elaine Zayas da Silva[1], Isobel Ronai[2,3], Emily Remnant[2], Benjamin Oldroyd[2], Klaus Hartfelder[1]

[1]Cell and Molecular Biology and Pathogenic Bioagents, Ribeirão Preto School of Medicine, University of São Paulo, Ribeirão Preto, SP, Brazil. [2]School of Life and Environmental Sciences, University of Sydney, Sydney, Australia. [3]Ecology, Evolution, and Environmental Biology, Columbia University, New York, USA.

Keywords: honey bee, gene body methylation, *Apis mellifera*

DNA methylation (DNAm) is an important regulator of gene expression, and has been considered a candidate mechanism for the regulation of behavioural plasticity in social insects, especially so the honeybee. However, recent studies have shown that DNAm patterns are typically sequence-specific, and are therefore not likely to directly regulate behaviours. To address this paradox, we manipulated the social environment within honeybee colonies and analyzed the workers' methylomes in two tissues at single base- pair resolution. We found no differences in the methylomes of workers living in queenright *versus* queenless colonies. This result is surprising, because the queen presence/absence greatly alters gene expression and the behaviour of workers. Furthermore, we found only 9 differentially methylated regions (DMRs) between the brain and ovaries, suggesting that DNAm is also not associated with differential gene expression between tissues. In contrast, we found thousands of DMRs for bees from genotypically different colonies. We further confirmed these results by amplicon sequencing on a MiSeq platform. Curiously, social environment, which did not alter worker methylomes, had a significant effect on DNA methyltransferase genes (*Dnmts*) in both tissues. Hence, we studied the expression of *Dnmt3* at the most important transition in a worker's life, the transition from nurse to forager. We found that *Dnmt3*, a gene that codes for an enzyme that promotes *de novo* DNAm, is upregulated in foragers compared to nurses. Surprisingly though, immunofluorescence confocal images revealed the DNMT3 protein is predominantly in the cytoplasm of both life cycle stages. This was confirmed using electron microscopy immunogold labelling, which showed that DNMT3 localizes inside lipid vesicles of fat body cells. Taken together, our results suggest that DNAm is not mediated by environmental change and does not regulate tissue-specific gene expression in honeybees. Furthermore, it is unlikely that DNAm is directly affected by *Dnmt3* expression, considering the subcellular localization of its protein. We therefore consider that many of the previous results proposing a major role of DNAm in honeybee biology need to be reinterpreted and we put forward new insights into the evolution and function of epigenetic systems in this social insect.

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THE ROLE OF HOTAIR IN THE ACTIVATION OF EPITHELIAL MESENCHYMAL TRANSITION GENETIC PROGRAM IN MELANOMA CELLS

Carlos Alberto Oliveira de Biagi Junior[1,2,3]; Ricardo Perecin Nociti[2]; Adamo Davi Diogenes Siena[1,2]; Josane de Freitas Sousa[6]; Floris Foijer[7]; Wilson Araújo Silva Jr[1,2,3,4,5]

[1]Department of Genetics-Ribeirão Preto Medical School, University of São Paulo, Ribeirão Preto, Brazil. [2]Laboratory of Molecular Genetics and Bioinformatics (LGMB), University of São Paulo, Ribeirão Preto, SP, Brazil. [3]National Institute of Science and Technology in Stem Cell and Cell Therapy, Center for Cell-based Therapy-CEPID/FAPESP, Ribeirão Preto, Brazil. [4]Center for Medical Genomics, Ribeirão Preto Medical School, University of São Paulo, Ribeirão Preto, SP, Brazil. [5]Center for Integrative System Biology-CISBi-NAP/USP, University of São Paulo, Ribeirão Preto, Brazil. [6]Laboratory of Evolution and Development, Institute of Biological Sciences, Federal University of Pará (ICB-UFPA), Belem, Brazil. [7]European Research Institute for the Biology of Ageing, University of Groningen, University Medical Center Groningen, Groningen 9713 AV, the Netherlands.

*biagi@usp.br

Key-words: single-cell RNA-Seq; melanoma; EMT

Studies show that HOTAIR plays a critical role because it acts as a trigger that activates the Epithelial-Mesenchymal Transition (EMT) and participates in the maintenance of stem cells in cancer cell lines. Overexpression of HOTAIR promotes changes in the gene expression profile of EMT and genes related to stemness. In addition, HOTAIR interacts with the PRC2 and LSD1/coREST complexes through their 5' and 3' binding domains, respectively. HOTAIR knockdown decreases the occupancy of the LSD1 complex and consequently a loss of H3K27 and the gain of H3K4me2/3 in the promoters closest to the region of the SNAI1 gene and, finally, the methylation of H3K27 and the demethylation H3K4me2/3 ends up influencing the epigenetic silencing of the CDH1 gene promoting the activation of the EMT pathway. These epigenetic changes result in the modification and alteration of the profile of a series of genes of positive or negative regulation of proliferation, invasion, apoptosis, and migration of cancer cells. In melanoma, HOTAIR leads to overexpression in metastatic tissue and promotes cell motility and invasion. Based on this, the elucidation of HOTAIR profile in scRNA-Seq view is crucial to clinical trials and diagnosis. In this project, we used the scRNA-Seq data provided by Tirosh, Itay, et al. Seurat package (v2.7) in R was used to perform the identification of a subpopulation of malignant cells that has an overexpression of HOTAIR. Checking the profile of the genes related to EMT pathway and stemness, it was able to identify that some genes like BMP1, CAMK2N1, CTNNA1, FN1, ICAM1, OCLN, SMAD5, SNAI1, TCF3, TWIST1, and VIM have a higher expression values than BMP7, CD24, ERBB3, GSK3B, KLF4, NANOG, POU5F1, SOX10, and TGFBR2. This demonstrates that the heterogeneity for the bulk of cells (RNA-Seq) changes when using single-cell data. Using AUCell package in R was possible to enrich the cells for EMT and stemness pathways and identify the same core of cells that has an overexpression of HOTAIR. Besides, we identified by coexpression the lncRNA TRHDE-AS1 and five coding genes (MAGEA4, NGEF, PAGE2, TERT, and C2orf82), which has the expression profile similar to HOTAIR. Finally, a pseudo time analysis using STREAM pipeline revealed distinct differentiation trajectories for EMT and stemness genes among melanoma tumors. In conclusion, our analysis pinpointed interesting expression patterns of HOTAIR and associated genes in melanoma cells and tumors and allowed us to identify TRHDE-AS1 and five coding genes as a potential EMT signature in malignant cells.

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QUANTIFICATION OF FETAL DNA BY INSERTION/DELETION MARKERS IN THE PLASMA OF PARTURIENTS

Aline Silva Paula Brazorotto[1], Maria Luisa de Barros Rodrigues[1], Marcela Dambrowski dos Santos[1], Cláudia Emília Vieira Wiezel[1], Aguinaldo Luiz Simões[1]

[1]Departamento de Genética, Universidade de São Paulo – Faculdade de Medicina de Ribeirão Preto, Ribeirão Preto, São Paulo, Brasil.

line.spaula@usp.br

Key-words: InDel; cffDNA; insertion-specific primer

Cell-free fetal DNA (cffDNA) in the maternal circulation is a source of fetal genetic material for noninvasive prenatal diagnosis. In some situations such as fetal aneuploidies and preeclampsia, it is not enough to detect the presence or absence of a fetus allele, making it necessary to accurately measure the concentration of circulating fetal DNA in maternal plasma. InDel markers are appropriate in this context because they allow analyses with short amplicons and amplification in samples with degraded or fragmented DNA. The present project aimed to demonstrate that primers complementary to exclusive fetal insertion sequences, together with flanking primers, allow the quantification of free fetal DNA by Real-Time PCR. We determined the laboratory conditions for Real-Time PCR quantification of three previously described markers (rs151001596, rs1610941 and rs148112183). All of them showed adequate efficiency and specificity for fetal DNA analysis. We measured the concentration of free fetal DNA in the plasma of 20 informative parturients, that is, those with *del/del* as maternal genotype and *in/del* as the fetal. The minimum fetal DNA concentration detected in maternal plasma was 0.086ng/µL. Free total DNA (maternal and fetal) was measured with primers for κ-actin gene sequences. The fetal DNA fraction ranged from 5.05% to 37.85% of total circulating plasma DNA, with average of 14.75% and median of 11.15%. Based on the same principle adopted in the quantification of fetal DNA with specific Y-specific sequences, in which primers complementary to only fetal sequences were used in order to avoid competition with maternal DNA, a simple and efficient method was demonstrated for quantification of cffDNA with markers InDels, applicable in all pregnancies, regardless of fetal sex. The methodology described here may be used in situations of non-invasive prenatal diagnosis or in gestational complications that require quantitative analysis of fetal DNA, without risks to fetus and mother.

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A SEX-RELATED PRELIMINARY ANALYSIS OF 90 CIRCULATING lncRNA LEVELS IN HIGH-PERFORMANCE ENDURANCE RUNNING ATHLETES

Caleb Santos[1,2]; Rodolfo Velasque[2]; Dailson Paulucio[2]; Rafael Xavier[2]; Camila Geyer[1]; Renato Alvarenga[2]; Fernando Pompeu[2]

Instituto de Biologia do Exército – IBEx[1]. Laboratório de Biometria – LADEBIO

calebguedes@gmail.com

Palavras-chave: lncRNA profile, Exercise Physiology, Circulating ncRNAs

A class of non-coding RNAs (ncRNA), the long non-coding RNAs (lncRNAs) are the RNA family with the highest functional diversity, ranging from 200 bp up to 100 kb. They plays an important role in up/down-regulation of genes involved in biological processes related to human physiology. Moreover they interact with other macromolecules such as proteins by direct structural recognition; this gives them a great functional diversity. Recently, interesting evidences of physiological roles of lncRNAs have been reported. However, data related to physical exercise as well as intervention strategies in humans are extremely limited. So, the aim of the study was to analyze the lncRNA profile differences of high-performance athletes from both sexes, before and after maximum aerobic exercise. Seven men (27.7 ± 6.6 years) and six women (28.2 ± 5.0 years) were selected. All of them were elite high- performance endurance running athletes with (140 ± 30) and (114 ± 26) km of weekly training volume, respectively. The subjects underwent a treadmill maximum endurance test to obtain the maximum oxygen uptake (VO_2máx). A peripheral blood extraction was realized before and after the test. Ninety circulating lncRNA extracted from plasma were tested using human lncProfiler qPCR Assay Kit (SBI) in a StepOnePlus Real- Time PCR System (Thermofisher). Every lncRNA was considered detectable if they appeared in at least 3 subjects. The VO_2máx of subjects were (76.2 ± 2.5) $mL.Kg^{-1}.min^{-1}$ in men and (65.3 ± 4.2) $mL.Kg^{-1}.min^{-1}$ in women. Our preliminary qualitative results showed GAS5 lncRNA detected in all women and only after exercise in some men. Curiously GAS5 has a strong connection with the suppression of breast cancer. Conversely, NEAT1 lncRNA was detected only in men. This lncRNA is crucial to nuclear paraspeckles formation. They are dynamic nuclear structures with reported roles in RNA transcription/processing. RoR, VLDLR and Y-RNA-1 lncRNA were detected in all groups independently of exercise moment. Moreover, 21A lncRNA regardless of sex was detected only after exercise in elite athletes and could be related to acute exercise response. The lncRNAs detected in plasma of elite athletes was slightly different in men and women using a preliminary qualitative analysis. A quantitative and functional broader analysis could better elucidate sex-specific lncRNA roles and contribute to precision medicine and fitness enhancement approaches based on personalized exercise training.

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ESTABLISHMENT OF A CELLULAR MODEL OF HEPATIC STEATOSIS AND MECHANISTIC EFFECTS OF MICRORNA 1914-5P IN LIPID METABOLISM

Thaís Porto Barbosa[1*]; Letícia Ferreira Ramos[2]; Karen Cristiane Martinez de Moraes[1,2,3]

[1]Pós-Graduação em Biotecnologia, UNESP - Instituto de Química (IQ) – Campus Araraquara. [2]Pós-Graduação em Biologia Celular e Molecular, UNESP - Instituto de Biociências (IB) – Campus Rio Claro. [3]Departamento de Biologia, Universidade do Estado de São Paulo (UNESP) – Campus Rio Claro, São Paulo - Brazil.

*thaisp.barbosa74@gmail.com

Key words: hepatic steatosis; lipid metabolism; microRNA

Hepatic steatosis is a metabolic dysfunction characterized by fat accumulation in the hepatocytes and despite been a physiological reversible condition, it may progress to steatohepatitis, cirrhosis and even hepatocellular carcinoma. Major changes in lipid metabolism, as increasing rates of cellular absorption or synthesis and decreasing rates of fat oxidation can support the development of hepatic steatosis. In this context, several studies have been demonstrated central roles of microRNAs in the development or controlling of hepatic pathologies. More recently, our laboratory demonstrated the effect of microRNA 1914-5p in lipid metabolism. Thus, this study aims to establish a pro- steatotic model to investigate the functional actions of the microRNA 1914-5p in that metabolism. For that, the human hepatic stellate cells, LX-2, and one of the three other different hepatocytes cellular lineages, HepG2, C3A, and Huh-7, were cultivated independently or in cocultures(1 LX2: 2 Hepatocytes). Those cells were stimulated with different concentrations of the fatty acids (FA) palmitic and oleic acids for inducing pro- steatosis in cell culture and cellular viability were accessed by the classical analysis of MTT using 2 x 10 4 cells. The results demonstrated that the presence of FA in 400 µM concentration did not affect the viability of the cellular cultures, which was mandatory for the futures analysis. Next, cells were incubated with 400 µM of FA mixture to evaluate the triglycerides and cholesterol productions by the different cellular groups, aiming to select the better cellular model to accomplish our goals. The results pointed higher levels of triglycerides and cholesterol production by the co-culture LX-2: Huh-7, when compared to LX-2: HepG2, after incubation of cells with 400 µM FAs. However, different studies demonstrated that carbohydrates control the strong lipid metabolism of Huh-7 lineage, which conducts to a different metabolic cycle from our goals. Therefore, based on literature directions, the cellular model selected for our analyses was the LX-2: HepG2 co-culture. Next, the co-cultures were transfected with different concentrations of miRNA 1914-5p mimic or inhibitor (mirVana™, Thermo Fisher Scientific™) to evaluate the best transfection condition using fluorescence microscopy and gene expression analyses. The results pointed the concentration of 40 mM of either miRNA 1914-5p mimic or inhibitor to be used in the transfections of cells incubated with 400 µM FA. Subsequently, fluorescence microscopy analyses using BODIPY TM 558/ 568 fluorophore were used for visualizing the lipid droplets in the investigated cellular groups. Currently, the effect of the miR-1914-5p in lipid metabolism has been investigating under gas chromatography-mass spectrometry (GC-MS) analyses, and Fourier Transformed – Infra Red (FT-IR) spectroscopy which allow quantitative and qualitative analyses of the effect of the miR in lipid metabolism.

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INTERACTION BETWEEN DUSP1 AND YAP1 IN PANCREATIC DUCTAL ADENOCARCINOMA

Ilze Mari Olivi Gomes[1]; Pamela Viani de Andrade[1]; Mariana Tannus Ruckert[1]; Gustavo A. Veiga Cruzeiro[2]; Kleiton S. Borges[2]; Vanessa S. Silveira[1*]

[1]Department of Genetics, [2] Department of Pediatrics. Ribeirão Preto Medical School, University of São Paulo, Ribeirão Preto, São Paulo, Brazil.

*vsilveira@fmrp.usp.br

Palavras-chave: PDAC, Hippo-signaling, Dual-specificity-phosphatase1

Pancreatic Ductal Adenocarcinoma (PDAC) represents 85% of the pancreatic cancers, being the most well-known histological subtype of pancreatic cancer. PDAC prognosis is extremely poor and presents a 5-year overall survival rate of only 7%. The highly malignant potential and aggressiveness of pancreatic adenocarcinoma cells is mainly promoted by oncogenic KRAS activation, which triggers innumerous signaling pathways and controls key processes for tumor progression, including acquired resistance. Among the several mechanisms involved in PDAC drug resistance, one of the most relevant is the amplification of the chromosome 9qA1 region, which leads to the upregulation of *YAP1.* The oncogenic function of this gene is well described in many cancers, and it was portrayed as a noteworthy determinant of clinical aggressiveness in PDAC patients. YAP1 activation is regulated according to its phosphorylation state at Serine residues and once activated control the Hippo signaling pathway. Giving the important role of YAP1 in PDAC this study aimed to identify key phosphatase molecules in YAP1 phosphorylation regulation. Public RNAseq data from 178 Pancreatic Adenocarcinoma samples (data generated by the TCGA Research Network: http://cancergenome.nih.gov/) was analyzed using the LinkedOmics (Vasaikar, S. et al. 2017) and GEPIA platforms (Tang, Z. et al 2017). Gene expression analysis and protein expression were performed by quantitative RT-PCR in pancreatic cell lines and non- tumoral pancreatic tissue. To address the role of DUSP1 in YAP1 phosphorylation pancreatic carcinoma cell lines PANC-1 and MIA PaCa-2 were treated with (E)-2- benzylidene-3-(cyclohexylamino)-2,3-dihydro-1H-inden-1-one (BCI) for DUSP1 targeted inhibition. Cell lines were treated for 2 to 24 hours. *In silico* analysis demonstrate that YAP1 has a significant correlation (in Pearson's correlation analysis) with the dual- specificity phosphatase DUSP1 (r-value = 0.3; $p<0.0001$). In addition, survival analysis of YAP1 normalized with DUSP1 gene expression revealed that patients with downregulation of YAP1 showed higher rates of overall survival ($p<0.02$), highlighting the clinical relevance of both YAP1 and DUSP1. Evaluating YAP1 targets we observed that CYR61 (the most important YAP1 target) showed a strong correlation with DUSP1 (r-value = 0.79; $p< 0.000001$). The same outcome has been observed for other important targets such as AXL and ITGB3 (r-value = 0.43 and 0.37 respectively; $p<0.001$). Interestingly, both DUSP1 and YAP1 are upregulated in PDAC samples compared to normal pancreatic tissue ($p<0.001$) as well as other YAP1 targets as CYR61. In vitro experiments to address DUSP1 role in YAP1 phosphorylation revealed that DUSP1 targeted inhibition upon BCI treatment increased YAP1 phosphorylation in a time- dependent manner suggesting that YAP1 might be regulated by DUSP1. Taken together, these data show for the first time that phosphatase DUSP1 can be a major player in YAP1 regulation in PDAC cells and elucidate this mechanism can contribute to new insights on pancreatic cancer biology field.

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Genetic population structure of the angular angelshark, *Squatina guggenheim*, an endangered endemic species from Southwest of the Atlantic Ocean

Ingrid Vasconcellos Bunholi[1,2*]; Rodrigo Rodrigues Domingues[1], Bruno Lopes da Silva Ferrette[1,3]; Matheus Marcos Rotundo[4]; Juan Martín Cuevas[5]; Mirta García[5]; Sebastián Gómez[5]; Renato Hajenius Aché de Freitas[6]; Claudio Oliveira[2]; Fausto Foresti[2]; Fernando Fernandes Mendonça[1].

[1]Laboratório de Genética Pesqueira e Conservação (GenPesC), Instituto do Mar, Universidade Federal de São Paulo (UNIFESP), Campus Baixada Santista, Brasil. [2]Laboratório de Biologia e Genética de Peixes, Instituto de Biociências de Botucatu, Universidade Estadual Paulista Júlio de Mesquita Filho (UNESP), Brasil. [3]Laboratório de Genética e Conservação, Universidade Santa Cecília (UNISANTA), Santos, SP, Brasil. [4]Laboratório de Pesquisas Biológicas (LAPEBio), Acervo Zoológico, Universidade Santa Cecília (UNISANTA), Santos, Brasil. [5]Facultad de Ciencias Naturales y Museo, Universidad Nacional de La Plata, La Plata, Argentina. [6]Laboratório de Biologia de Teleósteos e Elasmobrânquios (LABITEL), Departamento de Ecologia e Zoologia, Universidade Federal de Santa Catarina (UFSC), Brasil.

*inngridbunholi@gmail.com

Palavras-chave: Cytochrome b, Genetic diversity, Conservation genetics.

The angel sharks of the *Squatina* genus comprise 22 valid coastal benthic shark species, which inhabiting tropical and subtropical waters in continental shelves worldwide. Among these species, the angular angelshark, *Squatina guggenheim*, is an endangered endemic species from Southwest Atlantic with a limited range distribution between Espírito Santo state, southeast Brazil and Central Patagonia in Argentina. It is caught as bycatch by small-scale industrial and artisanal fisheries. However, its population genetic structure remains unclear. Therefore, this study aimed to assess the genetic population structure and genetic diversity of the angular angelshark, based on 781 bp of the mitochondrial DNA cytochrome *b*. A total of 61 individuals were sampled from 9 areas along the Southwest Atlantic. The mitochondrial sequences revealed 32 polymorphic sites, 45 haplotypes, with overall haplotype and nucleotide diversities of 0.623 (± 0.040) and 0.00099 (± 0.00012), respectively. In order to a wider Analyses of Molecular Variance (AMOVA), haplotypes (JX312439 - JX312507) from Garcia et al. (2014) were coupled to our sequence dataset, totalizing 129 sequences. The AMOVA showed the existence of at least three genetically distinct populations in Southwest Atlantic: Southeastern Brazil; South of Brazil and region of Rio de la Plata Estuary (K_{ST} = 0.17418, P < 0.00001). The F_{ST} pairwise differences ranged from -0.00558 to 0.44120, suggesting the existence of restricted gene flow between geographically distant groups. In addition, the median-joining haplotype network revealed a star-shaped pattern. The H2 was the more frequent haplotype, and it was present in all populations identified. The genetic relationships of the haplotype network do not express the presence of structured groups for cytochrome *b* gene. However, 39 haplotypes found only in the Rio de la Plata region indicate a high genetic diversity to this region and high indices of genetic divergence among the groups. These outcomes suggest that management and conservation plans for the angular angelshark should consider the genetic heterogeneity information in order to safeguard the evolutionary potential of this imperiled endemic shark species.

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DEVELOPMENT OF A GENE PANEL FOR THE DETECTION OF BIODEFENSE AND PUBLIC HEALTH PATHOGENS THROUGH MASSIVELY PARALLEL SEQUENCING

Victor Hugo Giordano Dias[1], Priscila da Silva Figueiredo Celestino Gomes[1], Bruce Budowle[2,3], Rodrigo Soares de Moura-Neto[4], Rosane Silva[1*]

[1]Instituto de Biofísica Carlos Chagas Filho, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil. [2]Center for Human Identification, University of North Texas Health Science Center, Fort Worth, Texas, US. [3]Center of Excellence in Genomic Medicine Research (CEGMR), King Abdulaziz University, Jeddah, Saudi Arabia,[4]Instituto de Biologia, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil.

*silvaros@biof.ufrj.br

Key-words: Biodefense; Bacterial detection; Pathogens DNA panel

The potential for a biological weapon is of great concern for the safety of the population and the adequacy of infrastructure for rapid response to an attack. Bioterrorism is defined as an intentional and planned release of pathogenic agents or toxins. The biological agents are used to promote epidemics, establish panic in the population and overload health systems. The accurate identification of these pathogens is important to distinguish the possibility of health hazard from bioterrorism. The use of Massively Parallel Sequencing (MPS) allows a deep analysis of DNA material present in unknown samples without a cloning step. The confidence of the analysis depends on the availability of data and as consequence many groups have been creating a variety of curated databases to improve the research on the bioinformatics field. This work aims to develop rapid identification protocols for important public health pathogens and bacterial species classified as a biological weapon through specific genes amplification and multiplex MPS. We selected thirty-six hazard bacterial species and designed fifty-three primers pairs, aiming specific 250 bp genes fragments of the target organisms. The selected bacterial species had more than one target-gene per strain We also design thirty-one synthetic gene (SG) fragments for the remaining primer's targets. Multiplexes amplifications reactions were performed using a mock of bacterial DNA samples and SG fragments, with different amounts of DNA for each assay (0, 1,000, 5,000 and 25,000 Genomic Equivalent - GE), using human or unknown microbial community DNA as background, in which contained template for the fifty-three primers target. After the sequencing on Illumina MiSeq, we mapped the reads against fifty-three gene references, corresponding to each primer pair set. It was possible to detect the 52 gene targets, including bacterial gDNA and GS fragments, due to the PCR optimization. With 0 GE of target DNA spiked-in, the number of reads mapped on the references genes sequences was higher for the environmental than the human background. However, when 5,000 or more GE of target DNA was added to the samples, the number of reads mapped to the references was similar between both backgrounds. The sensitivity of MPS approach, and a panel of 53 primers of target genes, demonstrates a promising technique to detect selected bacteria of health and biodefense interest. A bioinformatics pipeline analysis of the metagenomic data will be set up, aiming to create an automated computational workflow of analysis that is fast and effective in the identification of pathogens of public health and biodefense interest.

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Mapping of biological pathways regulated by microRNAs based on differential modulation of target gene expression level

Alves, LAC[1]; Oliveira, AC[1]; Bovolenta, LA[2]; Figueiredo, L[1]; Ribeiro, AO[1]; Campos, VF[3]; Lemke, N[2]; Pinhal, D[1].

