MANA 2021

Virtual Conference | Oct 18 - 21

Conference Program and Abstracts

Hosted by:

FFH Foods For Health Research Initiative

The Ohio State University

MANA 2021

Virtual Conference | Oct 18 - 21

Thank you to our sponsors

Agilent

Avanti® Polar Lipids
Part of Croda International Plc

CHENOMX
NMR Mixture Analysis

biocrates
The future of research and health

BRUKER

ThermoFisher
SCIENTIFIC

CIL

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™

MilliporeSigma

SCIEX

Metabolon

IROA TECHNOLOGIES
...making metabolic profiling easy®

Metabolomics Association of North America (MANA)
Schedule of Virtual Events
October 18-21, 2021

Monday October 18 – Instructional Workshops

Time	Topics	Presenters
10:30am – 3:30 pm	COLMAR NMR (Complex Mixture Analysis by NMR)	Rafael Bruschweiler, Abigail Leggett, Ohio State
11:00am – 1:00pm	Skyline	Will Thompson, Duke University
1:00 – 3:30pm	Global Natural Products Social Molecular Networking (GNPS)	Ming Wang & Pieter Dorrestein, University of California San Diego
1:30 – 4:00pm	Microbiome Metabolomics	Ken Liu, Emory University
4:30 – 7:30pm	NMR wine tasting	Lloyd Sumner, University of Missouri

MANA Day 1 – Tuesday October 19

Time	Activity	Presenter(s)
9:00 – 10:45	Mass Spec Mornings (OMSS)	
9:00	Triboelectric Nanogenerator Ion Mobility-Mass Spectrometry	Facundo Fernández, Georgia Tech
9:45	Spatial SILAC, establishing selective pulse chase-SILAC labeling of three-dimensional multicellular spheroids	Nicole Beller, Ohio State
10:05	Combining Electron Activation and Collision-Induced Dissociation Yields Massively Enriched Mass Spectra for >3,000 Metabolites	Uri Keshet, UC Davis
10:25	Combinational open-source software application for unknown feature analysis for LC-MS based untargeted metabolomics	Yongseok Kim, Ohio State
MANA		
11:00 – 11:10	**Welcoming Remarks**	
11:10 – 12:00	**PLENARY:** Marine microbial metabolomics: Investigating the chemical currencies of the surface ocean microbiome	Elizabeth Kujawinski
12:00 – 1:00	**Corporate Events and Interactive Forums**	

	WomiX Interactive Forum: Best Practices for Effective Science Communication		
	Agilent: Lipidomics Workflows- From Discovery Experiments to Quantitative Analysis		
	Waters Corporation: Advances in high-resolution LC-MS and Mass Spectrometry Imaging, for deeper and more confident measurement of the metabolome.		
	IROA: Why not make your data worth working up? The IROA way to better data quality.		
	MilliporeSigma: Food lipidomics in health and disease		
	Thermo Fisher		
1:00 – 2:40	**Oral Presentations**		
	Biomedical 1	*Computational*	*Food/Nutrition*
Moderator	Djawed Bennouna	Ewy Mathe	Daniel Quiroz Moreno
1:00	**Priyanka Baloni, Institute for Systems Biology** Multiomic analyses of sphingolipid pathway identifies potential drugs for Alzheimer's disease	**Hayden Johnson, Univ Memphis** Robust and reproducible time-domain NMR metabolomics of hepatic tissues using CRAFT	**Jacob Folz, UC Davis** In-vivo sampling of healthy human intestinal tract regions using a novel ingestible sampling device with comprehensive metabolomics
1:25	**Abigail Leggett, Ohio State Univ** Unique metabolite and pathway differences between planktonic and biofilm states in Pseudomonas aeruginosa by NMR-based metabolomics	**Yuri Corilo, PNNL** CoreMS: Mass Spectrometry software framework and acquisition-time data analysis	**Robert Hood, Emory** Pesticide residue intake from fruits and vegetables and alterations in the serum metabolome of women undergoing infertility treatment
1:40	**Nicole Prince, Harvard Univ** Steroid metabolite profiles at age 1 are indicative of immune-related outcomes in children through age 6	**Xinmeng Li, Tufts** Exploring improved graph neural networks with topic modeling and attention for spectra prediction	**Rosalie Zhong, Ohio State Univ** A metabolomics approach to iron chlorophyll derivative identification from kale leaf (Brassica oleracea L. var. sabellica) following moderate electric field (MEF) treatment
1:55	**Si Wu, Stanford Univ** Integrated multi-omics analysis of thyroid cancer reveals key molecular pathways involved in tumor formation and metastasis	**Gayatri Iyer, Univ of Michigan** Applications of data-driven network analysis in metabolomics and lipidomics data	**Haley Chatelaine, Ohio State Univ** 1H-NMR and LC-MS untargeted metabolomic profiling of mouse colon in response to probiotic yogurt consumption