[1]Laboratório de Genômica e evolução Molecular, Departamento de Genética, Instituto de Biociências de Botucatu, UNESP, Botucatu, SP, Brasil. [2]Laboratório de Bioinformática e Biologia Computacional, Departamento de Física e Biofísica, Instituto de Biociências de Botucatu, UNESP, Botucatu, SP, Brasil. [3]Laboratório de Genômica Estrutural, Centro Tecnológico de Desenvolvimento, Universidade Federal de Pelotas, Pelotas, RS, Brasil

Palavras-chave: MicroRNA, Gene Regulation, Functional Genomics

MicroRNAs (miRNAs) are small non-coding RNAs (~22 nts) that post-transcriptionally modulates gene expression levels of the protein-coding genes of virtually every biological process described. Knowing that a single miRNA is able to simultaneously control several biological functions, it would be expected that they have the ability to properly sort among distinctive biological process in order to regulates the protein production of its target messages RNAs (mRNAs), required by the correct functions of the cells. In this work, we performed a functional enrichment analysis by clustering protein-coding genes according to the intensity of changes in their expression levels after the overexpression of ten miRNAs. Doing this, we demonstrated that miRNAs are capable of distinctly modulate gene expression according to the biological function their targets are assigned to. Moreover, computational predictory analysis suggests that such miRNA regulatory modus operandi is an evolutionarily conserved phenomenon within vertebrates. We discovered novel a complex regulatory cluster-circuit approach performed by miRNAs that is dependent on the intensity of the repression performed on its targets. This data helps to clarify both the modus operandi and evolution of regulatory mechanisms underlying the functional activity of different miRNAs, as well as opens new doors to future analysis aimed at the determination of the specific impact of miRNAs in distinct biological processes.

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Action of lignans extracted from *Piper nigrum* on cervix cancer

Mayra Carolina da Silva Ferreira[1]; Luana Pereira Cardoso[1] Stefanie Oliveira de Sousa[2]; Rosangela da Silva de Laurentiz[3] Flávia Cristina Rodrigues Lisoni[1]

[1]São Paulo State University (UNESP), Faculty of Engineering Ilha Solteira (FEIS), Department of Biology and Animal Science, Ilha Solteira, SP, Brazil. [2]São Paulo State University (UNESP), Institute of Bioscience, Humanities and Exact Science (IBILCE), Department of Biology, São José do Rio Preto, SP, Brazil. [3]São Paulo State University (UNESP), Faculty of Engineering Ilha Solteira (FEIS), Department of Physic and Chemistry, Ilha Solteira, SP, Brazil

mayra.carolina.sf@hotmail.com

Palavras-chave: cell culture; natural compound; alternative therapy.

Piper nigrum, popularly known as black pepper, is one of the most known species due to its high commercialization and important medicinal properties, has aroused scientific interest to possess antitumorigenic, anti-inflammatory, antihypertensive, antioxidant, antimicrobial and antidepressant potential. However, these effects have been explored in the fruit and seed of the plant, while in the leaf there is little research, but it is already known that the P. nigrum leaf contains lignans such as cubebina that showed in numerous works properties that makes it a herbal medicine with potential antitumorigenic effect. Therefore, this work aimed to evaluate the potential antitumorigenic effect of lignans in cervical cancer cells, observing how this herbal medicine acts and how these alterations can participate in the tumor process. For this, the cell line HeLa (adenocarcinoma of the cervix) treated with the total extract of P. nigrum was used in three different concentrations (10, 50 and 100ug / mL) for 4, 24, 48 and 72h to evaluate the cell morphology, cell proliferation, cytotoxicity and cell migration. The results showed that the treatment with the total extract of the P. nigrum leaf did not alter the cellular morphology, diminished the cellular proliferation, altered cell viability, but it was not considered toxic and reduced the migration in a dose dependent of time. These results indicate that the extract acted in the inhibition of proliferation from mechanisms that may involve cell death, either by blocking cell division or by apoptosis. From these results, new assays become necessary, such as cell cycle and apoptosis analysis by flow cytometer. Thus, Piper nigrum can be considered a potential target for new molecular genetic assays, evidencing a possible use of this natural compound as a therapeutic alternative in cervical cancer.

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IN VITRO EVALUATION OF GENOTOXIC POTENTIAL OF AMPHOTERICIN B ENCAPSULATED IN NANOESTRUTURED LIPID CARRIERS

Matheus Reis Santos de Melo[1]; Fernanda Santos Fernandes[1]; Iara Silva Squarisi[1]; Lucas Teixeira Souza de Oliveira[1]; Tábata Rodrigues Esperandim[1]; Bianca Silva Alves[1]; Maraine Catarina Tadini[2]; Franciane Marquele-Oliveira [2], Denise Crispim Tavares[1]

[1]Universidade de Franca, Franca, São Paulo, Brazil. [2]ELEVE SCIENCE Research and Development, Ribeirão Preto, São Paulo, Brazil

Palavras-chave: Amphotericin B; Genotoxicity; Lipid carrier

Amphotericin B (AmB) is an active and intravenous substance, obtained from cultures of *Streptomyces nodosus*. AmB is widely used for treatment of serious systemic fungal infections, as well as for secondary treatment of visceral and cutaneous leishmaniasis. However, the treatment with AmB presents some side effects such as fever, chills, tremors, nausea, vomiting, headache, cardiovascular disorders, hypokalemia, hypernatremia, increased diuresis, hypomagnesemia, renal dysfunction and toxic effects on the bone marrow. In this sense, there is a search for new routes of administration of this substance, reducing its toxicity. Therefore, the present study aimed to evaluate the genotoxicity of AmB encapsulated in nanostructured lipid carriers (AmB-NLC). For this purpose, the micronucleus test was used in Chinese hamster lung fibroblasts (V79 cells). The treatment groups were designated as negative control (no treatment), AmB (10, 20 and 40 µg/mL), nanostructured lipid carrier (NLC - 1,250; 2,500 and 5,000 µg/mL), AmB- NLC (1,250; 2,500 and 5000 µg/mL) and positive control (methyl methanesulfonate, 44 µg/mL). For the evaluation of the genotoxic potential, 1,000 binucleate cells were analyzed by culture, totaling 3,000 binucleate cells per treatment. Regarding cytotoxicity, the nuclear division index (NDI) was calculated by analyzing 500 cells per culture, totaling 1,500 cells per treatment. The results showed that the groups AmB, AmB-NLC and NLC did not present frequencies of micronuclei that differed significantly from those observed in cell cultures not submitted to any treatment. No significant differences were observed in the NDI values between the treatment and negative control groups. Therefore, AmB, AmB-NLC and NLC did not demonstrate genotoxic or nor cytotoxic effects, under the experimental conditions used.

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Mitochondrial phylogeny of the yeast CTG clade

Gabriel Antônio Mendes de Brito[1]*; Thiago Mafra Batista[2]; Carlos Augusto Rosa[3]; Glória Regina Franco[1]

[1]Departamento de Imunologia e Bioquímica, Instituto de Ciências Biológicas - Universidade Federal de Minas Gerais, Belo Horizonte, Minas Gerais, Brasil. [2]Centro de Formação em Ciências Ambientais, Universidade Federal do Sul da Bahia, Porto Seguro, Bahia, Brasil. [3]Departamento de Microbiologia, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais - Belo Horizonte, Minas Gerais, Brasil

*gabrielmendes@ufmg.br

Keywords: CTG clade, mtDNA, phylogeny

The CTG clade is a taxon of ascomycetous yeasts possessing a large biotechnological potential, with several members capable of synthesizing industrial enzymes. These organisms display a unique characteristic of genetic encoding: they translate the CUG codon predominantly as Serine (97%) and rarely as Leucine (3%). This ambiguity in CUG encoding enhances protein diversity, allowing these organisms to have an expanded capacity of adaptation, what assurance their presence in different biomes. However, the evolutionary relationship of the CTG clade members is still under debate, as new species are being described and previous models are rendered incomplete. In order to clarify these relationships, we aim to reconstruct the clade's phylogeny using orthologous mitochondrial genes from publicly available genomes and from genomic data of new CTG species generated by our research group. We will then compare our model to a previous reconstruction which used 1:1 nuclear orthologous genes shared among these yeasts. We have assembled and annotated 29 new mitochondrial genomes of the CTG clade corresponding to yeasts from 14 genera, including *Scheffersomyces*, *Metschnikowia* and *Candida*. The reads of these organisms were obtained from NCBI Sequence Read Archive (SRA) and from our own sequencing project. Assembly was performed with paired and single-end reads, from different sequencing technologies, using the software NOVOPlasty. Annotation of protein-coding, tRNA and rRNA genes were executed with the tool Mitos2 Web Server. In the next steps, the individual genes will be aligned, and the evolutionary relationships will be reconstructed using maximum likelihood, maximum parsimony and Bayesian inference methods implemented in MEGA software.

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CELLULAR EFFECTS OF DNA DAMAGE IN CRISPR/CAS9 GENERATED COCKAYNE SYNDROME CELLS

Gustavo Satoru Kajitani[1]; Alexandre Teixeira Vessoni[2]; Carlos Frederico Martins Menck[3]; Camila Carrião Machado Garcia[1*]

[1]Universidade Federal de Ouro Preto. [2]Washington University School of Medicine. [3]Universidade de São Paulo

*carriao.camila@gmail.com

Keywords: Cockayne syndrome; Crispr/Cas9; DNA damage responses

Cockayne Syndrome (CS) is a rare, autosomal recessive disease, characterized by an early aging-like phenotype, including progressive neurodegeneration, cachectic dwarfism, retinal atrophy and lipodystrophy. CS is caused by mutations in genes related to transcription-coupled nucleotide excision repair (TC-NER), a molecular pathway that repairs bulky DNA lesions in transcriptionally active genes. Cells that lack TC-NER are known to be sensitive to ultraviolet radiation (UVR), a well-established exogenous source of lesions that distort the DNA molecule. However, it remains unclear whether endogenous sources of DNA damage, such as reactive oxygen species (ROS), are able to generate differential cellular responses in CS cells. This is mainly due to a variation in genetic background when investigating these types of responses in different cell lines. Thus, in this project, we aim to use CRISPR/Cas9 gene editing in order to resolve this issue of different genetic backgrounds and test whether deficiency in CS genes (CSA and CSB) is related to an increased DNA damage response to oxidatively generated DNA lesions. Using CRISPR/Cas9, we have generated HEK 293FT cell line clones that mimic mutations in CS patients. These clones are being tested for their sensitivity to UVR in order to confirm the CS phenotype. The clones will then be sequenced and evaluated for their cellular DNA damage responses to ROS, such as cell death and cell cycle arrest. These analyses will help to elucidate the specific role of CS genes regarding oxidatively induced DNA lesions, and thus help us understand the premature aging-like CS phenotype.

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Molecular identification of the protozoan coccidian parasite *Isospora sepetibensis* from black-goggled tanagers *Trichothraupis melanops* in southeastern Brazil

Saulo T. Abreu[1]; Jhon Lennon G. de Oliveira[1]; Águida A. de Oliveira[1]; Viviane M. de Lima[1]; Bruno P. Berto[1]

[1]Departamento de Biologia Animal (DBA), Instituto de Ciências Biológicas e da Saúde, Universidade Federal Rural do Rio de Janeiro (UFRRJ), Seropédica, Rio de Janeiro.

abreu.saulo.st@gmail.com

Key words: Molecular biology; Coccidia; Neotropical birds

Coccidia are obligated intracellular parasites which can be associated with enteritis and death in all classes of vertebrates, mainly Aves. The aim of this study was to identify genotypically *Isospora sepetibensis* parasitizing *Trichothraupis melanops* in Itatiaia National Park (INP). Four expeditions were conducted in INP and a total of fifteen *T. melanops* were captured with mist nets. They were kept in individual boxes and feces were collected immediatelly. Ten of them shed oocysts in their feces. The oocysts were isolated by flotation in Sheater's sugar solution and identified morphollogically by light microscopy. Posteriorly, fifteen oocysts with the same characteristic features were isolated and resuspended in PBS. DNA was extracted from the oocysts using the Qiagen DNeasy Blood and Tissue Kit. The PCR amplification for the mitochondrial cytochrome *c* oxidase subunit 1 (*cox1*) gene was by nested PCR. Products were sequenced by Ludwig Biotechnology, where an ABI-Prism 3500 Genetic Analyzer was used. Sequence was compared with *Isospora* spp. available on the GenBank using BLAST. Phylogenetic trees were constructed for *Isospora* spp. at the *cox1* sequences for additional isolates from GenBank. Sequence obtained from *T. melanops* was 98.1% similar with an *Isospora* sp. reported as pseudoparasite of bank voles *Myodes glareolus.* In this analysis, *Isospora sepetibensis* sat in the same clade of *Isospora* spp. from Neotropical passerines, with the highest similarity of 98.5% with *Isospora lopesi*. Based on all the results reported in the current work, *T. melanops* is recorded as a new host for *I. sepetibensis* in the Itatiaia National Park in Southeastern Brazil, as well as being the first coccidian parasite of a New World tanager to have its *cox1* sequence deposited in the GenBank database.

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MICRORNAS miR-19A-5p AND let-7-3p REGULATING SP7 GENE IN EPIMORPHIC REGENERATION IN *Danio rerio* CAUDAL FINS

Felipe dos Santos Pereira[1]; Beatriz Jacinto Alves Pereira[1]; Natascha Mozaner Nitzsche[1]; Amanda de Oliveira Ribeiro[1]; Danillo Pinhal[1]

[1]Instituto de Biociências de Botucatu – UNESP

felipe.pereira@unesp.br

Palavras-chave: Epimorphic regeneration; dedifferentiation; microRNAs

Regeneration is an important biological process conserved in vertebrate organisms. Although widely investigated, some of its primordial processes remain unknown at the molecular level. Zebrafish (*Danio rerio*), a fish with an extraordinary ability to regenerate, has been widely used as a model organism for the study of epimorphic regeneration. The fins of this species show a remarkable ability to regenerate after injury / amputation, from the activation of several mechanisms that include migration, dedifferentiation and proliferation, in order to recover the damaged limbs. The dedifferentiation of mature osteoblasts into osteoprogenitor cells is an important step that occurs within the first 72 hours post-amputation. This event is dependent on the correct expression of a gene signaling network in which transcription factors such as sp7, a marker of mature osteoblasts is highly expressed in the region adjacent to the lesion, act. MicroRNAs are notable for performing fine-tuning regulation of gene expression in multiple biological processes, but their role in dedifferentiation needs to be elucidated. The present study aims to identify microRNAs involved in sp7-mediated cell dedifferentiation during the epimorphic regeneration process in zebrafish. To do so, we performed in silico predictions of miRNAs with interaction sites of the sp7 gene using the miRNDA tool. From this analysis we selected the miR-19a-5p and let-7c-3p miRNAs as potential candidates for sp7 regulation. Then, we performed amputation of the caudal fin and collected tissues of 60 zebrafish subdivided into 6 different experimental groups: 0, 12, 24, 36, 48 and 72 hours post-amputation. After quantification and verification of the integrity of the total RNAs extracted, we constructed cDNA libraries that were used in RT-qPCR for amplification of the sp7 gene and the selected miRNAs. We calculate the relative fold change using the delta-delta Ct calculation. The sp7 gene qPCR revealed a peak expression in the period of about 12hpa of approximately 1,000% compared to the 0hpa period (control). We also observed a gradual decline in sp7 expression, reaching 7% in the 72 hpa period. Among the miRNAs analyzed, let-7c-3p showed decreased expression in 59% in the period of 12hpa, coinciding with the high expression of sp7 and both miRNAs showed increase of expression in the period of 72hpa, simultaneously to the decrease of sp7 expression. These miRNAs are potential regulatory agents and are described as relevant for the maintenance of homeostasis of bone tissue (mir-19a), and having tumor suppressor function and potential inhibitor of bone formation and differentiation (let-7c). In the context of epimorphic regeneration in caudal fins of zebrafish, let-7c-3p and miR-19a miRNAs possibly interfere with sp7 gene expression during cell dedifferentiation. Additional experiments are being developed to validate potential predicted interactions and also to determine the biological functions of miRNAs in dedifferentiation.

From nature to application – improved xylose uptake and molecular transport mechanisms for 2G ethanol production in engineered Saccharomyces cerevisiae

Bueno, J.G.R[1,2]; Borelli, G.[2]; Corrêa, T.L.R.[1]; José, J.[2]; Pereira, G.A.G.[2]; Santos, L.V. [1,2]

[1]Brazilian Biorenewable National Laboratory (LNBR), Brazilian Center for Research in Energy and Materials (CNPEM), Zip Code 13083-970, Campinas, Sao Paulo, Brazil. [2]Institute of Biology, State University of Campinas, Campinas, SP, Brazil

Key words: 2G ethanol, xylose, sugar transporter

The need to restructure the world energy matrix based on fossil fuels stimulated the development of new technologies based on renewable energy. One promising and cleaner alternative towards energy restructuration is the second-generation (2G) fuels, produced from lignocellulosic biomass. A major challenge on 2G technology establishment is the inefficient assimilation of the five-carbon sugar xylose by engineered *Saccharomyces cerevisiae* strains, increasing the fermentation time. The xylose uptake by *S. cerevisiae* strains occurs by endogenous sugar transporters which have low affinity to xylose and strong glucose repression, impairing fermentation. By assessing microbiomes such as the digestive tract of plague insects or several biomasses, we isolated several yeast species capable of using xylose. Comparative fermentations select the yeast *Candida sojae* as a potential source of high-affinity transporters. Therefore, comparative genomic analysis elects four xylose transporters - Cs186, Cs2608, Cs3894, and Cs4130 - whose performance properties were evaluated in the transporter-free EBY.VW4000 strain carrying the xylose pathway integrated into the genome. Surprisingly, while the best xylose heterologous transporter in literature, *GXF1*, is inhibited at concentrations above 10 g/L, the strains containing Cs3894 and Cs4130 show superior xylose uptake, not affected at concentrations up to 50 g/L. Considering that xylose concentrations in 2G hydrolysates reaches high values, these new transporters are profitable candidates for xylose uptake due to natural loss of inhibition. The modelled structure of Cs4130 shows the typical fold of membrane transporters belonging to the Major Facilitator Superfamily (MFS) with the extracellular, 12 transmembrane segments and intracellular domains. The superimposition with a notorious glucose/xylose transporter (*XylE*) structure harboring a D-xylose as ligand shows that the tyrosine 324 (Y324) residue in Cs4130 is dislocated when compared to *GFX1*, adopting an orientation that could act trapping the xylose molecule, preventing the scape to the extracellular side. This peculiar structural disposition illuminates interesting aspects of xylose transport mechanisms and will be used for rational design procedures aiming improved uptake kinetics. Here we demonstrate a novel eukaryotic transporter protein that are not inhibited in high xylose concentrations and can be used as a promising target towards efficient pentose utilization in engineered yeasts.

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PRODUCTION AND *IN VITRO* ANTIGENICITY OF RECOMBINANT PROTEINS FROM *ERYSIPELOTHRIX RHUSIOPATHIAE* CANDIDATES FOR VACCINAL ANTIGENS

Yuri N. Fuzissaki*[1]; Vinícius Marquioni[1]; Adilson J. Silva[1]; Teresa C. Zangirolami[1]; Fernanda F. Anibal[1]; Maria T. M. Novo-Mansur[1]

[1]Federal University of São Carlos, São Carlos, SP, Brazil.

*yurinakau@gmail.com

Key words: swine erysipelas; recombinant protein; antigenicity.

Erysipelothrix rhusiopathiae is a Gram positive, bacilliform and encapsulated bacterium, which causes swine erysipelas. Infected animals may have skin lesions and other signs such as septicemia, arthritis and endocarditis. Vaccines from attenuated or inactivated *E. rhusiopathiae* cells are commercially available but are not effective in preventing the chronic form of the disease and may aggravate symptoms of arthritis in vaccinated animals. Extracellular proteins from *E. rhusiopathiae* were detected as being potentially antigenic in previous immunoproteomic-based experiment performed in our research group, and we cloned 11 genes corresponding to these proteins. The present work aims to give continuity by the heterologous expression of four of these proteins, denominated P1, P3, P6 and P7, to investigate the antigenic characteristics *in vitro* and its protective character against *E. rhusiopathiae* in murine model. P1 and P7 proteins were produced in the recombinant form in *Escherichia coli* Rosetta (DE3) and P3 and P6 proteins in *E. coli* BL21 (DE3). After purification of the recombinant proteins by affinity and molecular exclusion chromatography, the antigenicity was evaluated by Western Blot and ELISA using serum from swine inoculated with the commercially available vaccine. The commercial vaccine used is composed of attenuated bacteria. All recombinant proteins were successfully expressed in *E. coli* and in quantity and purity considered sufficient for the next steps, also exhibiting immunoreactivity with the sera of pigs. Proteins will be tested for their protective effect in mice, which will subsequently be challenged with *E. rhusiopathiae* and monitored for survival percentage. The most promising recombinant protein will have its expression determined in bioreactor to study the viability of production on a larger scale for eventual commercialization as a subunit vaccine against *E. rhusiopathiae* and subsequently, the immunogenic profiles and avidity of these proteins will be determined for protection against *E. rhusiopathiae*.

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Association study of polymorphisms in *LRRK2* gene with leprosy

Isabela Espasandin Martins[1]; Fernanda Souza Gomes Kehdy[1]; Ohanna Cavalcanti de Lima Bezerra[1]; Thadeu Cordeiro Rezende Santos[1]; Thyago Leal Calvo[1]; Fernanda Saloum Neves Manta[1]; Rafaela Mota Dias[1]; Milton Ozório Moraes[1]

[1]Laboratório de Hanseníase – Instituto Oswaldo Cruz (Fiocruz - Rio de Janeiro, Brazil)

isabela.espasandin@gmail.com

Key-words: polymorphisms; leprosy; association

Leprosy is a chronic infectious disease caused by the intracellular pathogen *Mycobacterium leprae*. It has already been shown that there is a low genetic variability of this pathogen. Thus, variations in the host would be a more prominent factor for genetic of leprosy *per se* susceptibility and/or its various clinical manifestations. Previous studies have demonstrated the association of single nucleotide polymorphisms (SNPs) in genes involved in the type-I interferon pathway, autophagy, energetic metabolism and lipid metabolism with disease outcome. Genome-Wide Association Studies (GWAS) studies identified *LRRK2* gene as associated with leprosy, although further confirmation in independent population has not yet been elucidated. Data from literature suggest that the protein encoded by this gene would be involved in several cellular processes, such as vesicular trafficking and endocytosis, regulation of the immune response and autophagy. Therefore, the main objective of this work was to perform a genetic analysis involving SNPs located in the *LRRK2* region, in order to evaluate the association of these polymorphisms with the development of leprosy in the population of Rio de Janeiro, Brazil. For the case-control study, samples of patients were recruited from the Fiocruz Clinic in Rio de Janeiro, Brazil. We selected candidate SNPs after screening genotypes in the *LRRK2* region from African, European and Amerindian populations from 1000 Genomes Project phase 3. Then, Principal Components Analysis (PCA) using EIGENSOFT 4.2 were performed. PCA identified the main clusters and the SNPs were sorted by decreasing values of "SNP weight" for the principal components 1 and 2 (PC1 e PC2) and the functional annotation of all SNPs was performed with ANNOVAR. From these tools and associations with infectious diseases already reported in the literature, the tag SNPs rs7308720, rs10878434, rs3761863 e rs7962370 in the *LRRK2* region were selected for genotyping and haplotype construction. Haplotypes inferences using the selected SNPs, haplotype frequencies and linkage disequilibrium (LD) analysis for all studied populations were performed by HAPLOVIEW. The samples were genotyped for the SNP rs7308720 by real-time PCR using Taqman system and the allele and genotype frequencies were calculated. The association analysis was performed using the R program by logistic regression models adjusted by sex and ancestry. In patients, the MAF (Minor Allele Frequency) of rs7308720 was 0.13 and the frequencies of CG and GG genotypes were 0.17 and 0.012, respectively. No statistically significant association of this SNP was detected with the development of leprosy *per se* (OR = 1.25; P-value = 0.1717). In silico analyzes also showed that rs7308720 is in strong LD with rs7133914 in Europeans, which data from literature indicate that it has an important functional role related to Crohn disease, although not confirmed in leprosy. Haplotype analysis and association of other SNPs in *LRRK2* is important for the elucidation of its role in leprosy.

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EXPRESSION PROFILE OF HEAT SHOCK PROTEIN GENES IN CARACU CATTLE DIFFERING IN FEED INTAKE

Nedenia B. Stafuzza[1*]; Bianca V. Pires[2]; Sergio B. G. P. N. P. Lima[1]; Claudia C. Paro de Paz[1,2]

[1]Instituto de Zootecnia, Centro de Bovinos de Corte, Sertãozinho, SP, Brazil. [2]Departamento de Genética, Faculdade de Medicina de Ribeirão Preto, Universidade de São Paulo, Ribeirão Preto, SP, Brazil.

nedeniabs@gmail.com

Keywords: *Bos taurus taurus*; *HSPD1*; qPCR

Feed efficiency is measured as the ratio of feed consumed to gain in weight and directly affects productive and economic efficiency and environmental sustainability in beef industry. Improvements in feed efficiency of beef cattle offers an opportunity to increase producer profitability and simultaneously reduced environmental impact of livestock production. As studies have been reported that heat shock protein genes play important roles in animal's metabolism, the aim of this study was to investigate the hypothesis that the expression profile of heat shock protein genes may be related to feed intake in beef cattle. A total of 40 Caracu steers at 13–15 months of age were evaluated during 88 days in collective feedlot pens equipped with automatic feeding system (Intergado). The RNA was extracted from blood samples using the PureLink RNA Mini Kit (Thermo Fisher Scientific). The RNA concentration, purity, and integrity were verified by Agilent 2100 BioAnalyzer (Agilent Technologies) and the cDNA was synthesized using the GoScript Reverse transcription System (Promega), according to the manufacturer's instructions. The relative expression of *HSPD1*, *HSPA1A* and *HSP90AA1* genes were determined by qPCR in triplicate experiments using PowerUp SYBR Green Master Mix (Thermo Fisher Scientific) on a 7500 Real-Time PCR system. The *GAPDH* housekeeping gene was used to normalize RNA recovery from each sample and the relative change in mRNA expression levels was calculated using the delta Ct method. Pearson correlations were estimated through procedure CORR (SAS v. 9.2) at 5% of significance. Feed intake showed significant negative correlation (-0.14) with *HSPD1* gene expression ($P<0.027$). The *HSPD1* gene was down regulated in high feed efficient animals when compared to low feed efficient animals, which indicated that *HSPD1* gene expression contributes to the feed intake phenotypic variation observed in Caracu beef cattle.

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CHROMOSOMAL MICROARRAY REANALYSIS WITH FOCUS ON VARIANTS OF UNKNOWN CLINICAL SIGNIFICANCE IN INDIVIDUALS WITH MULTIPLE CONGENITAL ANOMALIES AND (OR) DEVELOPMENTAL DELAY

Samira Spineli-Silva[1]; Ilária Cristina Sgardioli[1]; Ana Paula dos Santos[1]; Vera Lúcia Gil-da-Silva-Lopes[1]; Társis Paiva Vieira[1]

[1]Department of Medical Genetics and Genomic Medicine, School of Medical Sciences, State University of Campinas, Campinas, São Paulo, Brazil.

samiraspineli@gmail.com

Keywords: Chromosomal Microarray Analysis, DNA Copy Number Variants, Developmental Delay.

Chromosomal Microarray Analysis (CMA) is the first test to the etiological investigation in individuals with multiple congenital anomalies (MCA) associated or not to developmental delay (DD) or intellectual disability (ID). However, the presence of Variants of Unknown Clinical Significance hampers the results interpretation. Currently, guidelines of the American College of Medical Genetics and the European Group of Human Cytogenetics classify the Copy Number Variants (CNVs) in Benign (class I), Likely Benign (class II), Unknown Clinical Significance (class III), Likely Pathogenic (class IV) or Pathogenic (class V), being the CNVs class III, IV and V reported in the final result. Besides, considering databases updates, the reanalysis of class III CNVs after some period of time can allow their reclassification and diagnostic conclusion. Thus, we reanalyzed the CMA data of individuals with MCA and (or) DD/ID, performed from 2012 to 2018 considering only CNVs bigger than 300 kb and mapped to hg19 genome version using CytoScan™ 750K or CytoScan™ HD Array (Affymetrix®, Santa Clara, CA, USA). The reanalysis was performed using the software Chromosome Analysis Suite (ChAS - version 4.0.0.385 (r28959) – Affymetrix®) (hg38) and an in house pipeline which included: interpretation according to international databases (DGV, Decipher, ClinGen), besides a database composed of 117 individuals from the Brazilian general population, used as an internal control group. The reanalysis of CMA data from 228 individuals showed 41 (18%) with the same pathogenic alteration (class V) previously reported. Among the remaining 187 cases, we found 132 (58%) with a CMA considered normal (class I, II and none CNV) and 55 individuals (24%) with class III CNVs, being one individual also with a class IV CNV. All class III and IV CNVs were investigated in the ClinGen Dosage Sensitivity Map tool. Six variants involved genes associated with autosomal recessive phenotype; one overlapped with an haploinsufficient gene with little evidence suggesting dosage sensitivity being associated with clinical phenotype (score 1); one contained an haploinsufficient recurrent region with emerging evidence suggesting dosage sensitivity associated with clinical phenotype (score 2). No other class V CNVs were found. Despite the low evidence of pathogenicity, these results might be relevant to direct further investigation of class III CNVs by other complementary methods, which may lead to an increase in diagnostic rate. In addition, this study highlights the importance of the reanalysis after some period of time, since our current knowledge about human genome is limited and the learning is constant.

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WHOLE EXOME SEQUENCING FOR PRECONCEPTIONAL SCREENING OF CONSANGUINEOUS COUPLES

Carolina Maria de Araujo dos Santos[1]; Ana Helena Heller[1], Heloisa B. Pena[1]; Betânia M.A. Pena; Sérgio D.J. Pena[1*]

[1]Laboratório GENE - Núcleo de Genética Médica. Belo Horizonte, MG, Brazil.

*spena@gene.com.br

Key-words: exome sequencing; consanguineous couples; preconceptional screening

Genetic studies performed in consanguineous couples suggest that the reproductive that distinguish them from other couples in the general population is related to autosomal recessive diseases. This risk is scattered among the thousands of diseases known. Thus, for the preconceptional screening of consanguineous couples it is necessary a study that encompasses the largest number of genes possible. For that reason, we decided to use whole exome sequencing. We sequenced completely the exomes of 22 consanguineous couples at high coverage (~100X). Applying bioinformatics filters, we could detect genetic variants that were simultaneously present in both members of the couple in 2,415 genes known to be causally related to autosomal recessive diseases. Shared variants were then assessed for pathogenicity. For non-truncating variants (missense and in-frame indels) we considered as "pathogenic" only the variants included as such in the ClinVar database. Shared truncating variants (frameshift, nonsense and canonical splice variants) were considered "likely pathogenic" when loss-of-function was a known mechanism of disease. The 22 consanguineous cases included two couples with a coefficient of relationship (CR) of 25%, 14 couples with a CR of 12.5% (first cousins), two couples with a CR of 6.25%, three couples with a CR of 3.125% and one couple with a CR of 0.78%. In 13 of the 22 couples (59.1%) we ascertained sharing of heterozygosis for a variant considered pathogenic for an autosomal recessive disease. In five couples we found sharing of heterozygosis for two pathogenic variants. Once the specific pathogenic variant was identified, it became possible for the couple to undergo prenatal diagnosis or, if desired, preimplantation genetic diagnosis (PGD) involving *in vitro* fertilization and embryo screening. In conclusion, our results demonstrate that preconceptional screening by exome sequencing is a useful new procedure that should be incorporated in the genetic counseling of all of consanguineous couples.

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Toxicity assessment of red propolis on zebrafish (*Danio rerio*)

Iara Silva Squarisi[1]; Karoline Soares de Freitas[1]; Saulo Duarte Ozelin[1]; Arthur Barcelos Ribeiro[1]; Lucas Henrique Domingos da Silva[1]; Lucas Teixeira Souza de Oliveira[1]; Natália Helen Ferreira[1]; Danieli Cristina Lemes; Rodrigo Cassio Sola Veneziani[1]; Jairo Kenupp Bastos[2]; Denise CrispimTavares[1]

1University of Franca, Franca, São Paulo, Brazil. 2Faculty of Pharmaceutical Sciences of Ribeirão Preto, University of São Paulo, Ribeirão Preto, São Paulo, Brazil.

iasquarisi79@outlook.com

Keywords: Red propolis; toxicity; zebrafish.

Propolis is known as a resinous substance with varying colors and consistencies prepared by *Apis mellifera* bees from several plant sources. Propolis has different therapeuticpropertiesthatmayberelatedtothevarietyofbiologicallyactivecompounds present, whose action has been widely studied. These properties comprise antioxidant, antimicrobial, anti-inflammatory, curative, anesthetic, anticariogenic, antifungal, antiprotozoalandantiviralactivities.AmongtheBrazilianpropolis,green,redandbrown are the most studied. More recently, red propolis has distinguished itself with a "natural remedy" for various diseases due to its pharmacological properties as antibacterial, antifungal, immunomodulatory, antioxidant, antiproliferative and anti-inflammatory. Despite the popularity of the use of propolis, there are few studies evaluating the safety of the consumption of red propolis. The objective of this study was to assess the toxic effect of the red propolis hydroalcoholic extract (RPHE) on zebrafish (*Danio rerio*), in acute exposures. The use of the species for toxicological evaluations is justified by the short reproductive cycle, 75% homology with the human genome and its great similarity in post-embryonic development with fetal and neonatal human development. In this sense,zebrafishwereexposedfor96hatsixconcentrationsofRPHErangingfrom3.12 to100mg/L.Theresultsshowedfishmortalityatconcentrationsof12.5,25,50and100 mg/L. However, the lowest concentrations (3.12 and 6.25 mg/L) were not lethal. The concentrationoftheRPHEinwaterthatwaslethalfor50%ofexposedpopulation(LC50) is 9.37 mg/L. However, additional studies are required to ensure safe use for human health.

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KNOCKOUT AND HERITABLE GENOME EDITING OF THE EYE COLOR SCARLET GENE FOR CRISPR/CAS9 IN AN IMPORTANT CROP PEST *SPODOPTERA FRUGIPERDA*

Thais P. Souza[1], Anne-Louise Doss[2], Baoju Wang[2], Linda L. Walling[3], Peter W. Atkinson[2] & Marcio C. Silva-Filho[1]

[1] Departamento de Genética, Escola Superior de Agricultura Luiz de Queiroz, Universidade de São Paulo, Piracicaba, Brazil, [2] Department of Entomology, University of California, Riverside, USA, [3] Department of Botany & Plant Sciences, University of California, Riverside, USA.

mdcsilva@usp.br

Key-words: CRISPR-Cas9; *Spodoptera frugiperda*; genome editing

Fall armyworm (*Spodoptera frugiperda*) is a polyphagous insect able to feed on more than 180 host plants. This pest which is originally from North and South America has become also a serious problem in the African continent. It is also expected that this pest reaches out Europe and Asia continents. *S. frugiperda* control relies on chemical pesticies and Bt crops, however, it has been shown that this pest developed resistance to transgenic Bt plants as well as to all major classes of pesticides. Currently, gene editing for CRISPR/Cas9 has appeared to be a potential alternative resource for control of insect pests. In the current work, we aim to define a CRISPR/Cas9 system to efficiently control the *S. frugiperda*. We used eye color mutations as visible markers for detection of successful transformation events to developing transformation techniques for this insect. By combination of sgRNA and Cas 9 protein injection in fresh laid eggs we have found that some of the injected insects showed either somatic phenotype or yellow eyes color. The mutations from *Sfscarlet* gene were transmissible through eight generations, demonstrating that CRISPR/Cas9 can be used for *S. frugiperda* gene editing. Developing strategies for species-specific pest management will potentially reduce environmental and human impacts.

Funding agency: FAPESP, CAPES, CNPq.

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INSIGHTS INTO THE REGULATION OF ALTERNATIVE SIGMA FACTOR EcfK FROM *XANTHOMONAS CITRI* SUBSP. *CITRI*: ROLE OF eSTK PknS

Lidia dos Passos Lima[1*]; Lucas de Moraes Ceseti[1]; Caio Vinicius dos Reis[2]; Dev Sriranganadane[2]; Katlin B. Massirer[2]; Rafael M. Couñago[2] and Cristina Elisa Alvarez-Martinez[1]

[1]Instituto de Biologia, UNICAMP, Campinas, SP. [2]Structural Genomics Consortium, UNICAMP, Campinas, SP.

*lima.lidiadospassos@gmail.com.

Key-words: *Xanthomonas*; kinase; sigma factor

Xanthomonas citri subsp *citri* (*X. citri*).is the causal agent of citrus canker, a disease that affects all economic relevant citrus cultivars. Ability to survive in the environment is an important aspect in Xanthomonads physiology, which promotes dissemination of bacteria from infected plants in the field. In a recent work, we have shown that *X. citri* can resist predation by the soil amoeba *Dictyostelium discoideum,* unveiling a new aspect of *X. citri* physiology that is expected to increase its environmental survival. Increased resistance to amoeba requires a type six secretion system (T6SS), a nanomachine used by Gram-negative bacteria to deliver effector proteins and toxins into target cells. The *X. citri* T6SS is regulated at the transcriptional level by the alternative sigma factor EcfK, which is required for the resistance phenotype and for induction of T6SS gene expression upon contact with *D. discoideum*. The Ser/Thr kinase PknS is encoded in a putative operon with EcfK and is essential for EcfK-mediated induction of T6SS gene expression. In this work, we hypothesized that PknS directly activates EcfK by phosphorylation. In order to test this, we have expressed and purified recombinant EcfK and the kinase domain of PknS in *E. coli* and performed *in vitro* phosphorylation assays. We show that the purified PknS kinase domain is functional and autophosphorylates in up to two residues. In addition, EcfK is directly phosphorylated by PknS in up to five residues. LC- MS^2 analysis of phosphorylation reactions after digestion with trypsin identified the EcfK phosphorylation sites. Interestingly, one of these sites (residue T51) is located in the σ^2 domain of EcfK, which is involved in recognition of -10 promoter elements and interaction with RNA polymerase. The other four phosphorylation sites are located in the linker region that separates the two functional domains of EcfK (σ^2 e σ^4). Altogether, these results suggest a new mechanism of regulation of ECF sigma factor activity.

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SCI1 (Stigma/style Cell-cycle Inhibitor 1) and its interaction partner, NtDDX41, are new plant spliceosome-associated factors

Pinoti, VF[1,2]; Strini, EJ[1,2]; Aziani, R[1]; Ferreira, PB[1,2]; Lubini, G[1,2]; Thomé, V[1,2]; Cruz, JO[1,2]; Quiapim, AC[1]; Goldman, GH[3]; DePaoli, HC[4]; Goldman, MHS[1].

[1]FFCLRP/University of São Paulo (USP), Ribeirão Preto, SP– Brazil; [2]PPG – Genética, FMRP/ University of São Paulo (USP), Ribeirão Preto, SP– Brazil; [3]FCFRP/University of São Paulo (USP), Ribeirão Preto, SP– Brazil; [4]Biological Systems & Engineering Division, Lawrence Berkeley National Laboratory, Berkeley, CA - United States.

vitorpinoti@usp.br

Key words: DEAD-box RNA helicase; U2a' protein; Y2H experiment

In previous studies we characterized the *SCI1 (Stigma/style Cell-cycle Inhibitor 1)* gene, which controls cell proliferation in the upper pistil of *Nicotiana tabacum* and *Arabidopsis thaliana*. Recently, we determined that *SCI1* is expressed at the whole floral meristem, since its establishment. The molecular mechanism(s) through which SCI1 controls cell proliferation is still unknown. Therefore, the screening of a *N. tabacum* stigma/style cDNA library in the yeast two-hybrid (Y2H) system, using SCI1 as bait, was performed. In the screening we identified the homolog of the human DDX41, a DEAD-box RNA helicase that acts as tumor suppressor. Human DDX41 is a component of the spliceosome and a second step factor of the splicing process. The interaction between SCI1 and NtDDX41 was confirmed by bimolecular fluorescence complementation (BiFC) and *in vitro* pulldown. Transient expression of SCI1-GFP and NtDDX41-GFP in *N. benthamiana* leaves revealed their nuclear localization. SCI1-GFP and NtDDX41-GFP co-localize with the nucleolus marker AtFIB1-mRFP, as well as the splicing speckles markers AtCypRS64-mRFP and AtRNPS1-mRFP. Additionally, BiFC experiments, coupled with co-localization, demonstrated that SCI1 and NtDDX41 interaction occurs within splicing speckles and nucleolus. BiFC experiments showed the interaction of SCI1 and NtDDX41 with the integral component of spliceosome U2a', validating their physical association with the splicing machinery. To gather more information about this novel plant protein, we conducted a Y2H screening using NtDDX41 as bait. In the screening, we found the homologs of the human second step splicing factor Cactin and of the human splicing related protein NF-κB activating protein (NKAP), as well as the putative homologs of the human splicing factors hnRNP Q and ZRANB2. Likewise, the homolog of the plant Cleavage and Polyadenylation Specification Factor 30 kDa (CPSF30) was identified in the screening. Taken together, our results unravel SCI1 and its partner NtDDX41 as novel splicing-related proteins in plants, demonstrate the association of NtDDX41 with splicing and RNA processing regulators and indicate that SCI1 may regulate cell proliferation through the splicing process.

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IN SILICO CHARACTERIZATION OF *POLE* p.Arg446Gln IN PATIENTS DIAGNOSED WITH ENDOMETRIAL CANCER DEFICIENT OF THE DNA MISMATCH REPAIR SYSTEM

Eduardo José Pereira Naves[1]; Adara Barbosa de Sousa[1]; Reginaldo Cruz Alves Rosa[1]; Victor Evangelista de Faria Ferraz[1]

[1]Department of Genetics, University of São Paulo - Ribeirão Preto Medical School. São Paulo - Brazil.

*adarasousaa@gmail.com

Keywords: Endometrial cancer; Lynch-Like Syndrome; Microsatellite instability.

Endometrial cancer (EC) is a gynecological neoplasia common in developed countries. Approximately 10% EC cases are related to hereditary causes, among them, Lynch syndrome (LS). LS is caused by germline mutations in genes related to the DNA mismatch repair system (MMR), resulting in tumors with microsatellite instability (MSI). Although MSI is a hallmark of LS tumors, more than 60% of all patients diagnosed with MSI endometrial cancer do not harbor a germline variant associated to LS, configuring Lynch-Like Syndrome (LLS). Another genetic disease associated with hereditary EC is Polymerase Proofreading-Associated Polyposis syndrome (PPAP), etiologically related to germline mutations in *POLD1* and *POLE* that encode the catalytic subunits of DNA polymerase alpha and delta, respectively. Germline *POLE* mutations can lead to MSI tumors, phenotype similar to LS (which configures LLS) in colorectal tumors, but the role of these variants in EC is still unclear. Three patients diagnosed with MSI EC were investigated for LS in a project conduced at University Hospital of the Ribeirão Preto Medical School (HCFMRP) through a multigenic panel that includes the LS genes, genes previously associated with LLS and other genes associated with EC predisposing syndromes. The panel includes the coding regions as well as the 5 'and 3' UTR regions and splicing processing sites of the *POLE* (NM_006231.3) that were captured using the SureSelectQXT library preparation kit (Agilent Technologies). The libraries were sequenced on the NextSeq platform (Illumina), and the variant was validated by Sanger sequencing. It was not identified any germline mutation in LS genes but a germline variant in *POLE* exonuclease domain (p.Arg446Gln – rs151273553) was identified. This variant is classified as Variant of Uncertain Significance (VUS) according to Clinvar. In order to characterize the variant, its frequency was checked in the population genomic databanks 1000 Genomes, Exac and ABraOM (Brazilian Genomic Variants) and its potential impact in the function of the protein was evaluated by in silico pathogenicity prediction tools (Mutation Taster, Sift, Polyphen, Provean and CADD). The population frequency of *POLE* p.Arg446Gln on 1000 Genomes and Exac are 0.0002794 and 0.0002562, respectively. This variant is not present on AbraOM databank. Reggarding to in silico prediction of pathogenicity, this variant as found as presenting tolerable effect based on Sift predictor (score: 0.073), probably damaging (score: 0.094) in Polyphen, pathogenic on Provean (score: -2.88) and CADD (score: 24) and regarding to MutationTaster prediction, the variant has pathogenic potential (score: 0.999). The present study reports the occurrence of *POLE* p.Arg446Gln in three patients with EC, two of whom are sisters, suggesting that the variant may be associated with the etiopathogenesis of the CE. Further studies involving segregation analysis in these families, as well as functional assay, can be useful to clarify the pathogenicity of p.Arg446Gln variant.

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EVALUATION OF THE MUTAGENIC/RECOMBINOGENIC EFFECT OF THE TALCC (TENSOACTIVE OF CASHEW NUT SHELL LIQUID) BY MEANS OF THE SMART TEST IN *DROSOPHILA MELANOGASTER* WINGS

Camila Mendes de Deus[1*]; Rosiane Gomes Silva Oliveira[1]; Bruna Cristina Silva[1]; Isabella Queiroz[1]; Juliana Miron Vani[2]; Denis Pires de Lima[2]; Adilson Beatriz[2]; Rodrigo Juliano Oliveira[2].

[1]Centro Universitário de Patos de Minas, Patos de Minas, Minas Gerais, Brazil. [2]Universidade Federal do Mato Grosso do Sul, Campo Grande, Mato Grosso do Sul, Brazil.

*camilamdeus@unipam.edu.br

Key words: TaLCC; SMART; *Aedes aegypti*.

The cashew nut shell liquid (LCC) is currently studied for its therapeutic and pharmacological properties, such as anti-inflammatory, antitumor and antioxidant activity. Besides these properties, it can also be used in the treatment of respiratory problems, asthenia, skin diseases and exert a strong larvicidal action against Aedes aegypti, the transmitter of Dengue fever, Chikungunya fever and Zika virus. Nowadays, dengue is an epidemiological disease of great concern to the Brazilian population. In this context, this study aimed to evaluate the possible mutagenic/recombinogenic effect of the Tensoactive of cashew nut liquid (TaLCC) by means of the Somatic Mutation and Recombination Test (SMART) in Drosophila melanogaster cells. To conduct the research the TaLCC was prepared by researchers from the Federal University of Mato Grosso do Sul, using absolute ethyl alcohol P.A. ACS VTEC 99% of purity, castor oil EXP type 01 lot M-05-04 Celtic, sodium hydroxide Dynamic P.A. ACS 97% of purity, distilled water, technical RESIBRAS LCC and natural LCC extracted by Soxhlet. In the Laboratory of Cytogenetics and Mutagenesis of UNIPAM (LABCIM), TaLCC was diluted in reverse osmosis water to obtain the concentrations 1.25, 2.5, 5.0, 10.0 and 20 ug / mL. Posteriorly, third stage larvae from the standard crossing (ST) between males "mwh" (mwh + / mwh +) and virgin females of the lineages "flr³" (flr³ / TM3, Bds) were treated with different concentrations of TaLCC, were also included doxorubicin as positive control at a concentration of 0.4 mM, and reverse osmosis water as negative control. The results revealed that only the concentrations of 20, 10, 5 and 2.5 µg / mL showed a statistically significant increase in the total of mutant stains when compared to the negative control. In view of the results we can conclude that the TaLCC, in the present experimental conditions exhibited mutagenic/recombinogenic effect, indicating that this can be one of the mechanisms by which the TaLCC exerts its larvicidal effects.

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FIRST REPORT OF EAST-CENTRAL-SOUTH AFRICAN LINEAGE OF CHIKUNGUNYA VIRUS IN THE ESPÍRITO SANTO, BRAZIL

Diego P Ventorim[1]; Fernanda MGS Rodrigues; Rebeca S Calasans[1]; Lucas A Vianna[1,2]; Raquel D Spinasse[1]; Luiz P Valli[†2]; Michelle Oliveira-Silva[1]; Iúri D Louro[1]

[1]Núcleo de Genética Humana e Molecular, Universidade Federal do Espírito Santo; [2]Laboratório Central do Espírito Santo – LACEN/ES.

*diego.pventorim@gmail.com

Key words: Chikungunya virus; Fylogeny; Lienage

Chikungunya fever is a highly debilitating arbovirosis caused by Chikungunya virus (CHIKV), which is transmitted by *Aedes* genus mosquitoes. In 2014, the first disease cases were registered in Brazil, showing the Asian genotype in Amazon region and East/Central/South African genotype in Bahia state, northeast of country. In the end of 2015, for the first time, cases were reported in Espírito Santo (ES) and between 2016- 2017 the state faced a disease outbreak. Despite of differences in epidemiological and pathogenicity among outbreaks involving distinct CHIKV lineages, few studies characterizing the diversity of strains have been reported. Thus, this study aims to identify the diversity of CHIKV strains circulating in ES state during the first chikungunya fever epidemic in 2016 and 2017. For this propose, positive samples for CHIKV by RT- qPCR tests were available from State Department of Health and the Central Laboratory of ES for PCR amplification of two envelope genes (E1 and E2) totalizing a fragment of 855 base pairs followed by Sanger sequencing. The six sequences obtained here were edited, aligned and analyzed with reference sequences from GenBank using MEGA 7.0 software. The analyzes showed that the circulating virus belongs to East/Central/South African lineage, which is responsible for outbreaks in Europe, Asia, Africa and in countries by the Indian Ocean. It was also observed that the ES virus clusters together with others of the same genotype circulating in Brazil. Moreover, molecular characterization of E1 and E2 protein fragments did not show the presence of adaptive mutations E1-K211E; E1-A226V; E2-L210Q and E2-I211T. This result suggests that circulating virus in ES has lower potential for dissemination compared to circulating viruses in recent major global epidemics. Furthermore, our data reveal a CHIKV-ECSA outbreak in Espírito Santo state which allows for the possibility of future infections by Asian-CHIKV strains also circulating elsewhere in Brazil. Lastly, due to the lack of an effective vaccine and the difficulty in mosquito vector control, genetic diversity studies are feasible alternatives for better understanding the chikungunya fever and help design efficient public health strategies for its control.

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Trypanosoma cruzi RNA binding protein 42: genotoxic stress induces aggregation into cytoplasmic foci

Daniela de Laet Souza[1]; Daniela Ferreira Chame[1]; Helaine Graziele Santos Vieira[2];Erich Birelli Tahara[1];Andrea Mara Macedo[1];Carlos Renato Machado[1]; Glória Regina Franco[1]

[1]Departamento de Bioquímica e Imunologia, Universidade Federal de Minas Gerais - Instituto de Ciências Biológicas, Belo Horizonte, Minas Gerais, Brazil. [2] Centre for Genomic Regulation, Barcelona, Spain.

danielalaet@gmail.com

Key-words: RNA binding protein; *Trypanosoma cruzi*, genotoxic stress

The parasite *Trypanosoma cruzi*, the etiological agent of Chagas disease, presents a remarkable resistance to stress conditions. The mechanisms underlying this resistance to stress are still unknown, but RNA binding proteins (RBPs) may play essential roles in stress response and cell recovery by aggregating into cytoplasmic granules. These granules could function as sorting centers for transcripts necessary for the parasite survival to stress. In this context, the main goal of this work was to study the involvement of an RBP, TcRBP42, in the *T. cruzi* stress response. Epimastigotes expressing 6-his- tagged versions of TcRBP42 and GFP (control) were treated with benznidazole (120µM and 240µM) and gamma radiation (500 Gy). The phenotype of the parasites expressing the tagged proteins was analyzed by their growth under normal and stress conditions. In all conditions tested, these parasites presented a similar growth pattern to that of wild-type (WT) cells. Additionally, both gamma radiation and benznidazole treatments arrested cell growth. Concerning the cellular localization of TcRBP42, immunofluorescence assays showed that this protein presents a differential localization in stress conditions. In normal conditions, TcRBP42 has a diffuse cytoplasmic distribution. However, both benznidazole and gamma radiation treatments cause the recruitment of this protein to cytoplasmic foci after 24 and 48 hours of treatment. The GFP protein remained distributed diffusely in the cytoplasm in all tested conditions. It was also analyzed the effect of the genotoxic stress induced by benznidazole and gamma radiation on RNA localization by labeling newly-synthesized RNAs with a uridine analog. At normal conditions, the newly-synthesized RNAs concentrate in the nucleus and kinetoplast. In contrast, 24 hours after parasite irradiation the newly-synthesized RNAs distributed diffusely in the cytoplasm, not being detected 48 hours after the gamma radiation exposure. For the benznidazole treatment, the newly-synthesized RNAs aggregated into cytoplasmic foci both after 24 and 48 hours of treatment. These results show that the distribution of newly-synthesized RNAs varies according to the stress type. In conclusion, the protein TcRBP42 and newly-synthesized RNAs present a differential distribution according to cellular conditions and may accumulate into cytoplasmic foci after stress induction.