2:10	**Oana Zeleznik, Harvard Univ** Metabolomics within-person stability over 10 years among women in two large datasets	**Yujue Wang, Rutgers** AccuCor2: isotope natural abundance correction for dual-isotope tracer experiments	**Michael Dzakovich, Ohio State Univ** Dietary tomato phytochemicals impact the mouse liver transcriptome and metabolome
2:25	**Oliver Fiehn, UC Davis** A Metabolome Atlas of the Aging Mouse Brain	**Yue Wu, Univ of Georgia** Automatic NMR spectral decomposition through computational fitting of time-domain signals	**Sneha Couvillion, PNNL** Multi-platform fecal metabolomics and lipidomics reveals significant differences between vegan and omnivore diets
2:40 – 2:55	Break (15 minutes)		
2:50 – 4:20	**Interactive Forums**		
	SODA (Software & Data Exchange)		
	Precision Medicine		
	Early Career Speed Networking and Virtual Job Fair		
4:20 – 4:30	Break (10 minutes)		
4:30 – 5:20	**PLENARY:** Machine Learning and Metabolomics	David Wishart	
5:20 – 6:50	**Poster Session 1**		
7:00 – 8:00	**MANA Members Business Meeting**		

MANA Day 2 - Wednesday October 20

Time	Activity	Presenter(s)
9:00 – 10:45	Mass Spec Mornings (OMSS)	
9:00	Developing Cross-linking Mass Spectrometry for Interactomics and Structural Biology	Lan Huang, UC Irvine
9:45	Achieving comprehensive lipid profiling with a CCS, retention time and MS/MS library	Naysha Munjoma, Waters
10:05	Direct mass spectrometry analysis of isomeric lipids and free fatty acids via online plasma-droplet derivatization	Alex Grooms, Ohio State
10:25	Development of a computational software for ultra-high precision collisional cross section measurements	Chris Harrilal, PNNL
MANA		
11:00 – 11:50	**PLENARY:** *Big Data, Metabolites and Health*	Michael Snyder