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GLOBAL GENETIC DIVERSITY *TRICHIURUS LEPTURUS* (ACTINOPTERYGII: PERCIFORMES)

Najila Nolie Catarine Dantas Cerqueira[1], Vanessa Paes da Cruz[1], Matheus Marcos Rotundo[2], Alexandre Pires Marceniuk[1], Claudio de Oliveira[1]

[1]Department of Morphology, Institute of Biosciences, Universidade Estadual Paulista - UNESP, Botucatu, SP, Brazil. [2]Acervo Zoológico of the University Santa Cecilia (AZUSC), State University Cecília (Unisanta), Santos, SP, Brazil.

catariny_24@hotmail.com

Key-words: swordfish, DNA *barcoding*, biodiversity, speciation.

The species *Trichiurus lepturus*, popularly known as swordfish, is considered a cosmopolitan species, that inhabits estuarine and marine areas aroud the world, forming large shoals. In the reproductive phase they perform vertical migration to spawn in estuaries and mangroves.The morphological identification of the group is complex, due to the few available morphological characters and the species being very similar with to the others of the genus, compromising the identification of the group, resulting in numerous synonymous species. Due to the difficulty of morphological identification and wide distribution of the species in the oceans, the main aim of the presente projetc was to analyze the Species *T. lepturus* the technique of DNA barcoding, through the sequencing of the gene Cytochrome c oxidase I (COI), and to test the hypothesis of occurrence of barriers that may influence the distribution of the species. In this study, 16 specimens were sampled from the north to the southeast coast of Brazil. A muscle tissue fragment was used for DNA extraction, then the genomic materials of the COI gene was amplified by PCR, the amplicons were sequenced resulting in 16 sequences. In order to obtain a better robustness in the data analysis, 108 sequences were included in the present study including other species of the same genus *T. nitens*, *T. japonicos* and *T. gangeticus*, obtained through the BOLD database (Barcode of Life Date), from the Pacific and Atlantic Oceans, resulting in 124 sequences. The intraspecific genetic distance analysis (K2P model) ranged from 0.000 to 0.005, interspecific values ranged from 0.029 to 0.161. The Neighbor-Joining (NJ) and Maximum-likelihood (ML) trees revealed the formation of 10 clusters. Considering the results, analyzes of species delimitation were also carried out, and ABGD and GMYC analysis assigned ten lineages, two lineages for the Atlantic and eigth for the Pacific. The analyze of PTP detected 11 lineages, suggesting two lineages of *T. lepturus* for the Atlantic Ocean and nine lineages for the Pacific. These results indicate that the diferente lineages of *T. lepturus* represent a species complex, with cryptic species of occurring in the Pacific and Atlantic oceans. The factors that contribute to the differentiation of *T. lepturus* may be related to physical and chemical oceanic barriers, making it necessary additional molecular studies and possibly taxonomic revisions for a better compression of the species diversity.

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ASSOCIATION AMONG *COL1A1* GENE CIS-REGULATORY VARIANTS WITH PRODUCTION TRAITS IN NELORE CATTLE

Karina S. de Oliveira[1]; Juliana Afonso[2]; Andressa O. de Lima[3]; Tainã F. Cardoso[4]; Jennifer J. Bruscadin[5]; Luciana C. de A. Regitano[6]

[1] Undergraduate student in Biotechnology, Federal University of São Carlos, São Carlos, SP. PIBIC/CNPq scholarship students of Scientific Initiation, Embrapa Southeast Livestock, São Carlos, SP, Brazil. [2,3,5] Federal University of São Carlos, São Carlos, SP, Brazil. [4,6] Embrapa Southeast Livestock, São Carlos, SP, Brazil.

karina.santos.oliveira11@gmail.com

Key-words: *Bos indicus*; SNP; Phenotype.

Feed efficiency and meat quality are important traits in the beef cattle industry. Genomic analyses by identifying genetic biomarkers may support breeding programs. Thereby, previous studies in our research group identified the *Collagen type I alpha 1 chain* (*COL1A1*) as differentially expressed among divergent animals for feed efficiency. Based on the biological role of *COL1A1*, we aimed to identify single nucleotide polymorphisms (SNPs) in transcription factors binding site (TFBSs) regions associated to meat quality, mineral concentration, and feed efficiency traits. SNPs were obtained by genotyping 800 Nelore steers in *Illumina BovineHD BeadChip* (770K), plus those SNPs imputed from the genome sequencing of 20 bulls, sires of the population. The SNPs were selected within a window of 1 Mb *upstream* and 2 Kb *downstream* of the *COL1A1* gene start site. The association between the SNPs and the traits was carried out by using the *GEMMA* software whereas the *PLINK* software was used to estimate the linkage disequilibrium (LD) among all SNP pairs. Functional annotation from those SNPs significantly associated and in LD was carried out through the *SNPEff* software. TFBSs were predicted from the *LASAGNA* software considering flanking regions of 25 bp before and after each SNP. From 303 selected SNPs and the association analysis, considering the adopted criteria of SNP pairs in LD potentially affecting TFBSs corresponding to transcription factors expressed in liver, four pairs of SNPs were associated with feed efficiency phenotypes; seven pairs of SNPs were associated with mineral concentration phenotypes, and three of them were associated with meat quality traits. The SNP pairs in the *COL1A1 gene* with cis-regulatory activity and LD related to economically important traits are potential biomarkers for trait selection in animal breeding.

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STUDY OF THE C.-14C>T MUTATION IN THE *IFITM5* GENE IN BRAZILIAN PATIENTS WITH OSTEOGENESIS IMPERFECTA

Maira Trancozo[1,2]; Jucimara Figueiredo Almeida[1,2]; Dalila Avila Silva[1,2]; Flavia Imbroisi Valle Errera[1,2] ; Akel Nicolau Akel Jr[3]; Valentin Sipolatti[3]; Vanda Regina Rangel Nunes[3]; Maria Regina Galveas Oliveira Rebouças[3]; Meire Aguena[4]; Maria Rita Passos Bueno[4]; Flavia de Paula[1,2].

[1]Núcleo de Genética Humana e Molecular, Departamento de Ciências Biológicas, Centro de Ciências Humanas e Naturais, Universidade Federal do Espírito Santo, Vitória, ES, Brazil; [2]Programa de Pós-Graduação em Biotecnologia, Universidade Federal do Espírito Santo, Vitória, ES, Brazil; [3]Hospital Estadual Infantil Nossa Senhora da Glória, Vitória, ES, Brazil; [4]Universidade de São Paulo, SP, Brazil.

flapvit@yahoo.com.br

Palavras-chaves: *Osteogenesis Imperfecta*; *IFTM5* gene; c.-14C>T mutation.

Osteogenesis Imperfecta (OI) is a heterogeneous Mendelian disease caused, in general, by mutations in genes that codify proteins related with bone formation. Although the wide clinical variability, patients with OI can develop recurrent fractures and bone deformities. Distinct patterns of heritance, as autosomal recessive, autosomal dominant and X linkage are described in OI families according to the mutated gene. Therefore, characterize the mutation profile of distinct genes related with OI can help to improve strategies of the molecular diagnosis contributing with the genetic counselling. Most of the patients carry mutations in the *COL1A1* or *COL1A2* genes, that contributed with 75% of the changes. Several works have been analyzed these genes. However, few studies analyzed the other genes related with OI in specific populations, like the gene that encoding the interferon-induced transmembrane protein 5 (*IFITM5*). The c.-14C>T pathogenic variation is a recurrent change in this gene and causes an in-frame translation start codon that add five amino acids to the terminus of the IFTM5. Mutations in this gene are related with autosomal dominant heritance. In the present work we screened the c.-14C>T mutation in the *IFITM5* gene in 28 unrelated patients with clinical diagnosis of OI from the Hospital Estadual Infantil Nossa Senhora da Glória, localized in the Vitória-ES, Brazil. The DNA sample of the patients were analyzed through single strand conformation polymorphism (SSCP) screening, next generation sequencing (NGS) and/or Sanger sequencing. The c.-14C>T change was found in heterozygous state in only one Brazilian OI patient. He is an isolated case that has severe symptoms. In conclusion, the results suggest that the proportion of the c.-14C>T change in *IFITM5* gene in Brazilian population is approximately 10%. However, is important replicate this study in other cities of the country to increase the number of patients in the analysis and confirm the frequency of this change in Brazilian population.

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GENETIC COMPOSITION OF MITOCHONDRIAL DNA OF COLOMBIAN ANDEAN POPULATION

Lara Deccache[1]; Adriana Castillo[2]; Humberto Ossa[3,4]; Elizeu Fernandes de Carvalho[1]; Filipa Simão[1], Leonor Gusmão[1*]

[1]Laboratório de Diagnóstico por DNA, State University of Rio de Janeiro, Brazil. [2]Genetics Laboratory, Industrial University of Santander, Bucaramanga, Colombia [3] Pontificia Universidad Javeriana, Facultad de Ciencias, Bogotá, Colombia. [4] Laboratório de Genética y Biología Molecular, Bogotá, Colombia.

leonorbgusmao@gmail.com

Key-words: mtDNA; Colombia; admixed population

The composition of most American populations involves the admixture between Native Americans, who inhabited the region before the European colonization, Europeans and Africans, who were brought to America as slave labor. The genetic composition of Colombian populations results from the admixture between these three population groups and tends to have a high genetic diversity. In the light of this, the aim of this study was to obtain an overview about maternal lineages composition, and diversity, from the Andean populations of Colombia. Then, a sample of 100 unrelated individuals from this region were selected for sequencing the control region of mtDNA,and to calculate haplotype diversity and haplogroup composition. Preliminary results from 38 samples were used to calculate FST genetic distances between our sample and published data from South American admixed populations from Colombia, Peru, Argentina, Paraguay and Brazil. No significant differences were observed between our sample and the Colombian population deposited on 1000 Genomes Project. On the other hand, high FST genetic distances were obtained in the pairwise comparison between our sample and Peru, Argentina, Paraguay and Brazil. A haplotype diversity of 0.9900 ± 0.0088 was observed with 4 haplotypes being shared between 2 individuals and one haplotype shared between 3 individuals. 95 % of the haplotypes belong to halogroups found both in central and south America (A2, B2d, C1, D1 and D4). The remaining 5% (2 samples) belong to the African haplgroups L3.

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ANALYSIS OF THE EFFECT OF THE microRNA-1914-5p IN THE CONTROL OF LIPID DROPLETS AND THE REACTIVE OXIGEN METABOLISM IN LIVER CELLS SUBMITTED TO PRO- STEATOTIC CONDITION

Ligia Pagliotto Marques Pereira[1]; Thaís Porto Barbosa[2]; Letícia Ferreira Ramos[3]; Karen Cristiane Martinez de Moraes[1,2,3]

[1] Departamento de Biologia, Universidade do Estado de São Paulo (UNESP) – Instituto de Biociências (IB) - Campus Rio Claro, São Paulo - Brazil. [2]Pós-Graduação em Biotecnologia, UNESP - Instituto de Química (IQ) – Campus Araraquara. [3]Pós- Graduação em Biologia Celular e Molecular, UNESP - IB – Campus Rio Claro.

ligiapagliotto@gmail.com

Key words: cell culture; hepatic steatosis; oxidative stress

Non-alcoholic hepatic steatosis (NAFLD) has been pointed as a serious health problem considering the increasing number of committed people worldwide. Besides, if the disease is not controlled, it may support the development of fibrosis and even hepatocarcinoma (HCC). It is known that at the early stage of NAFLDs, cells have remarkable increase in triglycerides and cholesterol, due to imbalanced liver metabolism. As a result, oxidative stress mechanisms is present, which contributes to the worsening of the disease. Thus, innovative ways to control the lipid metabolism in the organ is mandatory, once no effective therapeutic strategy is available so far. Recently, the microRNA-1914-5p was characterized as a modulatory element of lipid metabolism in the hepatic stellate cells, LX-2. miRNAs play major role in the controlling of gene expression and cellular homeostasis. Considering that in the liver the hepatocytes represents more than 80% of the cells, the main goal of this study was to investigate the effect of microRNA-1914-5p in controlling the deposition of the lipids in cells, cultivated under pro-steatotic conditions and evaluates its effect on the oxidative stress mechanisms. For the analyses, HepG2 cell line (ATCC® HB-8065™) was cultivated in DMEM (Dulbecco's Modified Eagle's Medium) medium containing 10% of fetal bovine serum (FBS).To simulate pro-steatotic conditions, 400 µM of fatty acids mixture (oleic and palmitic) were added to the culture medium and cells were transfected with either 40 nM of miR-1914-5p mimetic or inhibitory molecules (miRVana™, Thermo Fisher Scientific™), according to the reagent supplier. All positive and negative assay controls were performed. Next, the lipid droplets were analyzed in all the investigated groups of cells by fluorescence microscopy, using Nile Red staining. The results indicated reduction of lipid droplets in cells transfected with the miR-1914- 5p inhibitory molecules. Moreover, the reactive oxygen species (ROS) and the expression of relevant genes connected to the ROS production such as *sod*, *rac-1*, *gpx*, among others were investigated using the cDNA of these cultures in conventional PCR reactions (RT-PCR). The results were quantified and submitted to statistical tests in the program GraphPad, version 5.0 (Prism Inc.). Analyzes revealed that there are no statistical differences in expression of the *sod-1* gene when subjected to pro-steatotic conditions in the presence or absence of miR-1914-5p. However, the *rac-1* gene showed a significant increase of approximately 50% in its expression in the pro- steatotic environment and a considerable decrease in its level, approximately 1.0 equaling the control, in the presence of the miR-1914-5p inhibitor. *Rac1* contributes to the establishement of the oxidative stress process, so it can be inferred that in steatotic conditions the inhibition of the miR-1914-5p contributes to the reduction of lipid droplets and the oxidative process in hepatocyte cells.

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PROSPECTION OF PHAGES FOR FIG FLIES' CULTIVABLE GUT BACTERIA

Mombach, Daniela M.[1*]; **Ardisson-Araújo, Daniel**[2]; **Loreto, Élgion L. S.**[2]

[1]Laboratório de Biologia Molecular e Sequenciamento – LabDros (UFSM). [2]Laboratório de Virologia de Inseto – LaVi (UFSM).

*danielamombach@gmail.com

Keywords: dysbiosis; phageterapy; fruit fly

The gut commensal microbial community plays an important role in shaping the health and fitness of animals, such as insects. Fruit flies are a valuable model for microbiome research that combines genetic and genomic resources with simple protocols to manipulate the microbiota. Furthermore, recent studies show that the gut microbiota is an important regulator of *Drosophila* immune cells (e.g.: crystal cells) and it participates in the activation of antiviral immunity in the flies' gut, supporting that the microbial community also plays a necessary role in *Drosophila* immune system. Symbiotic relationship between phage and metazoan hosts provide anti-bacterial immune defense. So, we hypothesize that phages may impact the flies' microbial community and protect the organism from opportunistic bacteria invasion. We aim to test this hypothesis by causing dysbiosis in flies based on phageterapy and study its effects. The shift in the flies' microbiota will be caused by a phage that infects a cultivable Gram-negative gut-isolated bacteria. For this project, we selected *Zaprionus indianus* (common name fig-fly) to be the target organism. *Z. indianus* was first reported in Brazil in 1999 and it became spread throughout the country. The flies were caught using banana baits in Santa Maria city, RS, the flies' gut were dissected and plated in LB plus sucrose medium (30°C pH 5). We have isolated two morphologically different colonies, which were further identified as yeast and Gram-negative bacterium. The next steps are to identify the bacteria through 16S DNA sequencing and prospect for phages that infect this specific bacterium. By feeding the flies on phage-containing substrates, we aim to study the impact of putative dysbiosis caused by phageterapy upon *Z. indianus* microbiota and evaluate the concept of non-host-derived immunity.

Unusual case of mosaicism for balanced robertsonian translocation associated with abnormal phenotype.

Lima IBF; Oliveira SFS; Cantanhede GN; Fonseca CR; Serrão ALV; Moraes LFM; Llerena Jr JC; Bastos EF

Cytogenetic Laboratory / Genetics Center Dr. José Carlos Cabral de Almeida / Fernandes Figueira National Institute / FIOCRUZ

Robertsonian translocations (ROBs) are the most common type of structural chromosome rearrangement in the general population with an incidence of 1/1000 births. The majority is presented as a balanced alteration, without phenotypic effect, being important in terms of genetic counseling. However, the association with abnormal phenotype has already been reported, having as one of the explanations a post-zygotic mosaicism event. At birth, ROB mosaics, even if balanced, are extremely rare. In this work we report a case of ROB mosaicism with a normal line and a balanced t(13; 21) associated with diaphragmatic hernia. The patient, male, was diagnosed with diaphragmatic hernia still intra-uterus; cesarean birth, non-consanguineous parents, 42 year old mother and father unknown age. The cytogenetic evaluation was performed according to standard protocols, from peripheral blood samples stimulated by phytohemaglunin; GTG banding and ISNC classification (2016). The CBG technique was performed according to standard protocol. The FISH technique was performed using the satellite alpha probe 13; 21 and the UBE3A probe (D15S10) according to the manufacturer's guidelines. The GTG analysis identified the presence of two cell lines, one containing 45 chromosomes, including a seemingly balanced robertsonian translocation between chromosomes 15 and 21 (45, XY, t (15; 21) (q10; q10)) and another containing normal karyotype (46, XY), with about 50% of each. A new sample was collected which confirmed the presence of two cell lines. The CBG technique identified only one centromere on the translocated chromosome, which was confirmed by FISH, suggesting centromeric fission or loss of one of the centromere. Mosaicism with the presence of a normal cell line and a structural alteration is extremely rare. This corresponds to one of the only cases in the literature of balanced mosaic robertsonian translocation. Two mechanisms were proposed for the origin of this type of mosaicism: A dissociation of translocation already present in the zygote; and a translocation of chromatids into an originally XY zygote. The origin of two independent cell lines can also be considered, as in the cases of chimeras. The latter possibility can be investigated by analysis of microsatellite polymorphisms. Congenital diaphragmatic hernia (HDC) is a malformation characterized by a defect in the diaphragm leading to protrusion of abdominal contentes into the thoracic cavity, corresponding to 8% of the congenital malformations. Most cases are sporadic, however, the genetic contribution seems to be important ranging from 2 to 33% of cases that vary enter monogenic causes and chromosomal changes. Considering reports of abnormal phenotype as a consequence of mosaic ROB, the existence of a causal relationship could be explained by mechanisms such as uniparental disomy, leading to the existence of recessive genes in homozygosis and / or imprinting of genes on chromosome 15 influencing the etiology of HDC.

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SNP CALLING AND IDENTIFICATION IN RNA-SEQ DATA FROM AFRICANIZED HONEYBEE (APIS MELLIFERA L.)

Luiz Afonso Glatzl Júnior[1*]; Raquel Morais de Paiva Daibert[1]; Amy Toth[2]; Jay Evans[3]; Marco Antônio Machado[1]; Marcos Vinicius Gualberto Barbosa da Silva[1]; Érica Weinstein Teixeira[4]

[1]Laboratório de Bioinformática e Genômica Animal, Embrapa Gado de Leite, Juiz de Fora, Minas Gerais, Brazil. [2]Iowa State University, Ames, Iowa, US. [3]Bee Research Laboratory, USDA, Beltsville, Maryland, US. [4]Laboratório Especializado de Sanidade Apícola, Instituto Biológico, São Paulo, São Paulo, Brazil.

*luiz.glatzl@engenharia.ufjf.br.

Keywords: bioinformatics; transcriptomics; genomics

Next-generation sequencing techniques are very used in genomics and transcriptomics, resulting in a large amount of data for research with reduced costs and faster than Sanger method. These techniques allow the comparison of gene expression in different conditions and detection of Single Nucleotide Polymorphisms (SNP) markers. The present study was carried out to identify SNPs in Africanized honeybee transcriptome. Experimental samples were collected in colonies under selection for hygienic behavior, which was classified into two groups according to the percentage of brood removed in 24 hours after pin-killing pinked-eyed pupae. After RNA extraction, samples were submitted to Illumina RNA-Sequencing technology on a HiSeq3000 machine. Quality control check of the reads was performed using FASTQC and low quality reads were trimmed using Trimmomatic software. Reads were aligned to *Apis mellifera* genome assembly v4.5 with STAR aligner. Samtools suite was used to convert SAM files to sorted and indexed BAM format. GATK and FreeBayes software were used to variant calling which were filtered using VCFTOOLS. A total of 23M 100bp reads per sample were generated for 12 samples. After quality control, 2,366,213 reads were trimmed. The average number of aligned reads was 85% when aligned to *Apis mellifera* reference genome. Variants identified were filtered for a coverage > 7 and quality score > 30. These analyses allowed the discovery of variants in Africanized honeybee transcriptome and identified 122,226 SNPs, 35,863 insertions and 32,867 deletions. These findings are important to the identification of candidate genes related to economic important traits in honeybees.

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IDENTIFICATION OF CATHELICIDIN SEQUENCES IN TAMBAQUI (*Colossoma macropomum*)

Susiana Ipuchima Lima[1]; José de Ribamar da Silva Nunes[1] *

[1]Instituto de Natureza e Cultura – Universidade Federal do Amazonas.

*ribamarnunes@ufam.edu.br

Key-words: Alignment; disease resistance; antimicrobial peptides.

In Brazilian aquaculture, the tambaqui (*Colossoma macropomum*) is farmed in extensive and semi-intensive production systems where there is not adequate control of environmental variables. Thus, it is important that these animals exhibit genetic characteristics for better performance and are disease resistant. The cathelicidins comprise a family of antimicrobial peptides (AMPs) that have been identified in epithelial tissues and some myeloid cells of humans and animals. It has activity against a wide range of bacteria, fungi, enveloped viruses, and protozoa. The cathelicidins identification in fish are recent and there no studies describing any cathelicidin family member in tambaqui. In this study we carried out on Genotyping by Sequencing (GBS) libraries obtained from 424 farmed and wild individuals from three different Brazilian regions to SNPs discovery. We perform a functional annotation in BLAST using 1,668 cathelicidin nucleotides sequences from GenBank (1,140) and RefSeq (528) data base and Discontiguous Magablast Algorithm (with adjusted parameters). A total of 182,416 GBS sequences was used. It generated 13 alignments in 15 animals' species. Six tambaqui sequences had positive alignment to cathelicidin antimicrobial peptide gene (Camp) from *Mesocricetus auratus*, *Echinops telfairi*, *Saimiri boliviensis, Aquila chrysaetos canadensis, Mus caroli* and *Haliaeetus leucocephalus*. The others sequences were aligned with cathelicidin sequences from *Gallus gallus, Rattus norvegicus, Amolops loloensis, Protobothrops mucrosquamatus, Protobothrops mucrosquamatus, Lepisosteus oculatus, Bubalus bubalis, Ovis aries* and *Bos taurus*. The large number of species related to tambaqui cathelicidin sequences was expected, once a mature cathelicidin sequence varies greatly, not only between species but also among the often multiple cathelicidin peptides within a single species. The identification of candidate loci of cathelicidin in tambaqui can provide a valuable tool for selection of individuals more resistant to bacterial invasion.

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EVALUATION OF CYTOTOXIC POTENTIAL OF FORMONONETIN IN HUMAN MELANOMA CELL LINE

Arthur Barcelos Ribeiro[1]; Pollyanna Francielli de Oliveira[2]; Denise Crispim Tavares[1]

[1] University of Franca, Franca, São Paulo, Brazil. [2] Federal University of Alfenas, Alfenas, Minas Gerais, Brazil

Key words: Formononetin; Melanoma; Cytotoxicity.

Melanoma (MLA) has a heterogeneous and complex etiology resulting from the interaction of genetic, immunological and environmental factors, which play essential roles in all phases of its development. The best therapeutic responses for the treatment of MLA it is based on the infusion of cytotoxic drugs, limited by immunosuppression, resistance and high toxicity. In the search for new molecules for treatment, as well as in reducing the side effects of existing treatments, natural products are highlighted in cancer chemotherapy and can be used in treatments alone or combined with other antineoplastics. In this sense, formononetin (FOR), as a chemical marker of extracts of Brazilian red propolis, presents promising antiproliferative activity. In addition to the various biological activities reported in the literature as anti-inflammatory, anti-diabetic, analgesic and antimicrobial, most studies with FOR are based on cytotoxic assays against a wide range of tumor cell lines. The present study aimed to evaluate the cytotoxic activity of FOR in human melanoma cell line (A-375) for 24, 48 and 72 hours by the XTT (2,3-Bis-(2-methoxy-4-nitro5-sulfophenyl)-2H-tetrazolium-5-carboxanilide salt) cell proliferation assay. Cell cultures were treated with FOR in concentrations ranging from 5 to 640 µM, including negative (no treatment) and solvent (0.8% dimethylsulfoxide) controls. Cisplatin (CDDP) and dacarbazine (DACA) were evaluated at the under the same experimental conditions of FOR for the comparative effect. The results showed that FOR was not able to reduce the proliferation of A375 cells at concentrations assessed by the XTT assay. However, CDDP and DACA demonstrated cytotoxic effect. The inhibitory concentrations of 50% cell proliferation (IC_{50}) were 6.95± 0.03, 6.20 ± 0.15 and 3.34 ± 0.26 µM for CDDP during 24, 48 and 72 hours of treatment, respectively. DACA showed IC_{50} of 556.96 ± 12.38, 29.12 ± 1.79 and 3.11 ± 0.29 µM for 24, 48 and 72 hours of treatment, respectively. Therefore, it can be concluded that FOR showed no inhibitory action of A375 cell proliferation, under the experimental conditions used.

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DESCRIPTION OF MITOCHONDRIAL GENOMES AND PHYLOGENETIC INFERENCE FROM FIVE MIGRATORY FISH SPECIES FROM THE GENUS *PROCHILODUS*

Rosiane P. Santos[1,2], Mateus S. Vidal[3], José Mauro Ribeiro[2], Gabriel M. Yazbeck[2] Francisco Prosdocimi[3], Daniel C. Carvalho[1]

[1]Laboratório de Genética da Conservação, Programa de Pós-Graduação em Biologia dos Vertebrados, Pontifícia Universidade Católica de Minas Gerais (PUC Minas), Belo Horizonte, MG, Brazil. [2]Laboratório de Recursos Genéticos, DEZOO, CTAN, Universidade Federal de São João del-Rei (UFSJ), São João del-Rei, MG, Brazil [3]Laboratório de Genômica e Biodiversidade, Instituto de Bioquímica Médica Leopoldo de Meis, Universidade Federal do Rio de Janeiro (UFRJ), Rio de Janeiro, RJ, Brazil.

rosianeps2007@yahoo.com.br

Keywords: phylogenomics, phylogeography, mitogenome

Current science has been revolutionized by technological advance that increasingly allows the massive acquisition of data and information. In biology, advances in the development of technologies that allow access to genetic material made the process much faster and more accessible, enabling a significant increase in information and creating the 'genomic era'. An example of this is how the description of genes and genomes can be used in phylogeny, that is, the study of the evolutionary relationship between groups of organisms. The mitogenoma has been used to solve phylogenies and to understand biogeographic patterns. Fish constitute the group of vertebrates with the largest number of species and represent one of the best groups of organisms for the identification of biogeographic patterns. The Characiformes order of fishes includes about 2,000 species, distributed in 24 families, with 19 being endemic to the Neotropical region. The genus *Prochilodus* is one of the most abundant and widely distributed, including 13 species of occurrence in the main basin of South America. The objective of this work was to use complete mitogenomas for the better understanding of phylogenetic and biogeographic patterns that gave rise to the biodiversity of *Prochilodus.* The analyzes started with the sequencing of mitogenomas, using a new generation sequencing approach (NGS), and assembled using MIRA and MITObim software. The annotation of the mitochondrial genomes was carried out with the program Mitos Web Server and Mitoannotator. We also used 42 publicly available mitogenomas, belonging to 17 Characiformes families, for the assembly of a dated phylogenetic tree. Our results corroborate the previous phylogenetic hypotheses of the genus Prochilodus and suggest its origin during late Pliocene and radiation around the Calabrian Miocene, possibly caused by the connection event in the headwaters of the basins. Intraspecific comparisons were made between mitogenomas of the species studied, corroborating the specific divergence attributed to the current taxonomic classification of some species and the results found in this work will allow the formulation of paleo-biogeographic hypotheses, such as events of past connection between the basins of the Jequitinhonha river and of the São Francisco, as well as of this with the Upper Paraná, where the species studied are found.

MIR 17-5P AND MIR137 MAY BE ASSOCIATED WITH ANDROGEN RECEPTOR REACTIVATION IN CASTRATION RESISTANT PROSTATE CANCER

Ruan Pimenta[1]; Vitória Ghazarian[2]; Poliana Romão[1]; Juliana Camargo[1]; Vanessa Guimarães[1]; Nayara Viana[1]; Gabriel Arantes[1]; Iran Silva[1]; Kátia Ramos[1]; Miguel Srougi[1]; Sabrina Reis[1].

[1] Laboratory of Medical Research (LIM55), Department of Urology, Faculty of Medicine (FMUSP), University of São Paulo, São Paulo, SP-BR. [2] University of Sao Paulo City, Sao Paulo, Brazil.

Keyword: Prostate Cancer; Coactivators; microRNAs.

Abstract: The molecular characterization of the Castration Resistant Phenotype in Prostate Cancer (CRPC) has been extensively studied. It is now known that androgenic signaling persists even after the transition from the of Hormone-Sensitive Prostate Cancer (HSPC) to the CRPC. Considering the importance of androgenic signaling in the transition from HSPC to CRPC, there is a need for identification of new components that may in future serve as biomarkers and also for therapeutic purposes in PCa. Therefore, we highlight the cofactors (coactivators) of the Androgen Receptor (AR) that may represent new therapeutic targets attractive for this pathology, since AR can recruit numerous transcriptional enzymatic cofactors. These cofactors interact with AR and increase their transactivation and promiscuity, thus responding to low levels of androgens. Thus, our group evaluated the role of 5 AR co-activating genes in CRPC cell line (PC-3M-luc-6) and also the action of two microRNAs that targets these genes directly. We studied miR-17-5p and its p300 target genes and the CBP / p300 complex and miR-137, and their target genes, SRC1, 2 and 3. Our objective was to demonstrate the expression levels of these genes and microRNAs in the cell line resistant to castration. The gene expression and miRNAs experiments were performed using the qRT-PCR technique, using triplicate of the cellular samples and the control group was composed of 6 tissue samples from patients with Benign Prostatic Hyperplasia (BPH). The expression data are represented by the triplicate average. In our results, we found low expression of miR-17-5p (0.013) and overexpression of p300 (2,900) and CBP / p300 (20,679) genes. For the miR-137, we found no expression in the PC-3M-luc-6 cell line, and in relation to its target genes, we found an overexpression of the three genes, SRC- 1 (3,425), SRC-2 (7,211) and SRC-3 (39,817). The results presented are preliminary and further studies will be carried out to confirm the direct interaction of these microRNAs with their target genes and also for analysis of cell assays after induction of these microRNAs in this lineage. Thus, we can conclude that the absence or diminution of the expression of these miRNAs and overexpression of the coactivators studied may be associated to the reactivation of AR and consequent reduction of response to the last generation therapies for the castration resistant phenotype.

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ANALYSIS OF ANTICARCINOGENIC POTENTIAL OF *SALVIA OFFICINALIS* EXTRACT, THROUGH TEST FOR DETECTION OF EPITHELIAL TUMOR CLONES (WARTS) IN *DROSOPHILA MELANOGASTER*

Isabella Queiroz[1]; Yulla Fagundes Severino[1]; Bruna Cristina Silva[1]; Camila Mendes de Deus[1]; Bethânia Cristhine de Araújo[1]

[1] Laboratório de Citogenética e Mutagênese (LABCIM) - Centro Universitário de Patos de Minas – UNIPAM.

isabellaqueiroz99@hotmail.com

Key-words: *Salvia officinalis;* anticarcinogenic; warts.

Cancer has emerged as one of the diseases with the highest mortality rate of the last decades. It is a multifactorial pathology initiated by the process of carcinogenesis, which involves the stages of initiation, promotion and progression and is characterized by uncontrollable growth and invasion of abnormal cells, causing a neoplasia. In addition, due to the high cost and side effects caused by conventional approaches, there is a great demand for alternatives that can also be used in the treatment of the disease, and this connection seems to be promising on the increase in the life quality of the cancer patients. *Salvia officinalis* has a high antiproliferative and neuroprotective potential, as well as an inhibitory effect on the cell division of tumor cells. The aim of this study was to evaluate the anticarcinogenic effect of the hydroalcoholic extract of *Salvia officinalis* on somatic cells of *Drosophila melanogaster* through the test for the detection of clones of epithelial tumors (warts). The *Salvia officinalis* extract was obtained from a solution of 500 mL of 70% (v/v) CH_3OH, filtered and evaporated under reduced pressure. The obtained powder was resuspended in 5% (v/v) C_2H_5OH until the test concentrations (400 $\mu g.mL^{-1}$, 500 $\mu g.mL^{-1}$ and 600 $\mu g.mL^{-1}$). Doxorubicin (DXR) was used as a positive control due to its previously proven carcinogenic properties and ethanol was used as a negative control. In order to obtain the wts/mwh lineage, the crossbreeding between virgin wts/TM3 females and mwh/mwh males occurred. After the treatment, adult flies were analyzed in terms of the tumor frequency on the different structures of the fly's body and the results were submitted to statistical analysis. Differences between tumor frequencies of the tested and control concentrations were calculated using the Mann Whitney U non-parametric test, with significance level $p < 0,05$. In the isolated treatments there was no statistically significant difference between the frequencies of tumors found in the concentrations of 400 $\mu g.mL^{-1}$ and 500 $\mu g.mL^{-1}$ and the negative control. In relation to the concentration of 600 $\mu g.mL^{-1}$ the tumor frequency was even significantly lower, showing a dose-dependent reduction. In the associations between DXR and *Salvia officinalis* an anticarcinogenic effect was observed in all the concentrations tested, when compared to the positive control. The reduction in tumor frequency for the associated concentrations was 89,5%; 94,7% and 92,8%, respectively. Therefore, the anticarcinogenic effects of the *Salvia officinalis* hydroalcoholic extract on *Drosophila melanogaster* have been reported, further studies are proposed to confirm the specific concentrations in which the extract has cytotoxic capacity for tumor cell lines.

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ASSOCIATION BETWEEN ANGIOSTESIN-CONVERTING ENZYME (ACE) I/D POLYMORPHISM AND ESSENTIAL HYPERTENSION IN VITÓRIA DA CONQUISTA-BA

Samuel dos Santos Oliveira[1]; Pedro Barros Cerqueira[1]; Mariane de Oliveira Barreto[1]; Poliana Souza Santos Campos[1]; Sandra Mara Bispo Sousa[1]; Patrícia Santos Pereira Lima[1].

[1]Universidade Estadual do Sudoeste da Bahia – UESB, *campus* Vitória da Conquista;

samuel_bio20151@outlook.com

Key words: Essential hypertension; genetic polymorphism; ACE.

Essential hypertension (EH) is a world spread health problem, being a multifactorial disease that affects between 20% and 30% of the global population. Many studies have being searching the association between genetic polymorphisms and HE, contributing to the knowledge on the disease. The insertion (I) and deletion (D) of 287 base pairs polymorphism in the 16th intron ot the Angiotesin-Converting Enzyme (ACE) gene it's being studied as possible risk factor for EH doe to his important function in the homeostasis of extracellular fluids and so the control of arterial pressure. The D alele is the most frequent according in the studied populations (European and Asiatic) and some researches are pointing to it as an risk factor for EH, however that relation are still not clear. The present study try to search the frequency of the ACE's I/D polymorphism in the population of Vitória da Conquista – BA and check for association between that polimorphism and EH. One hundred fifteen subjects where genotyped through Polymerase Chain Reaction (PCR), being 65 cases with EH and 52 controls. For this study we consider hypertensive subjects that declareted use of antihypertensive drugs. The Hardy-Weinberg equilibrium (HWE)and the genetic differentiation were checked trough Genepop 4.2 software. In the case group the I and D alleles were respectively 41.5% and 58.5%. with the genotipic frequency for DD, ID and II being 33.8%, 49.2% and 17% respectively. In the case group the frequency of I and D were 38.2% and 62.8% respectively, with the frequency for DD, ID and II being 37.25%, 49% and 13.75%, respectively. Both groups where in HWE. There was no association between the I/D polymorphism and EH (gene differentiation analysis, p = 0.69029, genotype differentiation analysis, p = 0.69077). The allele frequency found in the present study do not differentiate from those see in most of the populations, although a bigger amount of samples will be added to the analyses and ancestry markers will also be utilized, since the population of Bahia are from very mixed races, which could differentiate from other studies in the area.

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Steatotic potential effects of the phytosanitary products ethiprole and fipronil in the hepatocyte cell line HepG2

Lara Cavalari Santello[1]; Thaís Porto Barbosa[2]; Letícia Ferreira Ramos[3]; Karen Cristiane Martinez de Moraes[1,2,3]

[1] Departamento de Biologia, Universidade do Estado de São Paulo (UNESP) – Instituto de Biociências (IB) - Campus Rio Claro, São Paulo - Brazil. [2]Pós-Graduação em Biotecnologia, UNESP - Instituto de Química (IQ) – Campus Araraquara. [3]Pós-Graduação em Biologia Celular e Molecular, UNESP - IB – Campus Rio Claro

*lara.santello@gmail.com.

Key words: cell culture, agriculture poisoning, hepatic steatosis.

Hepatic steatosis has been considered a serious health problem by the World Health Organization (WHO). The pathology is characterized by an excessive fat accumulation in the liver, which is able to damage the tissue and, in some cases, the organ is able to develop cirrhosis and even hepatocarcinome. The hepatic steatosis, in its initial phase, presents a chronic inflammatory state of the hepatocytes and the lipid accumulation in the cells is able to induce mitochondrial dysfunction, which connects to the disruption of homeostasis of the entire body. Unhappily, hepatic steatosis can be induced by several different ethological agents and considering the intense use of agriculture pesticides, also called phytosanitary, it is mandatory to investigate the negative cellular and molecular processes in the liver that are connected with the effects of the pesticides. Based on the above observations, the present study aims to evaluate the cellular and molecular effects of the organophosphorus pesticides fipronil and ethiprole present in the composition of the commercial reagents REGENT® 800 WG and CURBIX 200 SC® in the hepatocyte cell line HEPG2 (ATCC® HB-6075™). This cell line was chosen, based on its high metabolic rates. For the analyses, MTT assays were performed using different concentrations of the phytosanitaires (0,2 - 200 μM). The results demonstrated any negative effect in cellular viability under 0,2 μM, and because of this we selected the concentrations of 0,2 μM and 2 μM of either fipronil or ethiprole to be further evaluated. Using the mentioned concentrations of the phytosanitaires, morphological changes in the cultures were observed, especially in ethiprole-treated cells. Next, cells were stained with Nile Red and fluorescence microscopy demonstrated cleared changes in lipid deposition in phytosanitary-treated cells, when compared to the untreated culture. In addition, to analyze molecular elements that support the effect of the investigated compounds in hepatic cells, semi-quantitative polymerase chain reactions (PCRs) were performed and the results demonstrated changes in relevant enzymes of the Reactive Oxygen Species (ROS) production, such as Rac1, GSR, SOD between others in phytosanitary-treated cells, when compared to the untreated groups. Actually, we are investigating elements that contribute to the lipid metabolism to address molecular elements that mechanistically contributes to the lipid accumulation in HEPG2 phytosanitary-treated cells.

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CHEMICAL ECOLOGY: EVOLUTION OF GLUTATHIONE S-TRANSFERASE D1 PROTEIN IN CACTOPHILIC *Drosophila*

Adriano Silva dos Santos[1], Silvana Giuliatti[1], Maura Helena Manfrin[1,2].

[1]Departamento de Genética – FMRP - Universidade de São Paulo – Ribeirão Preto, São Paulo, Brazil. [2]Departamento de Biológicas – FFCLRP - Universidade de São Paulo - Ribeirão Preto, São Paulo, Brazil.

adrianosantos@usp.br

Key-words: *Drosophila* cactophilic; GSTD1 protein; chemical evolution.

The *Drosophila buzzatii* cluster (*D. repleta* group) consists of seven cryptic species that use necrotic tissues of cacti as breeding sites. Cacti species consist of complex chemical groups, such as the alkaloids. The alkaloids alter the metabolic pathway in flies, as showed by differences in gene expressions of some cactophilic species. In *D. buzzatii*, the *GstD1* gene is overexpressed in larvae as they develop in secondary host cacti, that can be related to metabolic detoxification function. In the present work, we isolated the complete sequence coding regions of *GstD1* from the seven species of the *D. buzzatii* cluster, with the objective of investigating the genetic variants and selection signal that result in structural and / or functional modifications of GSTD1 protein. We sequenced 630 bp of the *GstD1* gene for each species and the proteins primary structures with 209 amino acids were defined by in silico analysis. In the Selecton program, the selection signals were evaluated and compared using M8 (beta, $\omega \geq 1$), M8a (beta, $\omega S = 1$), M7 (beta, $\omega < 1$). Three positive selection signals were detected for the following species and codons with the respective amino acid residues: *D. buzzatii*, *D. gouveai*, *D. koepferae*, *D. serido*, *D. borborema* and *D. antonietae* (Gln-39- hist); *D. koepferae* and *D. serido* (Leu-117-Val); *D. seriema* and *D. borborema* (Thr-133-Ala); *D. buzzatii* (209-Asp). These substitutions represent changes in the chemical structure of proteins. Among these substitutions, residue 39 (hist), positive charge, represents a gain in the function of chemical interaction and catalysis within the G-site. The selection signals at residue 117 (val) represent a gain in the recognition of hydrophobic substrates, such as lipids. Residue 209 (Asp), of negative charge and reactive with non-protein atoms, may represent a gain in the chemical interaction capacity with metallic ions, such as zinc. Later, we performed homology modeling using the *D. mojavensis* GSTD1 tertiary structure model (GenBank: 3MAK) to model the proteins of each species of the *D. buzzatii* cluster, and in the PyRx program, we performed protein docking with the mescaline linker (alkaloid with toxic potential, present in several host cacti). The proteins of *D. seriema* (-5.9 kcal/mol), *D. gouveai* (-5.9 kcal/mol) and *D. koepferae* (-5.6 kcal/mol) presented the best chemical interactions, with lower costs energy. For *D. buzzatii*, the highest energy expenditure (-4.8 kcal/mol) was demonstrated. Likewise, *D. koepferae* and *D. buzzatii* show plasticity when using the cactus *Trichocereus terscheckii,* with *D. buzzatii* presenting less adaptation to this cactus. Therefore, the genetic variants in *GstD1* codons with selection signals of *D. gouveai*, *D. seriema* and *D. koepferae* represent a gain in the catalytic function of their proteins in the detoxification process.

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GENOMIC SIGNATURES OF RECENT SELECTION IN THE BRAZILIAN MANGALARGA MARCHADOR HORSE

Wellington Bizarria dos Santos[1]*; Guilherme Luis Pereira[1]; Júlio Cesar de Carvalho Balieiro[2]; Guilherme de Camargo Ferraz[1]; Henrique Nunes de Oliveira[1]; Rogério Abdallah Curi[1]

[1]São Paulo State University – UNESP. [2]University of São Paulo – USP.

* wellington.bizarria@unesp.br

Keywords: JNK; iHS; ROH

In this study, two methodologies - integrated Haplotype Score (iHS) and Runs of Homozygosity (ROH) - were used to capture, and validate as much as possible the evolutionary scenario for recent positive selection in Mangalarga Marchador (MM) horses. In order to achieve this objective, 240 animals were genotyped using two platforms - Axiom ® Equine Genotyping Array - 670k SNP (n=192) (Thermo Fisher, EUA), and Equine SNP70 BeadChip - 54k SNP (n=48) (Illumina, Inc., EUA), with a final density of 570,401 SNP after imputation. In the sequence, complementary quality control was performed in Plink and R software, with thresholds for samples (Call Rate<0.90) and SNP (Call Rate<0.95), Hardy-Weinberg (P<1e-8), and MAF (iHS=0.01; ROH=0.005). At the end of this process, it remained 440,580, and 465,127 SNP. Thus, 34 candidate regions were identified in the iHS method (P<0.0001). Based on the MM genome linkage disequilibrium, 250kb windows were applied for annotation using the biomaRt package and 113 candidate genes were mapped. Of these, 70 genes are characterized, and 46 were tagged as uncharacterized proteins. For the ROH results, 476 SNP were located in hotspot regions with mean occurrence > 50% among individuals. Using the same annotation criteria above, 476 candidate genes were identified in runs of homozygosity, of which 247 are known. By performing a comprehensive summary of the compiled into the two methodologies, it was possible to access 335 different genes on the MM genome, being 27 of them common in both methods. Complementary functional enrichment analysis was applied to the 27 common candidate genes, characterized in this study as the progress of recent positive selection. Protein networks corresponding to the 27 genes, presented 11 interactions (P<0.04540), is that 92.24% showed co-expression, 5.60% co-localization, and 2.16% share protein domain. Five genes with major relevance for the MM were identified on chromosomes 1 (BUB1B), 2 (MAP3K6; WASF2) and 23 (CCL19; GALT). Therefore, the biological process signals associated with the CCL19 and MAP3K6 genes presented potential signatures of recent selection, these process correspond to the activation, regulation and positive regulation of the c-Jun NH2 kinase activity (JNK), which represents an intrinsic response to muscular contraction (studies have found that JNK activity increase only in leg exercises, which may be associated with the performance of the two gaits modalities performed by MM). In addition, the JNK activity, for being composed of a group of mitogen-activated protein, participates in several signal transduction events mediating specific cellular functions. The other potential signatures are associated with energy metabolism, cytoskeleton, and immunological system.

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HISTONE MODIFICATIONS IN RESPONSE TO SODIUM VALPROATE (VPA) TREATMENT IN HeLa CELLS

Marina Amorim Rocha; Maria Luiza Silveira Mello

Institute of Biology, University of Campinas (Unicamp), Campinas, Brazil.

marinaamorimro@gmail.com

Key words: Histone methylation; Epigenetics; Sodium valproate (VPA).

Histones are chromatin proteins that are subject of a wide variety of post-translational modifications thus playing a central role in gene activation and silencing. These modifications may be affected in response to histone deacetylase (HDAC) inhibitors. Valproic acid/sodium valproate (VPA), a well-known antiepileptic agent, has been characterized as a direct inhibitor of class I and II HDAC, inducing histone hyperacetylation and DNA demethylation in several cell types. In a few cell models, it has also been reported to affect the histone methylation profiles. The aim of the present study was to determine whether VPA would affect histone methylation in HeLa cells. To assess histone methylation, immunofluorescence assays and Western blotting were performed in HeLa cells subjected to a 2 mM VPA-treatment for 24 h and 48 h. Results demonstrated that VPA increased the methylation levels in di- and tri-methylated lysine 4 (H3K4me2 and H3K4me3) and in mono- and tri-methylated lysine 27 at histone H3 (H3K27me and H3K27me3). In contrast, a decrease in mono- and di-methylated lysine 9 at histone H3 (H3K9me and H3K9me2) was observed. Recent studies have demonstrated that HDAC inhibitors, including VPA, can trigger increased levels of H3K4 methylation in all cell types tested so far, including HL60 cells, mouse embryonic stem cells, and primary blast cells from patients with acute myeloid leukemia. On the other hand, a decrease in H3K9me mono-methylation has been reported for the neuroepithelium of mouse embryos after treatment with this drug. Once H3K9 methylation is associated with transcriptional silencing, whereas H3K4 methylation is correlated with gene transcription, the changes in methylation levels in lysine residues at histone H3 detected in HeLa cells suggest a temporary global shift towards gene expression in these cells. Further studies are still required to elucidate the meaning of these VPA-induced changes in histone modifications with regards to specific genes.

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CHANGES IN THE REPULSIVE GUIDANCE MOLECULE A (RGMA) EXPRESSION IN SKELETAL MUSCLE CELLS AND MUSCLE STEM CELLS OF A PARKINSON'S DISEASE MURINE MODELL

Júlia Meireles Nogueira[1], Iago Cunha Lage[1], Pedro Henrique Moura Prazeres[1], Alinne do Carmo Costa[1], Alexander Birbrair[1], Erika Cristina Jorge[1]

[1]Department of Morphology, Institute of Biological Sciences, Federal University of Minas Gerais, Belo Horizonte, Brazil.

*jumeirelesn@gmail.com

Palavras-chave: muscle development; RGMa; muscle atrophy.

During development, neural circuits are established by a set of axon guidance molecules that play roles as attractive or repulsive clues that guide the neuron outgrowth to their targets. Changes in the expression or function of these molecules have been associated with a number of neurological diseases, including Parkinson's disease. The Repulsive Guidance Molecule a (RGMa) is one of these clues, found as upregulated in the substantia nigra of patients with Parkinson's disease, which is a neurodegenerative disorder characterized by the progressive loss dopaminergic neurons. Curiously, RGMa was also found to be expressed in some skeletal muscle cells of different muscle groups, in an expression pattern similar to sympathetic fibers. In this work, we have used 6-OH- Dopamine to induce selective neurotoxic disruption of dopaminergic pathways in mice and evaluate the possible association of RGMa with sympathetic muscle fibers. The 6- OH-Dopamine was administrated in two doses by intraperitoneal injections (100mg/kg on day 0 followed by 250mg/kg on day 2). Control mice were obtained by the injection of saline. Treated and control mice were euthanized after three days of the last injection. Samples of EDL, soleus, gastrocnemius and tibialis anterior were harvested to be analyzed by morphometry, RT-qPCR and immunofluorescence using an anti-RGMa antibody. The morphometry analysis revealed that the EDL and soleus muscles were atrophied by the 6-OH-Dopamine treatment; no differences in fiber size were found in the gastrocnemius and tibialis anterior muscles. RGMa transcripts were upregulated only in atrophied muscles (EDL and soleus); while no differences in RGMa transcript levels were found in non-atrophied muscles. However, the immunofluorescence data showed no difference in the RGMa expression pattern in the atrophied muscles; while in the treated tibialis anterior samples, we could detect a completely different RGMa expression pattern, with this molecule expressed only in the nuclei of muscle satellite cells. The control samples revealed RGMa expression as expected. Although we have not detected an association of RGMa with sympathetic fibers, this work revealed that RGMa is induced in atrophied muscle cells and that, for some unknown reason, the dopaminergic depletion induces the expression of RGMa in muscle stem cells.

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SODIUM VALPROATE DOES NOT INDUCE LYSINE ACETYLATION IN HISTONES H3 AND H4 OF THE HETEROCHROMATIN OF *TRIATOMA INFESTANS*

Alessandra Bassani; Marina Amorim Rocha; Maria Luiza Silveira Mello

Institute of Biology, University of Campinas (Unicamp), Campinas, Brazil.

mlsmello@unicamp.br

Key words: *Triatoma infestans*; Epigenetics**;** Sodium valproate (VPA).

Valproic acid/sodium valproate (VPA), a drug widely prescribed for seizure control, is a classic histone deacetylase (HDAC) inhibitor. Reduction in HDAC abundance and activity promoted by VPA is often accompanied by chromatin remodeling and by acetylation induction in lysine 9 at histone H3 (H3K9) and lysine 8 at histone H4 (H4K8) in several cell types. In Malpighian tubules of VPA-injected *Triatoma infestans* specimens, heterochromatin unraveling revealed in large chromocenters affects only some cell nuclei, a fact that was suspected to result from the lack of drug assess to all the organ cells. Because of this, we decided to revisit the effect resulting from the VPA action in these cells under tissue culture conditions. *T. infestans* Malpighian tubules were cultivated *in vitro* for various periods in presence of different VPA concentrations. Three to four specimens were used for each assay. Cells subjected to the Feulgen reaction were examined for chromatin remodeling whereas cells subjected to immunofluorescence assays were used for investigation of H3K9 and H4K8 acetylation. Just as the finding reported for cells from VPA-injected insects, not all of the cell nuclei were found to exhibit chromocenter heterochromatin decondensation when the tubules were cultivated in presence of 0.05 and 0.5 mM VPA for 1 to 4 h. An elevated portion of the cellular population subjected to 10 mM VPA concentration underwent cell death. Fluorescence signals that could reveal H3K9 and H4K8 acetylation were not induced by treatment with 0.5 and 1.0 mM VPA for 4 h in the chromocenter heterochromatin of all the cell nuclei examined. On the other hand, an increased abundance of fluorescence signals related to H3K9 acetylation was detected in the euchromatin encircling the large chromocenter body, where rDNA presence is revealed by FISH. The unraveling response of the chromocenter heterochromatin to VPA in this model thus depends on a mechanism action other than that associated with H3K9 and H4K8 acetylation.

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ANALYSIS OF SOMATIC MUTATIONS IN OSTEOSARCOMAS

Sara Ferreira Pires[1*]; Silvia Souza da Costa[1], Daniel Onofre Vidal[2], André van Helvoort Lengert[2], Érica Boldrini[3], Sandra Regina Morini da Silva[3], Luiz Fernando Lopes[3], Carla Rosenberg[1],Mariana Maschietto[4], Ana Cristina Victorino Krepischi[1]

[1] Instituto de Biociências, Universidade de São Paulo (São Paulo, SP, Brazil), [2] Pediatric Oncology Laboratory, Molecular Oncology Research Center, Barretos Cancer Hospital (Barretos, SP, Brazil), [3] Barretos Children's Cancer Hospital (Barretos, SP, Brazil), [4] Centro Infantil Boldrini (Campinas, SP, Brazil)

* saraferreirap@ib.usp.br

Keywords: pediatric cancer, osteosarcoma, genomic variants

Pediatric cancers are among the leading causes of childhood mortality, osteosarcomas being the most prevalent bone tumors, characterized by an aggressive clinical course. However, knowledge about the molecular basis of osteosarcomas is still limited, hampering advances in diagnosis and treatment. In this context, we characterized the genomic landscape of somatic mutations in 28 osteosarcomas (24 pediatric patients and 4 adults) aiming to delineate the panel of prevalent mutations in Brazilian patients. Genomic libraries of these osteosarcoma samples were constructed using the TruSight One panel (Illumina), covering 4,813 clinically relevant genes, including known cancer genes. Generated data at high coverage (median >100x depth coverage) were annotated based on public databases containing population variant frequencies (including ABRAOM), as well as clinical information. For further analysis, we filtered only coding non-synonymous variants absent from both population databases and an additional pool of non-related germline samples. In total, 728 variants were identified mapping in 606 genes, with an average rate of 41 mutations per tumor. The panel of variants was composed by 93 loss-of-function mutations (38 frameshift, 25 splicing, and 30 premature stop codon), 635 missense (43 inframe insertions/deletions, and 592 single nucleotide variations). Some of these variants were already reported in cancer databases (27 in COSMIC and 39 in ICGC). From 606 genes, 25 were previously associated with osteosarcomas, and they are related to apoptosis, cell growth and differentiation, DNA repair, transcriptional regulation, and tumor suppression mechanisms. The most frequent mutations were detected in *RB1* and *TP53*; other recurrently mutated genes were mostly linked to biological pathways potentially related to the disease onset, as hormonal response (*AR*), transcriptional regulation and muscle differentiation (*FRG1*), DNA repair (*HERC2*), cell signaling (*KIR2DL4*), and cell proliferation/differentiation (*PTPRQ*). Functional studies can be further used to better understand the tumor biology as well to identify novel biomarkers for early diagnosis of the disease.

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Machine learning for histological classification of the Head and Neck Cancer

Tiago Henrique[1]; Vinicius Winck Goes[1]; Ana Carolina B. Stefanini[1]; Eduardo C. N. Costantino[2]; Eloiza H. Tajara[1,3*]

[1]Laboratory of Molecular Markers and Bioinformatics - School of Medicine of São José do Rio Preto - FAMERP. [2]Laboratory of Pathology - Hospital de Base/FAMERP. [3] Graduate Program, Department of Genetics and Evolutionary Biology, Institute of Biosciences, University of São Paulo.

* tajara@famerp.br

Head and neck cancer; cancer diagnosis; Artificial intelligence

Head and neck squamous cell carcinoma (HNSCC) is the sixth most common type of tumor in the world, with 600,000 cases diagnosed annually. These neoplasms are derived from the epithelial cells lining oral cavity, pharynx and larynx and have a large phenotypic, clinical, and genetic heterogeneity, being therefore considered as a group of several tumors. HNSCCs are clinically and pathologically staged by tumor size, lymph node status and distant metastasis, and grouped according to anatomical location, with subgroups presenting different prognosis and response to treatment. Histopathological assessment of neoplastic tissue specimens is a time-intensive and complex process mainly due to several immunohistochemical markers to be analyzed, and to the increasing number of quantitative parameters that have to be extracted for grading systems. The development of the slide scanner technology has allowed the digitization of histological slides, which has greatly facilitated image sharing for collaborations or second opinions. Second opinions in pathology reduce diagnostic errors in challenging cases and lead to more appropriate clinical decisions. However, this conduct results in higher costs, which is a difficult task for small laboratories. The objective of the present study was to develop a machine learning approach to improve the objectivity and efficiency of cancer diagnosis. The rationale is that localizing the origin of tumors with unknown primary site due to invasion of surrounding sites might lead to more specific prognoses and treatments. We postulated that the computerized evaluation and scoring of histological variables by quantifying pixels in images of hematoxylin and eosin-stained sections capture the differences between tumors from different subsites. The machine learning tool was developed in Python programming language using pandas, numpy, matplotlib and scikit-learning and tensorflow libraries, and a support vector machine followed by a random forest classifier. One pathologist manually annotated 207 histologic images of different subsites of HNSCC tumors obtained from the database The Cancer Genome Atlas/TCGA. The images were split into the training (n=166) and test (n=41) sets. The results showed that the algorithm has a sensitivity of 72% for the identification of HNSCC subsites, which indicate that it may be useful in helping to elucidate cases with unknown primary site. Although it will be necessary to carry out more tests with a greater number of histological images, our results showed a reasonable performance in the classification of the HNSCC tumors and constitute the first step for future machine learning studies.

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SALICYLIC ACID-MEDIATED DEGRATION OF THE NIG (NSP INTERACTING GTPASE), A VIRAL MOVIMENT COFACTOR

Gabriel Angelo Saraiva Raimundo[1]; Ruan Maloni Teixeira[1]; Christiane Eliza Motta Duarte[1]; José Cleydson da Silva[1]; Virgílio Adriano Pereira Loriato[1]; João Paulo Batista Machado[2]; Elizabeth Pacheco Batista Fontes[1]

[1]Universidade Federal de Viçosa (BIOAGRO). [2]Universidade Federal de Viçosa *campus* Florestal.

*gabriel88saraiva@gmail.com

Key-words: NIG; salicylic acid; begomovirus.

The *Begomovirus* genus (*Geminiviridae* family) comprises a large number of virus species that can affect subtropical and tropical crops, such as tomatoes, beans and soybeans. The viral DNA transport from the nucleus to the cytosol is accessorized by NSP (*Nuclear shuttle protein*), which along with MP (*Movement protein*), moves the viral DNA to neighbor cells through plasmodesmata, for the establishment of a systemic infection. Among other host proteins, NIG (*NSP-interacting GTPase*) has been found to Interact *in vitro* and *in vivo* with NSP, facilitating the release of the NSP- viral DNA complex from the nuclear pore complex to the cytoplasm. Although NIG is a cytosolic perinuclear GTPase, confocal microscopy showed that Salicylic Acid (SA) treatment induces its translocation to the nucleus. NIG can also be trapped in nuclear bodies formed by its partner WWP1 (*WW domain-containing protein 1*). To confirm the influence of SA in NIG trafficking, we treated NIG-GFP-overexpressing *Arabidopsis thaliana* with SA, with or without MG132, a proteasome inhibitor. Nuclear fractionation assay was performed, and the samples were evaluated through western blotting. Only plants exposed to SA and MG132 accumulated NIG-GFP in the nuclear fraction, suggesting a proteasome-mediated degradation of NIG. We then investigated whether SA could affect NIG homeostasis. NIG-GFP-overexpressing *A. thaliana* were treated with cycloheximide and SA, with or without MG132, and the amount of NIG-GFP was measured through western blotting. SA induced depletion of NIG-GFP levels over time, which was prevented with simultaneous treatment with MG132. In order to verify if begomovirus infection could lead to increased SA levels, we infected *A.thaliana* Col-0 with *Cabbage leaf curl virus* (CaLCuV) DNA through biobalistics. The phytohormones were extracted and then quantified through LC/MS UHPLC QqQ. *A. thaliana* showed higher levels of SA at 10 days of infection as compared to healthy plants. Furthermore, analysis using the linear mixed model allowed the establishment of a direct correlation between SA content and the number of symptomatic chlorotic leaves (NCL). Accordingly, additional RNAseq analysis demonstrated that begomovirus-infected plants displayed upregulation of genes linked to SA biosynthesis and accumulation. To study the WWP1 role in NIG nucleocytoplasmic trafficking, six independently transformed *atwwp1* lines overexpressing YFP-NIG were obtained. Seedlings of *atwwp1*/YFP-NIG were treated with SA and the localization of YFP-NIG was evaluated through confocal microscopy. Even in the absence of AtWWP1, SA treatment redirected YFP-NIG to the nucleus of root cells. At higher SA concentration, we observed YFP-NIG degradation in cotyledon epidermal cells. Collectively, our results indicate that SA identifies NIG as a recessive resistance gene against begomoviruses, leading to its translocation to the nucleus and subsequent degradation, a mechanism apparently independent on WWP1. Therefore, the SA-mediated degradation of NIG might constitute a plant defense mechanism against begomoviruses.

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DISCOVERY OF FUNCTIONAL VARIANTS IN TYPE-2 DIABETES GENES IN MECAN AMERICANS

Marcio Almeida[1], Juan Peralta[1], Rector Arya[1], Farook Thameem[2], Joanne E. Curran[1], Christopher P. Jenkinson[1], Donna M. Lehman[3], Ralph A. DeFronzo[3], John Blangero[1] and Ravindranath Duggirala[1]

[1]South Texas Diabetes and Obesity Institute, Department of Human Genetics, School of Medicine, University of Texas Rio Grande Valley, Brownsville, Texas, USA; [2]Department of Biochemistry, Faculty of Medicine, Kuwait University, Kuwait City, Kuwait; [3]Department of Medicine, School of Medicine, University of Texas Health San Antonio, San Antonio, Texas, USA.

* Marcio.Almeida@utrgv.edu

Palavras-chave: Type-2 Diabetes; Whole Genome Sequencing; T2D-GENES

Type 2 diabetes (T2D) is a complex blood glucose-homeostasis disorder characterized by both insulin resistance and pancreatic ĸ-cell dysfunction. During the last decade, many studies were conducted for the identification of SNVs (Single Nucleotide Variants) associated with susceptibility to T2D. These studies identified numerous common SNVs associated with T2D, but their combined contribution substantially fail to account for the total T2D's heritability estimate. Rare genetic variants are considered good but unexplored candidates to explain the missing heritability. Our project (part of the AMP T2D-GENES Consortium) uses an extended pedigree-based study design to evaluate rare genetic variants contributing to T2D's risk. In fact, estimates, obtained on our data, suggest that 25% of total gene expression control is likely due to rare genetic variants, which highlights their potential to explain the phenotypic variability of traits of interest. The project is composed of 2,620 complete genomes obtained from Mexican American individuals living in San Antonio, Texas, USA. Mexican Americans are a minority population with a high burden of T2D, obesity and cardiovascular diseases. We identified a large number of 29 million SNVs, and nearly sixty percent of them were rare or family- specific. Data collection included measurements of fasting glucose, fasting insulin, 2- hours glucose and 2-hours insulin. T2D diabetes status for each participant was defined following the ADA (American Diabetes Association) diagnostic guidelines. We tested the association of each SNV with T2D, using a linear mixed model (SOLAR). We identified a genome-wide significant association for the marker rs80228500 ($P=1.81x10^{-09}$) located in an intergenic region of Chromosome 8 near the long non-coding RNA *LINC00293*. The analysis of a large number of genetic variants requires a stringent genome-wide significance threshold. The integration of gene annotation data and the computational prediction of SNVs effect would improve the detection of reliable genetic associations. We defined a set of 19,235 high-deleterious Non-Synonymous SNVs as defined by Polyphen2 and SIFT computational routines. We identified a promising association for the marker rs144189912 located at the *FLG* gene ($P=2.4x10^{-07}$). This SNV triggers an amino acid substitution with deleterious consequences for the predicted tertiary protein structure. The filaggrin (*FLG*) gene encodes an intermediate filament-associated protein that aggregates keratin filaments with a central role for the protective skin barrier. Our results are currently being independently validated and, if confirmed, will greatly improve the current understanding about the role of rare genetic variants in T2D risk in the Mexican American population.

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Integrated genome-wide association and pathway analyses reveal insights into the heifer early calving until 24 months in Nelore cattle

Alejandro Barrera Carvajal[1], Lúcio Flávio Macedo Mota[2], Fernando Sebastian Baldi Rey[3], Raysildo Barbosa Lôbo[4], Danísio Prado Munari[1].

[1]Departamento de Ciências Exatas, Universidade Estadual Paulista – Faculdade de Ciências Agrárias e Veterinárias, Jaboticabal, São Paulo, Brasil. [2]Department of Agronomy, Food, Natural Resources, Animals and Environment (DAFNAE), University of Padua, Legnaro, Padua, Italy. [3]Departamento de Zootecnia, Universidade Estadual Paulista – Faculdade de Ciências Agrárias e Veterinárias, Jaboticabal, São Paulo, Brasil. [4]Associação Nacional de Criadores e Pesquisadores, Ribeirão Preto, SP, Brasil.

*alejandrobarreracarvajal@gmail.com

Keywords: beef cattle; reproductive trait; SNP-windows

Heifer early calving (HC) has been recognized as an important factor in herd fertility in beef cattle. The knowledge about the genetic control of sexual precocity allows to select animals more precocious, which will contribute to increase profit in the beef production. The present study was aimed to integrate genome-wide association (GWAS) with pathway analysis as a strategy to uncover regulatory genes set for HC at 24 months (HC24) in heifers Nelore cattle. The HC24 was evaluated in 27,606 Nelore heifers, from which 8,652 were genotyped with 454,236 SNP markers. In order to determine the sexual precocity, the heifers were exposed in a breeding season after weaning. The HC24 was defined attributing a value of 1 (success) to heifers calving until 24 months of age and 0 (failure) otherwise. The single-step GBLUP approach was used to estimate the amount of genetic variance explained by SNP-windows of 100 adjacent and deemed significant when genetic variance was higher than 1%. Pathway analyses were carried out on the gene set identified using the R packages ReactomePA and clusterProfiler. The GWAS identified genomic regions on BTA 2, 3, 4, 6, 7, 8, 9, 14, 16 and 22, explaining 19.36% of the additive genetic variance. These regions harbor the major genes, such as *LEP, GNRHR, PLAG1, CHCHD7, PENK, KISS1* and *GHRL* that play an important role in processes related to the control metabolic, endocrinological and reproductive, as well as growth functions. These genes set lead to better understanding of interaction of genetics factors and body energy reserves required to sexual precocity. Gene set enrichment analysis showed that pathways connected with neuroendocrine mechanism which links the body homeostasis by metabolic substrates providing that heifers are able to achieve early calving until 24 months. The identification of genomic variants associated with metabolic hormone and functions can contribute to the development of strategies for improving early calving in Nelore heifers.

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ROLE OF XPG ENDONUCLEASE IN REDOX STRESS

Davi Mendes[1]; Giovana da Silva Leandro [1]; Pilar Tavares Veras Florentino [1], Clarissa Ribeiro Reily Rocha[1], Carlos Frederico Martins Menck [1]

[1] Department of Microbiology, Institute of Biomedical Sciences, University of Sao Paulo.

davi2.mendes@usp.br

Key-words: XPG, redox stress, XP/CS, neurodegenerative diseases

Several mutations in genes related to nucleotide excision repair (NER) culminates in two human syndromes such as Xeroderma Pigmentosum (XP) and Cockayne syndrome. Patients portraying a mutation in that truncates the NER-endonuclease XPG (ERCC5) develop a syndrome that is composed by the UV hypersensitivity of Xeroderma Pigmentosum combined with neurodegeneration and premature aging from Cockayne syndromes, called XP/CS. Otherwise, mutations that inactivates the endonuclease activity cause only the Xeroderma Pigmentosum syndrome. It was demonstrated by several groups that, XP/CS cells have increased death after redox stress, while the XP cells does not demonstrate the same behaviour. Thus, our goal is to verify the role of XPG endonuclease in redox protection. For that, we over expressed the wild type XPG allele in immortalized cells from a patient with XP/CS and whether the complementation could improve the response to reactive oxygen species (ROS). After re-expressing XPG, we observed an increased in cell viability and a decrease in sub-G1 population after ROS induction by hydrogen peroxide and photoactivated methylene blue (pMB) as well was observed in response to UVC induced damage. Moreover, we observed an increase on repair capacity in lesions caused by ROS from pMB. However, the levels of genotoxic marker γH2AX, decrease after UVC and pMB treatment, whereas with hydrogen peroxide the levels slightly change. Together these data confirms that XPG protein is important to deal with DNA lesions provoked by ROS, preventing cell death.

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THE INTERPLAY BETWEEN MUSASHI AND NOTCH PATHWAYS IN PEDIATRIC MEDULLOBLASTOMAS

Pablo Ferreira das Chagas[1]; Ricardo Bonfim-Silva[3]; Gustavo Alencastro Veiga Cruzeiro[2]; Luciana Chain Veronez[2]; Luis Fernando Nagano[2]; Kleiton Silva Borges[2]; Karina Bezerra Salomão[2]; Rosane de Gomes de Paula Queiroz[2]; Silvia Regina Brandalise[4]; Carlos Alberto Scridelli[1,2]; Luiz Gonzaga Tone[1,2]; Elvis Terci Valera[2].

[1] Department of Genetics, [2] Department of Pediatrics; [3]Department of Surgery and Anatomy Ribeirão Preto Medical School, University of São Paulo – USP; Ribeirão Preto, SP, Brazil. [4]Centro Infantil Boldrini, Campinas, São Paulo, Brazil.

pablochagas@usp.br

Keywords: Medulloblastoma, Musashi-1; Nocth, brain cancer

Medulloblastoma (MB) of Groups 3 and 4 are genetically less characterized and do not have a specific deregulated signaling pathway to date. These 2 MB subgroups constitute a challenge in relation to diagnostic and treatment approaches. It has been described that the Notch pathway is deregulated in the MB 3 and 4 subgroups. The Notch pathway is responsible for controlling important cellular processes during the early stages of development of the central nervous system. Although little is known about how Notch deregulation occurs, there is evidence of NUMB involvement as a Notch pathway repressor. Moreover, the Notch repressor NUMB is targeted by Musashi-1 (MSI1) gene. MSI1 is a highly conserved RNA binding protein (RBP) that modulates post-transcriptional expression of several target mRNAs and is recognized as a neural stem cell biomarker. MSI1 overexpression is strongly associated with poor prognosis in several human cancers yet, its role in MB still is ill defined. The aim of this study was to investigate whether MSI1 correlates to the downregulation of the Notch pathway in MB Group 3 e 4. The MSI1 gene expression was evaluated in a public MB database and validated in a Brazilian cohort of MB patients through RNA-seq and qRT- PCR. *In silico* analyzes were performed using the GSE85217 database (n=763) to evaluate the expression profile of NUMB, NCOR2 (Notch repressors), Notch 1-2 (Notch receptors) and MSI1. As we hypothesed, Notch-1 and Notch-2 expressions were found diminished, while the expressions of NUMB and NCOR2 were higher in Groups 3 and 4 MB ($p < 0.001$) when compared to the WNT and SHH subgroups. RNA-seq data and qRT-PCR validation with 63 samples confirmed the highest expression of MSI1 in Group 3 and 4 MB ($p < 0.001$). These preliminary results suggest that MSI1 possibly contributes as a negative regulator of Notch pathway in Groups 3 and 4 MB by inducing NUMB and NCOR and reducing Notch1 and 2 expressions.

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A Simple PPI-lncRNA Network Based Approach to Visualize and Identify Differentially Expressed Posttranscriptional Regulation Related Genes and Pathways in Hepatocites Treated with Anticancer Drugs

Giordano B. S. Seco[1]; Agnes Alessandra Sekijima Takeda[1], José Luiz Rybarczyk-Filho[1]

[1]Dept Physics and Biophysics - Institute of Biosciences of Botucatu – Univ. Estadual Paulista "Júlio de Mesquita Filho".

*giordano.bruno@unesp.br

Palavras-chave: Bioinformatics, Networks, lncRNA

In recent years many studies have demonstrated how transcripts such as lncRNAs (long non-coding RNAs) might act in posttranscriptional regulation of gene expression. Here we make use of this assumption to create a lncRNA-PPI (Protein-Protein Interaction) network to study expression data of 2 anticancer drugs: etoposide and lomustine. Both drugs are known to induce apoptosis in cells through different mechanisms. Microarray data for both drugs was prospected from the OPEN TG- GATES Project which used Affymetryx chips to measure gene expression in normal hepatocites (control) and drug treated hepatocytes (case) in high, middle and low doses for 3h, 8h and 24h. The raw data was pre-processed in R enviroment with Affy package from bioconductor repository and normalized with robust multi-array average (RMA) method. DEGs (differentially expressed genes) were defined as genes with | logFC|>2 and adjusted p-value < 0.001 (benjamini-hochberg method), lncRNAs were then searched in the DEG list. In total, 5 lncRNAs were found as DEGs: Dancr, EGOT, GAS5, MALAT1 and TUG1. For each of the selected lncRNAs, a RBP-lncRNA (RNA binding proteins) network was prospected in the Starbase database. The mRNAs in each of these networks were then used to prospect 5 PPI networks in the STRING database, using 'Database' and 'Experiment' as interaction types and with a score higher than 0.7 in order to avoid false-positive interactions. The 5 RBP-lncRNA and 5 PPi networks were concatenated into a single network and duplicated interactions were removed. The final network was renderized in Cytoscape, where MCODE plugin was used to derive clusters/modules from the network, with degree cutoff of 2, node score cutoff 0.2, K core 2, depth 100 and loops included. The BiNGO plugin was used to perform functional enrichment of the whole network and the 12 modules. As expected the network and clusters are highly enriched for GO (Gene Ontology) terms such as mRNA catabolic process, ncRNA metabolic process, posttranscriptional gene silencing by RNA, nuclear mRNA splicing (via spliceossome), etc. We took the ratio between the high-24h cases expression for both drugs and plotted it over the network. SRSF10 seems to play a key role in the cell fate determination as it is highly downregulated for both drugs (which induces apoptosis). The remaining results show a drastic downregulation of transcription initiation, nuclear mRNA transport and spliceossomal- mediated mRNA splicing.

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CHARACTERIZATION OF THE FUNGAL GUT POPULATION (MYCOBIOTA) IN AN EXPERIMENTAL MURINE MODEL OF CANCER CACHEXIA

David Aciole Barbosa[1]; Fabiano B. Menegidio[1];Yara N. L. F. de Maria[1]; Rafael dos S. Gonçalves[1]; Kalti naitis B. N. H. dos Santos[1]; Miguel L. Batista Jr. [1]; Regina Costa de Oliveira[1]; Daniela L. Jabes[1]; Luiz R. Nunes[2]*

[1] Universidade de Mogi das Cruzes. [2] Universidade Federal do ABC.

*Luiz.nunes@ufabc.edu.br

Key-words: Cachexia; Cancer; Mycobiom e.

Cachexia is a complex wasting syndr om e, associ ated with a mar ked detr imental effect upon life quali ty and survival in pati ents with cancer and other underlying diseases. Cancer- induced cachexia (CC) is characterized by sever e loss of fat and skeletal muscle mass, often accom pani ed by inflammati on, and cannot be reversed by conventional nutr itional support. The human gut is a robust ecosystem, composed of a dynamic microbial community, which plays important rol es in the acquisition of energy fr om feedstocks and in the regul ation of host physi ology. So far, ther e is a limited number of studies investi gati ng the associ ation between the gut microbiome and cachexia, and, so far, all such studies have focused solely on the bacterial gut community during the developm ent of CC. To obtain informati on regar ding alterations (dysbi osis) that may occur in the fungal gut population (mycobiom e) during the developm ent of cachexia, this work descr ibes an analysis perform ed with C57Bl /6 mice that were injected with Lewis Lung Carci noma (LLC) tumor cells to induce CC. Thus, stool samples of mice were obtained at day 0, to establish a common reference for the study (C0). A subgroup of these animals was inoculated with LLC cells, whil e the rem aining mice were inoculated with saline, to establish a contr ol group (SC) . All animals were kept under the same conditions unti l day 28, when a series of the LLC- injected animals developed cachexia (group CQ) , whil e others did not (group TB). DNA was extracted fr om stool samples obtained fr om all these animals at day 0 and day 28 and used to construct amplicon libr ar ies of the Internal Tr anscr ibed Spacer 1 (ITS1) of the fungal population present in these samples. These libr ar ies were sequenced in an Ill umi na MiSeq NGS sequencer and population analyses, conducted with an ITS1 rRNA pipel ine, speci fi call y developed by our group, allowed the identificati on of mycobiom e dysbi oses speci fi call y associ ated with the developm ent ofCC.

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SCI1 expression in Nicotiana tabacum floral meristems is regulated by the transcription factors WUSCHEL, AINTEGUMENTA-LIKE6 AND AGAMOUS

Cruz, J.O[1, 2]; Lubini, G[1,2]; Strini, E.J[1,2]; Ferreira, P.B[1,2]; San Martin, J.A.B[1]; Pinoti, V.F[1,2]; Thomé, V[1,2]; Quiapim, A.C[1]; Goldman, G.H[3]; Goldman, M.H.S[1]

[1]FFCLRP/University of São Paulo (USP), Ribeirão Preto, SP– Brazil. [2]PPG – Genética, FMRP/ University of São Paulo (USP), Ribeirão Preto, SP– Brazil. [3]FCFRP/University of São Paulo (USP), Ribeirão Preto, SP– Brazil

joelmacruz@usp.br

Keywords: cell proliferation; floral meristem determinancy; promoter analysis.

We previously described a gene that controls cell proliferation in the stigma and style of Nicotiana tabacum (DePaoli et al, 2011), which was named SCI1 (Stigma/style Cell-cycle Inhibitor 1). Recent analyses by in situ hybridization and transgenic plants expressing SCI1prom::SCI1-GFP showed that SCI1 is expressed since the specification of floral meristem and is temporally and spatially regulated. In the end of floral development, its expression is restricted to the dividing cells at stigma, style and ovary. Our results indicate that SCI1 expression is restricted to floral meristematic cells. To investigate how SCI1 expression is regulated, we initially analyzed SCI1 promoter sequence using the softwares PlantPan and PlantRegMap. We identified putative target sequences for transcription factors that regulate floral meristem initiation and specification of whorls, such as the Arabidopsis WUSCHEL, AINTEGUMENTA-Like 6 genes and AGAMOUS. Therefore, we cloned their N. tabacum homologues, NtWUS, NtAIL-6 and NAG1, respectively. In situ hybridization experiments performed with SCI1, NtWUS and NAG1 have demonstrated that they are expressed spatiotemporally in the same cells/tissues. Yeast one-hybrid (Y1H) experiments confirmed the binding of NtWUS and NAG1 transcription factors to the proximal region of SCI1 promoter (443bp upstream of the initial ATG) and of NtAIL-6 to a further fragment of SCI1 promoter. These 3 transcription factors are involved in different aspects of the regulation of meristematic cell proliferation, as well as cell differentiation in flowers. Considering that SCI1 is an inhibitor of cell proliferation, it may act as an effector of floral meristem determinancy, a hypothesis we are currently investigating. Taken together, our results point toward the regulation of SCI1 expression by the transcription factors NtWUS, NtAIL-6 and NAG1 during flower development.

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S-GOAT: A NEW WEB-BASED TOOL FOR TRANSCRIPTOME DATA ANNOTATION AND POST-FILTERING SEQUENCE ANALYSIS

Luan Pinto Rabelo[1]*; Carlos Murilo Tenorio Maciel[2]; Cristiana Ramalho Maciel[3]; Marcelo Vallinoto[1]

[1,2] Laboratory of Evolution; [2] Biodatta; [3] Laboratory of Aquaculture;

*luanrabelo@outlook.com

Key words: Transcriptome; bioinformatics; open source.

The characterization of transcriptomes and the annotation of their genes permit reliable inferences on their function based on comparisons with homologous genes deposited in public databases, although this approach generates an enormous quantity of data. The reliable interpretation of these data is unviable without automated bioinformatic data mining tools. The data obtained from a transcriptome permit a large-scale comparison of the genomic sequences expressed in different organisms or closely-related taxa, which allows for the identification of common functions. A number of databases, such as *NCBI, UniProt, InterPro, KEGG*, and *KOGG*, can be consulted to enrich these data. The paid software *BLAST2GO* is useful for functional genomic research through the data mining of different databases, although its high price means that it is used only by licensed research groups, while alternative freeware, such as *AmiGO*, *GOrilla*, *REVIGO*, *QuickGO*, *NaviGO*, and *GOFeat*, are used for annotation. These tools nevertheless have a number of limitations, ranging from less than intuitive interfaces, problems with the installation and the procedures used to search for a specific sequence in the results, to the difficulties of exporting the results for analysis in other software. Given these problems, the present study aimed to develop a freeware for transcriptome analysis that has a simple and intuitive interface which permits the filtering of sequences through a gene ontology-based function. The *s-GOat* freeware was developed in *PHP*, a programming language that allows the user to access the tool remotely, while reducing the demand for specific hardware. The *MySQL* database was chosen for the storage of the data resulting from the consultations. The *s-GOat* freeware accepts files in *FASTA* format as input, and uses *blastx* to compare results with homologous sequences found in the different databases selected by the user. This permits the storage of the results, which can subsequently be connected with public databases through the internet, to search for information on the specific function of each transcript, and to locate and export the information on the genes related to a given function, for analysis in *WEGO*. The *s- GOat* freeware also permits the classification of the genes for comparisons among different transcriptomes, this means that the freeware presented here provides a more complete analysis of the transcriptomes than existing options, as well as being available to be run remotely by any user.

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THE LOSS OF A LONG NONCODING RNA CAN LEAD TO DELAYED NEURAL DEVELOPMENT

Wilson Araújo Silva Junior[1,2,3,5]; João Monteiro de Pina Neto[1]; Greice Andreotti de Molfetta[3]; Simone da Costa e Silva Carvalho[1,2]; Maria Florencia Tellechea[1, 2]; Kamila Chagas Peronni[2,3]; Ádamo Davi Diógenes Siena[1,2]; Carlos Alberto Oliveira de Biagi Júnior[1,2]; Aline Fernanda de Souza[4]; Cibele Cardoso[2];Amanda Cristina Corveloni[1,2]; Jessica Rodrigues Plaça[2,5]; Reginaldo Cruz Alves Rosa[1,3]; Patrícia de Cassia Ruy[3]; Isabela Ichihara de Barros[1]

[1]Department of Genetics at Ribeirão Preto Medical School, University of São Paulo, Ribeirão Preto, Brazil. [2]Center for Cell-Based Therapy (CEPID/FAPESP); National institute of Science and Technology in Stem Cell and Cell Therapy (INCTC/CNPq), Regional Blood Center of Ribeirão Preto, Ribeirão Preto, Brazil. [3]Center for Medical Genomics, HCFMRP/USP, Ribeirão Preto, Brazil. [4]Faculty of Animal Science and Food Engineering of USP, USP, Brazil. [5]Regional Blood Center of Ribeirão Preto, Ribeirão Preto, Brazil.

wilsonjr@usp.br

Key words: Long noncoding RNA, Intellectual disability, Neural differentiation.

Long noncoding RNAs (lncRNAs) have been demonstrated to play crucial roles as regulatory molecules in brain diseases. Recently, we have found a lncRNA, RP11- 1082A3.1, with unknown function that was lost in a patient with intellectual disability. This observation made us wonder if it is involved in neural development. Previous data from our group showed that this lncRNA is expressed exclusively in testis, and also in several cancer cell lines and spermatogonial cells. *In silico* analysis also showed that RP11- 1082A3.1 is predicted to interact with some RNAs involved in the development, diseases, and genetic syndromes. In this context, our goal is to investigate if the lncRNA RP11- 1082A3.1 defective is involved with abnormal neural differentiation, and with the intellectual disability phenotypes. For this, we collected peripheral blood from the patient and a control individual and separated mononuclear cells by Ficoll gradient. From this fraction, erythroblasts were expanded and then nucleoporated with five episomal plasmids (OCT, P53, SK, UL, and EBNA) for reprogramming in induced pluripotent stem cells (iPSCs), which were then induced to differentiate into neurons with specific culture media. Cells were collected at day 12 of the differentiation into neural precursor cells (NPCs) and later at day 18 of the mature neurons induction protocol. We performed High Content Screening analysis of specific markers and qPCR to verify the expression of specific genes of each stage. Image processing was performed with CellProfiler and ImageJ, and gene expression was analyzed with 7500 Fast Software 2.0.6. Statistical analysis was done with Excel and GraphPad Prism 5. In culture, it was possible to observe differences between control and patient along with the differentiation experiment, what was noticed by the faster growth of the patient cells. Also, we observed that control cells were differentiating in higher proportions than the patient cells, presenting large neurite-like structures, while patient cells had shorter ones and a higher number of precursor-like cells in the wells. This tendency was also noticed in the later stages of differentiation. Quantitative PCR of NPC markers showed that, at day 12 of differentiation, the cells showed SOX2 and PAX6 expression, while nestin was not detected, which indicates the early stage of neuroectoderm specification. The expression of these markers was higher in control cells, which might mean that patient cells are delayed in the differentiation process. These data may indicate that the lncRNA loss may be influencing the capacity of the patient cells to differentiate into neurons.

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USING A DETECTIVE GAME TO TEACH AND COMMUNICATE METHODS AND CONCEPTS IN GENETICS AND BIODIVERSITY

Izadora de Souza[1*]; Ana Paula Carmignotto[1]; Renato Kenji Kimura[1], Maria Elena Infante Malachias[2]; Ana Claudia Lessinger[1]

[1]Departamento de Biologia, Centro de Ciências Humanas e Biológicas - Universidade Federal de São Carlos/UFSCar, Campus Sorocaba. [2]Escola de Artes, Ciências e Humanidades – Universidade de São Paulo/USP.

*iza_valentim@hotmail.com

Keywords: Educational; games; science.

Educational games can be used to engage students and increase learning outcomes, as to provide teachers with new tools for exploring complex concepts. However, game- based learning faces challenges as: being accessible for the students, suitable for use in the classroom, based on low cost materials, aligned with learning objectives and feasible to allow adaptations by teachers. In this study we report an integrated approach that use a detective game as a tool for teaching and communicating scientific concepts, methods and technologies in diverse scenarios: undergraduate genetic classes and public events of science communication. The main idea to be delivered is that "DNA sequences hold biological information". The game "Detetive Na Aula" was designed to support practical classes of the discipline Molecular Genetics for undergraduate students in the Biological Science Course at UFSCar. Lab procedures include micropipettes manipulation, DNA extraction, electrophoresis, PCR amplification and DNA purification and sequencing; followed by bioinformatics classes for DNA sequences analyses. This classes integrate the scope of a DNA-based species identification project that supports the forensic appeal of the game. The game presents a case that requires the identification of a killer using a series of clues, retrieved by rolling dice and advancing steps on a board, which final clue reveals a DNA sequence that matches a species identification in a database (BOLD). In alternative scenarios - on communicating science for elementary and high school students at Universidade Aberta/2018 and Projeto Pequenas Cientistas/2019 - the game was online: clues were retrieved by a code available next to answering general questions on biodiversity and genetics. Additionally, students went to a brief lab experience by a guided tour to five experimental stations showing equipment and on-going procedures at the Molecular Genetics Teaching Lab. The Projeto Pequenas Cientistas also went to an extra guided tour at the Zoology Lab for exploring concepts based on morphological diversity. Concepts and methods shown in the lab sessions were required to answer code-to-clue questions. By playing the game, students were able to work as teams, actively participate by answering questions, create explanations along with the clue's findings, revise initial hypothesis, relate game content with lab contexts and speculate about the nature of genetic information. We consider that the "Lab experience tour" can be replaced by illustrated cards with friendly info about lab devices and protocols (no need of a well-equipped lab) and the case/clues online displays can be organized as a poster (no need of a computer lab) for becoming a suitable and low-cost tool for teachers in the classroom. In addition, the game has some level of "tolerance" that allows teachers to shape clue-access questions to meet the students' needs and interests. This approach is currently under testing at a public school.

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INVETIGATING THE MOLECULAR BASIS OF NATURAL PIGMENT BIXIN IN *Bixa orellana* L. BY PROTEOMIC APPROACHES

Monique Reis de Santana[1*]; Carlos Priminho Pirovani[2]; Virgínia Lúcia Fontes Soares[2];

[1] Programa de Pós-Graduação em Genética e Biologia Molecular, Universidade Estadual de Santa Cruz, Ilhéus, Bahia, Brazil.[2] Centro de Biotecnologia e Genética, Departamento de Ciências Biológicas, Universidade Estadual de Santa Cruz, Ilhéus, Bahia, Brazil.

* monique.bio12@gmail.com

Keywords: carotenoid; mass spectrometry; proteomic profile.

It is estimated that a considerable fraction of plants genome specifies biosynthetic pathways of natural low molecular weight products used in the food, cosmetic and pharmaceutical industries. Most of the genes and enzymes that regulate the biosynthesis of these compounds are still unknown. The species *Bixa orellana* L., known as annatto, is one of these plants, that has great economic importance because it is the only species available as a source of the natural pigment bixin. Bixin is the pigment that gives a red color to annatto seeds, which has been extracted and widely used as an additive dye by the textile, cosmetic, pharmaceutical and food industries. From an economical point of view, bixin is the second most used dye in the industry. However, very little is known about biosynthesis, catabolism and regulation of pigment deposition in annatto seeds. Two-dimensional electrophoresis (2-DE) is a very efficient technique predominantly used for plant proteomic studies, which along with mass spectrometry allows the identification of proteins with differential accumulation and the definition of their potential functions. The main problem related to obtaining annatto seed proteins is due to the high levels of interfering compounds which prevent extraction, solubilization and resolution of the proteins in two-dimensional gel (2D-SDS- PAGE). This may explain the lack of data in proteomic studies of annatto seeds. With an optimized protocol for protein extraction it was possible to isolate a high yield of high-quality proteins from annatto seeds. To assure the quality of the extraction, unidimensional electrophoresis was performed showing well defined protein bands from seeds and flower bud. Also, the quantification of these proteins showed a satisfactory yield of proteins to establish the protein profile of the annatto organs studied in 2D-SDS-PAGE. Thus, the 2D-SDS-PAGE from three stages of seed development, selected based on length and diameter of the fruit, and flower bud was obtained. Differentially expressed proteins at the different stages of seed development and flower bud, isolated in 2D-SDS-PAGE, will be identified by mass spectrometry and might be related to bixin biosynthesis pathway. Also, from the data obtained it might be possible to generate a protein-protein interaction network. Therefore, the present study aims to broaden the understanding of the molecular base involved in the accumulation of bixin pigment in annatto. This is the first report of a *B. orellana* 2D-SDS-PAGE proteomic profile.

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THE GENOME OF SUN CORAL *TUBASTRAEA* SPP. AS A TOOL FOR THE DEVELOPMENT OF BIOTECHNOLOGICAL SOLUTIONS

Danielle Luciana Aurora Soares do Amaral[1*]; Giordano Bruno Soares-Souza[1]; Daniela Batista[2,3]; Marcelle de Queiroz Guimarães[3]; Andre Luiz Quintanilha Torres[2,3]; Luciana Leomil[1]; Anna Carolini Silva Serra[2,3]; Liza Fernandes Moutinho[1]; Aline Silva Romão Dumaresq[1]; Mauro de Freitas Rebelo[2,3]

[1]Instituto Senai de Inovação, SENAI CETIQT, Rio de Janeiro, RJ, Brazil. [2]Instituto de Biofísica Carlos Chagas Filho, Universidade Federal do Rio de Janeiro – Centro de Ciências da Saúde, Rio de Janeiro, RJ, Brazil. [3]Bio Bureau Biotecnologia, Polo de Biotecnologia - Parque Tecnológico UFRJ, Rio de Janeiro, RJ, Brazil

*DLAmaral@cetiqt.senai.br.

Key-words: Sun Coral; genome; transcriptome.

The Sun Coral (*Tubastraea* spp.) are invasive species in Brazil and native to the Indo- Pacific Ocean with an estimated arrival in the 1980s. The invasion reached six Brazilian states: Rio de Janeiro, Bahia, São Paulo, Santa Catarina, Ceará, and Sergipe. There is evidence of the reduction of corals, crustaceans and other Brazilian native species, and incrustation on drilling rigs, oil production facilities, various types of vessels, port facilities, shipyards, and terminals, leading to environmental and economic losses. The traditional approaches have been inefficient and expensive giving rise to a national control plan to deal with the infestation. The high invasiveness of Sun Coral has been hypothesized as due to the high regeneration, adhesion, growth capacity, and reproduction success. Nevertheless, there are still many knowledge gaps related to the genetic architecture of *Tubastraea* spp. invasiveness and on how to improve monitoring. To address these issues we are sequencing the genome and transcriptome of two species: *Tubastraea tagusensis* and *Tubastraea coccinea*. We expect that the data generated in this project helps to better understand which genes are related to its success in invasion and to develop biotechnological routes to control their establishment and expansion. The specimens' collection was done in 5 localities of the Rio de Janeiro State coast. The genome size from *T. tagusensis* and *T. coccinea* were estimated by flow cytometry and chromosome number determined by cytogenetics. DNA from *T. tagusensis* was sequenced using 2nd (Hiseq X, Illumina) and 3rd generation sequencing (Sequel System, PacBio) and *T. coccinea* using only PacBio. *T. tagusensis* genome assembly was done using Masurca, in a hybrid approach, and *T. coccinea* genome assemblywas done with Falcon unzip. Total RNA from both species was sequenced, with 30 million reads for each species, using Illumina (Hiseq X, Illumina). The genome size determined by flow cytometry was approximately 1.3 Gb for *T. tagusensis* and 0.9 Gb for *T. coccinea* and chromosome number of *Tubastraea* sp. was estimated in 40. The mitochondrial genome from both species was recovered and phylogenetic analysis corroborated morphological identification. *Tubastraea tagusensis* and *T. coccinea* transcriptome presented, respectively, a BUSCO completeness of 98.1% and 89.4%, alignment rate of 94.81% and 88.94%. The complete genomes and transcriptomes assembled and annotated, together with karyotype determination will provide us information to understand the invasiveness process of sun coral and develop biotechnological routes to control it.

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STAGE-SPECIFIC RESPONSE OF DEVELOPMENTAL GENES TRIGGERED BY MORPHOGENETIC HORMONES IN APIS MELLIFERA

Thiago Depintor[1]; Flavia Freitas[2]; Zilá L. P. Simões[3*]

[1*] Faculdade de Medicina de Ribeirão Preto – USP, Depto. deGenética. [2] Instituto de Ciências Biomédicas da Universidade Federal de Alfenas. [3] Faculdade de Filosofia Ciências e Letras de Ribeirão Preto – USP, Depto. de Biologia.

*Thiago_depintor@usp.br

Key words: Metamorphosis, signaling pathway, miRNA.

The development in honeybees is mainly controlled by the action of two major hormones, juvenile hormone (JH) and 20-hidroxyecdysone (20E). These hormones trigger gene cascades, which results in phenotypic, physiological and behavioral changes. Besides hormones, a class of non-coding RNAs, the microRNAs, regulates gene expression at a post-transcriptional level during insect development. In this study we aimed to analyze the relationship between developmental genes and morphogenetic hormones, in final stages of the development of *Apis mellifera*. The expression profile of the orphan nuclear receptor *Usp, ftz-f1, EcR, chd64, inr2, Kr-h1), gce, early-trypsin*, and their putative regulators miRNA-34, miRNA-281, miRNA-252a and miRNA-252b were assessed from 5º instar larvae to newly emerged adults by qPCR. The effect of exogenous doses of both hormones applied on white eyed pupae (Pw) and brown eyed pupae (Pb) was also tested. Most of the genes seem to respond to hormonal variation in pupal stages as they do in larval stages. However, *gce* and *chd64* showed a different response to hormonal treatment in pupal states, thus suggesting they play different roles in final stages of development. Unexpectedly *gce*, which is a nuclear receptor of JH in insects, showed a quick response to 20E treatment and no response to JH in pupal stages of honeybees, as well as chd64 which also responded only to the 20E treatment. In addition, we recognized *Usp* as an Immediate Early Gene, for it responded rapidly to hormonal treatments and quickly restored its level. In addition, we find the miR-34 and miR-281 as strong candidates of regulators since they presented many putative interactions in the 3'UTR of the candidate genes and showed to be affected by the hormonal treatment. This study describes new components to the regulatory network that regulates bee development. We thank the São Paulo Research Foundation (FAPESP) for the scholarship during this study (Process number: 2016/13854-6).

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TRACKING *FBN1* AND *FBN2* EXPRESSION DURING IPSC DIFFERENTIATION: DOES *IN VITRO* CARDIOMYOCYTES AND ENDOTHELIAL CELLS GENERATION RECAPTULATE THE EMBRYONIC DEVELOPMENT?

Juliana Borsoi[1,2]; Bianca Portela[1]; Alexandre Butenas[1]; Raquel Sarafian[1], Mariana Morato-Marques[1], Luis Ernesto Farinha-Arcieri[1], Diogo Mosqueira[2], James Smith[2], Chris Denning[2], Lygia da Veiga Pereira[1*]

[1]National Laboratory for Embryonic Stem Cells (LaNCE), Institute of Biosciences, University of São Paulo.[2]Wolfson Centre for Stem Cells, Tissue Engineering and Modelling (STEM), University of Nottingham.

* lpereira@usp.br

Keywords: iPSCs; fibrillins; cardiovascular development

FBN1 and *FBN2* encode the two main members of the fibrillins family. Both fibrillin-1 and 2 are extracellular matrix components that, in association with elastin, form the elastic fibres. The elastic fibres confer elasticity and resilience to connective tissues and perform a very important biomechanical role in blood vessels, heart, ligaments and skin. The dynamics of *FBN1* and *2* expression during the human cardiovascular system development remains unclear, but most gene expression data points *FBN1* as the main expressed isoform in the adult heart and vascular tissues. Considering the ethical issues and technical difficulties of using human embryos, a suitable *in vitro* model that recapitulates the cell-type specific gene expression patterns that govern cardiovascular differentiation would be of great value for both normal embryonic development studies and disease modelling. Induced pluripotent stem cells (iPSCs) can be cultured for long- term, and differentiated to virtually any cell type, and have been used successfully as models for many diseases. However, the differentiated cells usually display immature embryonic/foetal characteristics, which makes difficult to model late stage disease phenotypes. Recent transcriptome data of both human and mouse hearts show that during development there seems to be a switch from *FBN2* to *FBN1* expression in both cardiomyocytes (CMs) and endothelial cells (ECs). Also, single cell RNA-seq data of iPSC-derived CMs and ECs show that on day 30 and days 5-15 of differentiation, respectively, these cell types tend to have a low *FBN1*/*FBN2* expression ratio, which reinforces their immaturity. To find if iPSC differentiation in CMs and ECs recapitulates the temporal expression pattern observed during human heart development, we analysed *FBN1* and *2* expression during different stages of differentiation, from day 0 to day 60 for CMs, and from day 0 to day 15 for ECs and noticed that, at later stages, the pattern seems to approximate the adult levels, but not enough for the foetal/adult switch to occur, at least for the CMs.

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TRANSCRIPTOMIC ANALYSIS OF MALE AND FEMALE GONADS OF THE INVASIVE GOLDEN MUSSEL *LIMNOPERNA FORTUNEI*

Luana Ferreira Afonso[1]; Juliana Alves Americo[2]; Giordano Bruno Soares-Souza[3], André Luiz Quintanilha Torres[2], Inês Julia Ribas Wajsenzon[1], Mauro de Freitas Rebelo[1]

[1]Instituto de Biofísica Carlos Chagas Filho, Universidade Federal do Rio de Janeiro – Centro de Ciências da Saúde, Rio de Janeiro, Rio de Janeiro, Brazil. [2]Bio Bureau Biotecnologia, Polo de Biotecnologia - Parque Tecnológico UFRJ, Rio de Janeiro, Rio de Janeiro, Brazil. [3]Centro de Tecnologia da Indústria Química e Têxtil, Rio de Janeiro, Rio de Janeiro, Brazil.

luana.afonso@biof.ufrj.br

Key-words: golden mussel; RNA-seq; sex determination.

The golden mussel (*Limnoperna fortunei*) is an Asian invasive bivalve that threatens aquatic biodiversity and causes environmental and economic damage in South America. Due to its high adaptation and reproduction rate, this mollusk has spread fast. Nowadays, more than 40% of Brazilian hydroelectric power plants report the presence of golden mussel, with no efficient control method for the infestation available so far. In order to develop a biotechnology-based control strategy, it is necessary to fulfill relevant knowledge gaps regarding the reproduction mechanisms of this mussel. Sex determination and differentiation are crucial stages of reproduction. Genes related to those processes have played important roles as targets for genetic engineering, aiming to control populations (e.g. disease vectors) such as mosquitoes. We explore the possibility that the same strategy could be applied to control the invasive golden mussel. However, sex determination mechanisms in bivalves have not been yet characterized. In this study, we present the gonadal transcriptomes of males and females specimens of *L. fortunei* in order to identify potential sex determination genes. We sequenced total RNA of 7 organisms with 65 million reads per sample, on average, with Illumina HiSeq 4000. A total of 200,185 contigs were generated using a concatenation of 3 different assembly strategies, resulting in N50 of 2,254 bp and a 99.3% complete transcriptome, according to BUSCO analysis with Metazoa dataset. We performed *in silico* annotation through similarity of golden mussel sequences against UniprotKB/Swiss-Prot and non- redundant sequences from NCBI databases, and identified 54.7 and 69.3% of hits, respectively. In addition, 13.1% of the transcripts were mapped to at least one Gene Ontology term using Blast2GO; 11.8% of protein sequences were assigned to a KEGG pathway through KAAS web server, and 48.4% to a conserved domain of Pfam or CDD databases. Differential expression between 3 male and 3 female specimens were performed with EdgeR software, in which 3,906 sex-biased transcripts were identified. Among 140 genes related to sex determination/differentiation described in other bivalves, 104 genes were identified, from which 12 transcripts showed sex-biased expression in golden mussel gonads transcriptome. Our analysis identified golden mussel orthologs of key genes involved in sex determination and differentiation in model organisms from vertebrates to the Pacific oyster *Crassostrea gigas*, such as the forkhead box transcription factor (FoxL2) and SRY (Sex determining region Y)-box 30 (SOX30).

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ASSESSMENT OF GENOTOXIC EFFECTS ON ELDERLY POPULATIONS EXPOSED TO HIGH TRAFFIC AREAS: RESULTS FOR SUPPORTING PUBLIC HEALTH SURVEILLANCE

Vanessa Santana Vieira Santos[1]; Boscolli Barbosa Pereira[1,2]

[1]Universidade Federal de Uberlândia, Instituto de Biotecnologia, Uberlândia, Minas Gerais, Brasil. [2]Universidade Federal de Uberlândia, Instituto de Geografia, Uberlândia, Minas Gerais, Brasil.

*vanessasvs2009@hotmail.com

Key-words: Genotoxicity; Pollution; Micronuclei.

In urban areas with intense vehicular traffic, particulate matter in suspension, especially the fraction of particles with ultra-fine diameter, has been regarded as the main problem of chronic diseases in susceptible populations, such as the elderly. This study aimed to determine the genotoxic effects of exposure to air pollution evaluating the association between the frequencies of MN and BN in exfoliated oral mucosa cells of elderly population and exposure conditions, considering the influence of traffic and concentration of PM in different aerodynamic diameters. Participants were asked to rinse their mouths with water and oral mucosa samples were then gently exfoliated with a swab and preserved in 5 ml of saline solution (0.9% NaCl). Cells from the saline solution were submitted to centrifugation for five minutes at 1500 rpm. The pellet of cells was smeared on a microscopy glass slide, fixed in methanol : acetic acid (3:1) solution for ten minutes and air-dried at room temperature. The dried slides were stained with Giemsa (4%), diluted in phosphate buffer (pH 8) for ten minutes. Cytological analysis were done under a light microscope (at 1,000X magnification) and the frequency of micronucleus and binucleated cells was investigated in a total of 2,000 cells of each subject. Traffic of passenger vehicles, heavy duty trucks and environmental concentrations of Particulate Matter were measured twice a day during 28 days before biological sampling of oral mucosa from 154 participants living in areas of distinct levels of urban traffic. Data from this study showed that the group of participants living near road traffic exhibited higher MN cell frequency, when compared to the other groups of subjects. In addition, a canonical correlation analysis between environmental and genotoxicity variables analysis revealed that high concentrations of the particulate matter were correlated with intense traffic and the genotoxicity in exfoliated oral cells. Routine air quality measurements usually monitor the concentrations of particulate matter ($PM_{2.5}$) in atmosphere, adopting as parameters values of daily and annual average of 25 and 10 Kg/m^3, respectively, as recommended by World Health Organization. However, different studies have reported results in which negative health effects of the exposed population were observed even at concentrations below this threshold. In conclusion, the results of this study imply to the need to implement environmental health surveillance actions with focus to populations living in areas of high vehicular traffic, especially on the roadsides, which present intense truck traffic. Thus, the use of biomarkers of genotoxicity, such as the MN test in exfoliated cells of the oral mucosa, concomitantly with the monitoring of concentrations of particulate matter in the atmosphere is encouraged, since it provides valuable epidemiological evidence for the prevention of chronic outcomes, including cancer.

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VARIATION OF ADRB3, ABCA1, TCF7L4, CAPN10 and FTO GENES ASSOCIATED WITH METABOLIC SYNDROME IN AN INDIGENOUS POPULATION OF THE STATE OF PARÁ: SURUÍ- AIKEWARA

Yure Jefferson da Cruz do Nascimento[1*]; Dennyson Leandro Mathias Fonseca[1]; Eliene dos Santos Rodrigues[1]; Ana Carolina Brito de Farias Hage[1]; André Monteiro Pinto[1]; Greice de Lemos Cardoso Costa[1]; João Farias Guerreiro[1].

[1]Laboratory of Human and Medical Genetics. Institute of Biological Sciences. Federal University of Pará.

*yure_jefferson@hotmail.com

Keywords: Aikewara; Indigenous; Metabolic Syndrom.

The metabolic syndrome is a set of conditions that include arterial hypertension, central obesity, altered glycemia, hypertriglyceridemia and low HDL cholesterol concentration, constituting a risk factor for heart disease, stroke and diabetes. Absence of physical activities, excess of weight and genetic constitution are factors that contribute to the development of the syndrome. Semi-isolated populations such as the indigenous ones are important for studies of genetic epidemiology, since the increase of contact with non- indigenous populations inserts into their routines habits and diet patterns that may interfere in the morbidity profile of the Indians with the increase in the prevalence of metabolic syndrome and associated comorbidities. In this work, we analyzed genetic variants associated with components of metabolic syndrome in the Suruí-Aikewara indigenous group, Tupi-Guarani language, located in the rural area of the municipality of São Geraldo do Araguaia, state of Pará. DNA samples were studied from 77 adult individuals, 50% male, who gave formal consent to the study. SNPs were analyzed in the TCF7L4 genes (rs7901695, T>C) and CAPN10 (rs5030952, C>T) associated with type 2 diabetes; ADRB3 (rs4994, A>G) and FTO (rs8050136, C>A), associated with weight gain; and the ABCA1 gene (rs9282541, G>A) associated with high density lipoprotein (HDL) levels. Genotyping of the variants was done by TaqMan® allelic discrimination (Life Technologies, USA) of Real Time PCR. Comparison of the data obtained with those described for Europeans, East Asians and Africans by the "1000 Genome Project", shows that in the loci associated with diabetes the observed frequency of the risk variant CAPN10-rs5030952-'C '(0.21) is lower than the mean value described for the three population groups (0.93, 0.76 and 0.47, respectively), whereas the frequency of the allele TCF7L4-rs7901695-'C '(0.08) is lower than the observed in European (0.34) and African (0.30), but similar to that described in Asians (0.02). Among the variants associated with weight gain, the ADRB3 -rs4994-'G 'variant was found with a frequency of 0.10, which is similar to those observed in European (0.08), Asian (0.13) and African (0.09), while the FTO-rs8050136-'A 'variant exhibited a surprisingly high frequency among Suruí-Aikewara (0.92), higher than that described for European, Asian and African (0.41, 0.17 and 0.43, respectively). This variant seems to be restricted to Amerindian populations, not being found in Europeans, Asians and Africans. On the variant associated with HDL levels, ABCA1-rs9282541-'A', the frequency of 0.13 found among Suruí-Aikewara is similar to the mean reported for other indigenous groups (0.096). The high prevalence of this variant indicates that other variants associated with this phenotype are investigated in this and other indigenous populations to better describe the genetic variability underlying the high prevalence of overweight and obesity observed in Brazilian indigenous peoples.

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MOLECULAR DOCKING INTERACTION BETWEEN PIPLARTINE AND IMMUNE-RELATED PROTEINS AS THERAPEUTHIC STRATEGY FOR DOWN SYNDROME

Victor Miranda Hernandes[1]; Marlon Fraga Mattos[1]; Lennon Pereira Caires[1], Tiago Henrique[2], Olívia Borghi Nascimento[1],Eny Maria Goloni-Bertollo[1],Eloiza Helena Tajara[2], Érika Cristina Pavarino[1]

[1]Genetics and Molecular Biology Research Unit - UPGEM, São José do Rio Preto Medical School- FAMERP – Brazil. [2]Laboratory of Molecular Markers and Bioinformatics, São José do Rio Preto Medical School FAMERP, SP – Brazil.

erika@famerp.br

Key words: Molecular docking, Immunological response, Down syndrome

The Down syndrome (DS) is the most frequent chromosomal anomaly in the world, with an estimated prevalence of 1 in 850 live births. Immunological disorders like autoimmune diseases and high frequency of infections, especially in the respiratory tract, are more common in individuals with DS than in the general population. The etiology of the immunological deficiency in DS is not completely known. Differential expression of genes involved in immunological processes has been reported in individuals with DS. Piplartine, also known as Piperlongumine, is a biologically active component of Piper longun (Piper longum L. – Piperaceae), which has multiple pharmacological activities including a potential role in immunological response. Previously, we identified five immune-related genes, four genes (CD40, NOS2, ITGAM and ITGB1) down-regulated and one gene (IL-10) up-regulated in DS individuals. In the present study, we evaluated by bioinformatics methods the potential interaction between piplartine and proteins encoded by differentially expressed genes in our previous studies in DS. The protein structures of the molecular targets CD40, NOS2, ITGAM, ITGB1 and IL-10 were obtained on Protein Data Bank (PDB ID: 5DMI, 4NOS, 1NA5, 4WJK, 2H24, respectively) and used as the receptors for docking simulation and the three-dimensional structures were prepared with the Autodock tools. The interaction of the ligand piplartine with the molecular targets was performed using AutoDock Vina program. To ensure reliability of this interaction, the docking parameters were validated using the redocking approach. Molecular docking experiments showed a binding energy of -6.2kcal/mol for CD40 and ITGAM, - 6.3kcal/mol for IL-10, -7.3kcal/mol for NOS2, -7.6kcal/mol for ITGB1. ITGB1 presented the lowest energy, therefore, showed the most efficient ligation prediction with the piplartine. Although the physical interaction between this natural compost and ITGB1 was not tested in vitro, in previous study we demonstrated that the piplartine reduced the expression of ITGB1 in laryngeal tumor cell line. Therefore, in vitro studies in trisomic cells are necessary to evaluate the action of piplartine in these cells. In addition, this previous study also showed that CD40, NOS2, ITGAM genes are up- regulated by piplartine and could be promising targets to improve the immunological response. In conclusion, our results showed that the piplartine has potential binding site for the proteins evaluated in this study. It is important to conduct further studies both in vitro and in vivo on piplartine and their active principles to validate our in silico prediction.

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DNA-BASED ID OF BATS (MAMMALIA: CHIROPTERA) FROM ESTAÇÃO ECOLÓGICA SERRA GERAL DO TOCANTINS: A MOLECULAR INVENTORY

Alessandra Vama Vieira[1]*; Ana Paula Carmignotto[1]; Renato Kenji Kimura[1]; Ana Claúdia Lessinger[1]

[1] Departamento de Biologia, Centro de Ciências Humanas e Biológicas - Universidade Federal de São Carlos/UFSCar, Campus Sorocaba.

*alessandravama@gmail.com

Keywords: DNA-barcodes, Chiroptera, bat inventory

The Cerrado Biome – that includes the Tocantins State – shows highly diversified habitats, promoting high levels of species richness of bats (Mammalia: Chiroptera). The Jalapão complex is the major preserved area of Cerrado from Brazil, included in te Uruçuí-Mirador biodiversity corridor. A molecular inventory focused on bats neotropical diversity could contribute to fill the gaps on sampling genetic data related to Chiroptera fauna from Tocantins. This study aims to provide a molecular inventory based on the characterization of DNA Barcode sequences of bats from Jalapão, increasing the representativeness of these species in international databases and boosting studies related to the identification of species complexes and discovery of new species. Samples from Jalapão were previously identified using morphological criteria by skilled taxonomists. Following field collection tissues were stored at -20ºC in ethanol until use in DNA extraction protocol. A fragment of 650 base pairs from the mtDNA COI gene (barcode region) of 20 specimens were successfully amplified using Folmer universal primers. PCR products were sequenced for both strand and consensus sequences were edited and retrieved using the software GENEIOUS. Species identifications were tested using the Identification Engine in BOLD database. "DNA-barcodes" analyses classified nine samples with a "match" status (45%), including *Nyctinomops laticaudatus* (n=2), *Epitesicus furinalis* (n=1), *Artibeus cinereus* (n=1), *Artibeus planirostris* (n=1), *Glossophaga soricina* (n=1), *Trachops cirrhosus* (n=1), *Micronycteris sanborni* (n=1), *Artibeus lituratus* (n=1), however two results do not recover the morphological id. Another eight sequences report ambigous results (40%), showing a "best match" status for *Molossus molossus* (n=1), *Lophostoma silvicolum* (n=1), *Lasiurus ega* (n=2), *Platyrrhinus lineatus* (n=2), *Artibeus glaucus* (n=1), *Carollia perspicillata* (n=1) - where four identifications disagree with previous morphological analysis. Three samples were classified as "no match" (15%) – two of the supposed species were not represented in the database. Taxonomic revisions are underway to confirm conflicting identifications.

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CHARACTERIZATION OF PROMOTER ELEMENTS RESPONSIVE TO THE QUORUM SENSING (QS) AUTO- INDUCER 2 (AI-2) IN *ZYMOMONAS MOBILIS*

Renata Ozelami Vilas Boas[1]; Valquíria Campos Alencar[1]; Juliana de Fátima dos Santos Silva [2]; Vinícius Manganaro Farnézio[2,] David Aciole Barbosa[1]; Fabiano Bezerra Menegidio[1]; Daniela Leite Jabes[1]; Regina Costa de Oliveira[1]; Luiz R. Nunes[2*]

[1]Núcleo Integrado de Biotecnologia, Universidade de Mogi das Cruzes (UMC), Av. Dr. Cândido Xavier de Almeida Souza, 200, Mogi das Cruzes, SP, CEP 08780-911. [2]Centro de Ciências Naturais e Humanas, Universidade Federal do ABC, Rua Santa Adélia, 166, Santo André, SP, CEP 09210-170.

*Luiz.Nunes@ufabc.edu.br

Key-words: *Zymomonas mobilis*, auto-inducer 2, quorum sensing

Zymomonas mobilis is an ethanologenic bacterium that displays many desirable attributes for industrial ethanol production, including elevated ethanol tolerance, capacity to grow at a broad pH range and high ethanol yield productivity, associated with anaerobic use of the Entner–Doudoroff pathway for glucose metabolism. In this scenario, Z. mobilis has been considered an important microrganism for several industrial activities, such as the production biofuels. Previous studies have shown that Z. mobilis is responsive to the quorum-sensing (QS) signaling molecule known as Autoinducer 2 (AI-2). Quorum-sensing systems have been used in metabolic engineering studies to synchronize gene expression across a population to reduce cell-to-cell variability, leading to an increase in yields of product molecules in engineered strains. Therefore, if genes of industrial interest were incorporated into the Z. mobilis genome under the control of AI-2 responsive promoters, it would be possible to induce their expression in a controlled method. Importantly, Z. mobilis does not possess the genes generally associated with sensing AI-2 in other organisms, and, consequently, most likely utilizes a new and novel quorum-sensing system that awaits elucidation. Thus, our objective is to elucidate the AI-2 signalling pathway using a transcriptomic approach and perform the characterization of AI-2 responsive promoters. Then, Z. mobilis cells were cultivated in minimal medium and synthetic AI-2 was added when the culture reached mid log phase. RNA was extracted after cultures reached stationary growth phase used to prepare RNA- seq libraries, according to the ScriptSeq v2 RNA-Seq Library Preparation Kit (Illumina). RNA-sequencing was executed on Illumina NextSeq, generating around 20 million reads for each library. The sequences obtained were processed by the Rockhopper software, using the reference genome of Z. mobilis ZM4. The genes identified as differentially expressed were automatically identified by the software and their mean RPKM (Reads Per Kilobase Million) values were used to calculate the magnitude of modulation for each gene. Results show a total of 1082 genes exhibited significantly differential expression patterns (q value ≤ 0.01), including 967 upregulated genes and 111 downregulated genes. Expression of some of these genes was validated by qRT-PCR. These studies led to the identification of chromosomal regions carrying AI-2-responsive promoter elements, which are currently being mapped and identified. Such inducible promoters may constitute an important tool for Z. mobilis manipulation, with possible application in a wide variety of industrial bioprocesses, allowing the expression of transgenes in this bacterium, under controlled and optimized conditions.

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DIFFERENTIAL EXPRESSION OF SUCROSE 6-PHOSPHATE PHOSPHOHYDROLASE FROM SUGARCANE

Vania Gabriela Sedano Partida*; Marie-Anne Van Sluys

Departamento de Botânica – Instituto de Biociências USP

*vsedano@usp.br & mavsluys@usp.br

Palavras-chave: sucrose metabolism; gene expression; *Saccharum.*

Sucrose, the main disaccharide produced by photosynthesis. It is synthesized in the cytosol and released by the action of the enzyme Sucrose 6-phosphatephosphohydrolase (S6PP), EC 3.1.3.24, PF 08472. Prior to entry to the vacuole of the parenchyma cells where is stored, the intermediate Suc6P is dephosphorylated by S6PP, providing the energy necessary for the entry. The enzyme S6PP is found in vascular and non-vascular plants and some cyanobacteria as an adaptation to osmotic stress. Due to the high economic value of sugarcane as a world commodity to produce sugar, renewable energy and other biomolecules, due to its ability to store large amounts of sucrose in the internodes of the culm as part of its developmental program, this work aims at the molecular understanding of the gene that encodes sucrose 6-phosphate phosphohydrolase (*S6PP*). The existence of a new isoform composed of two S6PP domains and further explored in the present study. In order to verify the transcription in sugarcane of the two isoforms, *S6PP.1* and *S6PP_2D*, we examined the expression pattern from two commercial hybrid varieties SP80320 and R570, as well as two progenitor species *S. officinarum* (Badilla) and *S. spontaneum* (IN8458). Different tissues (leaves, culm, meristem and roots) and different development time points (3, 6, and 9 months) were addressed. Total RNA from tissues was isolate and cDNA was synthesized and qPCR was carried out. The results supports that expression pattern is variable over time in both cases. We observed greater expression of both enzyme transcripts in tissues at 3 months of age and a notable decrease over time. At early stages of the culture when plant growth is accelerated, there is a greater requirement of sucrose to carry out the vital functions of the plant. Evidence by the expression of the transcript, we supposed that the enzyme is involved in constant activity allowing the cyclic balance between synthesis, storage, use and re-synthesis. Otherwise, the sugar cane tends to accumulate more sucrose when it has a low growth rate, when reaches a mature age, between 10 and 14 months. *In vitro* essays suggest that biochemical catalytic activity of S6PP enzyme is downregulated in the presence of sucrose, in natural conditions in commercial sugarcane varieties the sucrose accumulation can reach high concentrations, up to 650 mM. Therefore, we speculate the reduction of the *S6PP* transcripts is due to the high accumulation of sucrose.

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EFFECTS ON THE BIOLOGICAL ACTIVITY OF THE LYSOSOMAL ENZYME GLUCOCEREBROSIDASE AFTER GENE MODIFICATION

Wilson Lau Júnior[1]; Cláudia Emília Vieira Wiezel[1]; Aguinaldo Luiz Simões[1]; Daniela Pretti da Cunha Tirapelli[2]; Vania D'Almeida[3]; Aparecida Maria Fontes[1]

[1]Departamento de Genética, FMRP – USP. [2]Departamento de Cirurgia e Anatomia, FMRP – USP. [3]Departamento de Psicobiologia, Escola Paulista de Medicina – UNIFESP.

* wilsonlau.junior@gmail.com

Key-words: transfection efficiency; GFP; glucocerebrosidase.

The delivery and the expression of a functional gene for a host cell are crucial steps for gene therapy assays. The success of this process relies on several factors, such as cell type, culture medium and transfection method. Thus, this study investigates these elements of transfection using the green fluorescent protein (GFP) as a biomarker; biological activity of the lysosomal enzyme glucocerebrosidase (GBA) and cell proliferative potential were analyzed for cell phenotypic characterization after gene modification. Firstly, the human embryonic kidney 293-FT cell line was transfected using DMEM, DMEM+2%FBS, OptiMEM and OptiMEM+2%FBS medium and Lipofectamine 3000 with 2 ug of the plasmid vectors containing the GFP gene under the control of human elongation factor 1α promoter (Hef-1α_GFP) to test the influence of medium composition on transfection efficiency; at 48 h post transfection, cells were harvested and analyzed by flow cytometer to evaluate the percentage of cells expressing GFP. Once the medium was set, two different transfection reagents were tested using the Hef-1α_GFP plasmid; 293-FT cells were transfected with Lipofectamine 3000 and Polyethylenimine 25-kDa (PEI) and flow cytometry analysis carried out; non transfected cells were used as control. After transfection reagent and medium were defined, 293-FT cells were transfected with 4-5 ug of Hef-1α_GFP plasmid; cells and culture medium were collected to analyze specific and secreted GBA activity 48 h post transfection and cell number were counted. Transfection efficiency using DMEM+2%FBS, DMEM, OptiMEM+2%FBS and OptiMEM medium resulted, respectively, 27.4%, 31.5%, 29.2% and 33.4% GFP^+ cells. In regard to the transfection reagent, it has been found that after 48 h, 61.20% of the cell transfected with PEI were expressing GFP while 61.76% were GFP^+ when transfected with Lipofectamine. As both transfection protocols had similar efficiency, Lipofectamine was applied for GBA activity analysis based on its practicality. Specific GBA activity of 293-FT_GFP and control were 66.01±10.53 U GBA/mg and 169.0±5.561 (p=0.0032) while secreted GBA activity were 20.15 ± 0.43 and 22.87 ± 0.58 U GBA/mL (p=0.0326), respectively. After transfection with Hef-1α_GFP plasmid, cell population increased only 2.21±0.14 fold and control increased 3.77±0.19 fold (p=0.0032). As transfection efficiency is affected by multiple factors, protocol standardization was crucial for achieving satisfactory levels of gene expression. The transfected cells showed lower specific and secreted GBA activity, probably affected by its lower cell population. The DNA delivered by lyposomal systems might be degraded in lysosomes and may somehow affect GBA production; however, further investigation is required to determine whether this decrease in GBA activity is specific for transfections using Lipofectamine or may also occur with other transfections reagents, such as polyethylenimine.

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WHOLE-EXOME SEQUENCING REVEALS THE IMPACT OF UVA LIGHT MUTAGENESIS IN XERODERMA PIGMENTOSUM VARIANT HUMAN CELLS

Natália Cestari Moreno[1], Tiago A. Souza[1], Camila C. M. Garcia[2], Nathalia Quintero Ruiz[1], Camila Corradi[1], Ligia P. Castro[1], Veridiana Munford[1], Susan Ienne[1] and Carlos F. M. Menck[1]

[1]Institute of Biomedical Sciences, University of Sao Paulo (USP), Sao Paulo, SP. [2]NUPEB & Biological Sciences Department, Federal University of Ouro Preto, Ouro Preto, MG

ncmoreno@usp.br

Key-words: UVA light, XP-V patients, mutagenesis.

The UVA light corresponds to 95% of UV wavelength that reaches the Earth surface and it is able to induce DNA damage, as well as, it participates of skin cancer formation. The DNA polymerase eta (pol eta) protects people against sunlight-induced tumors. The importance of pol eta in suppressing mutagenesis is evidenced in Xeroderma Pigmentosum Variant (XP-V) patients, who present increased frequency of skin cancer. However, the role of UVA-light in the carcinogenesis of these patients is not completely understood. Thus, the goal of this work was evaluating the UVA-induced mutagenesis in normal and XP-V patient cells through whole-exome sequencing. This work provides a detailed evaluation of the UVA-induced increase in point mutations in human cells, especially in cells from XP-V patients, which may help us to understand the increased frequency of skin tumour formation in these patients. The absence of pol eta contributed to higher accumulation of mutations, mainly due to C-containing pyrimidine dimers (C>T or CC>TT). The results also demonstrate the role of pol eta in correctly bypassing oxidatively generated lesions, suppressing C>A mutations, even in the absence of irradiation, which could be related to internal tumours in XP-V patients. Additionally, we observed low levels of mutations at T-containing dimers, due to A insertions opposite these lesions, as suggested by the "A-rule", but these mutations seem to be highly independent of pol eta. On the other hand, pol eta seems to be more important in the insertion of a G (this would be the "G-rule") at C-containing dimers, preventing mutations. The examination of mutational signatures revealed that a single UVA irradiation of XP-V cells, corresponding to 30 minutes of sunlight exposure, was enough to allow the detection of mutational signatures related to cutaneous skin melanoma in the human population. Thus, this work provides data for better understanding the skin cancer initiation process related to DNA damage in XP-V patients as well as healthy individuals.

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DOES CENTROMERE DRIVE EXPLAIN HIGH DIVERSIFICATION OF CENH3 CENTROMERE PROTEIN?

João Pedro do Carmo Filgueiras[1]; **Iderval da Silva Júnior Sobrinho**[1]

[1]Universidade Federal de Jataí - GO.

iderval_jr@yahoo.com

Key-words: molecular evolution; histone; positive selection

Centromeres are essential to chromosome segregation during cell division. Centromere formation is epigenetically marked in nucleosomes by the presence of CENH3 histone. Differently from its paralogous copy (histone H3), CENH3 shows a history of rapid evolution inside lineages. This rapid evolution is mainly observed in the Loop-1 and N-terminal tail domain of CENH3 and is explained by the Centromere Drive Hypothesis (CD Hypothesis). According to this hypothesis, CENH3 high evolutionary rate would be triggered by expansions via duplications of centromeric chromatin, which should favor a "competition" among chromosomes to be included in the unique viable egg in asymmetric female meiosis. In this scenario, chances in CENH3 which would enhance the affinity of this protein to the centromeric region would be positively selected, since they would compensate for the meiotic distortion caused by the expansions in the centromeric region. Considering that the core assumption of CD Hypothesis to explain CENH3 rapid diversification is the occurrence of asymmetric meiosis, one way to test the general validity of this theory could be the study of CENH3 evolution in lineages that have symmetric meiosis (zygotic meiosis). Then, this work aims to test the CD Hypothesis as a general hypothesis by studying the evolution of CENH3 in the Apicomplexa, which is a lineage with symmetric meiosis. We collected CENH3 coding sequences from genomes of four Apicomplexa lineages (Cryptosporida, Eimeriidae, Haemosporida e Sarcocystidae) obtained by BLAST software from GenBank. We aligned the sequences using WebSoftware T-Coffee and estimated a Maximum Likelihood phylogeny using MEGA7 software. We tested for signals of diversification by positive selection in the four Apicomplexa lineages using the Branch-site Test implemented in PAML software. In case we would accept CD Hypothesis, we would expect to find a low evolutionary rate in CENH3 in lineages where meiosis is symmetric, like in the Apicomplexa lineage. However, our results evidenced a high evolutionary rate for CENH3, promoted mainly by positive selection. The signals of positive selection were detected in all tested Apicomplexa lineages and were identified in 19 sites, from which nine were found in secondary structures responsible for protein-protein interactions, such as Loop-1, alpha helix-2, and alpha helix-N. We conclude that CD Hypothesis fails as a general explanation of the rapid diversification of centromere protein CENH3. Considering that some diversifying sites were located in protein-protein interaction regions, we propose a new hypothesis in which the rapid diversification of CENH3 is better explained by a coevolution among CENH3 and kinetochore proteins that interact with it.