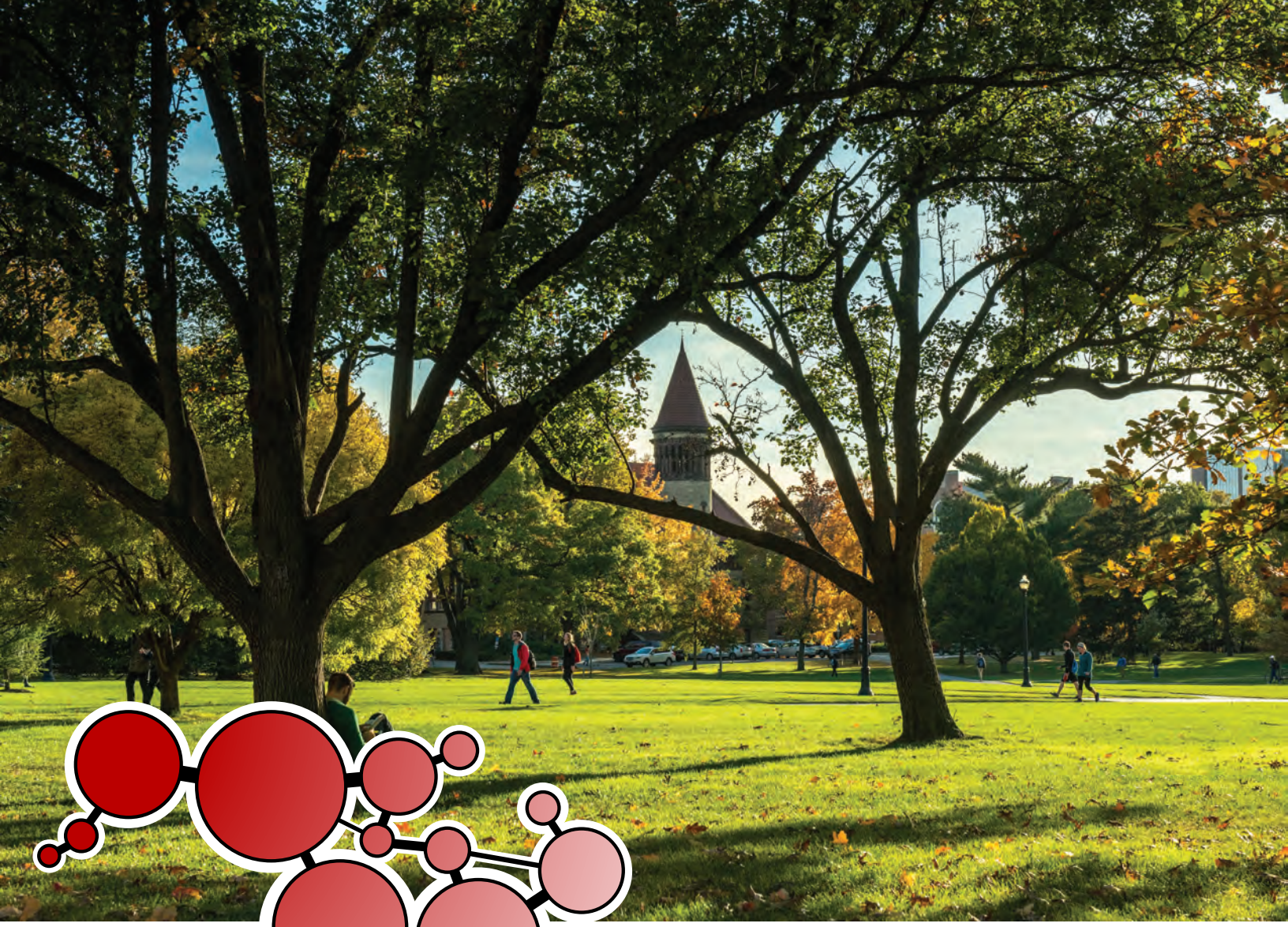
11:50 – 12:00	BREAK (10 minutes)		
12:00 – 1:40	**Oral Presentations**		
	Agriculture, Ecology, & the Environment	*Biomedical 2*	*Metabolite ID*
Moderator	Tracey Schock	Chris Zhu	Tytus Mac
12:00	**Oliver Baars, North Carolina State Univ** Root exudation of secondary metabolites by three tomato varieties in response abiotic and biotic stressors	**Laura-Isobel McCall, Univ of Oklahoma** Chemical cartography-based metabolomics to guide rational drug development	**Vasuk Gautam, Univ Alberta** NP-MRD: The World's Largest NMR Database for Natural Products
12:25	**Christopher Anderton, PNNL** High spatial resolution laser ablation electrospray ionization mass spectrometry for target single cell analysis and imaging application	**Fouad Choueiry, Ohio State Univ** Metabolomics integration with gene expression profiling elucidates IL4I1 as modulator of ibrutinib resistance in ABC-diffuse large B cell lymphoma	**Jessica Bade, PNNL** Drift time shift modeling in IMS-MS/MS fragmentation matching
12:40	**Amanda Bayless, NIST** The Influence of Caging on the Dreissenid Mussel Metabolome	**Xin Ma, Georgia Tech** Ultrahigh resolution imaging mass spectrometry reveals lipidome alterations in early-stage ovarian cancer	**Goncalo Gouveia, Univ of Georgia** Building a fraction library for metabolomics
12:55	**Maris Cinelli, Michigan State Univ** Discovery of indole-tropane hybrid alkaloids from *Datura stramonium*	**Rachel Kelly, Harvard Univ** Metabo-endotypes, Multi-Omic Endotypes and Precision Medicine: An Example from Asthma	**Wenyun Lu, Princeton Univ** Experimental approaches for confident annotation of ammonium adducts in LC-HRMS metabolomics data
1:10	**Pawanjit Kaur Sandhu, Clemson Univ** Mapping the cellular physiology of glyphosate resistance in Palmer amaranth using global metabolomic approaches	**Boryana Petrova, Harvard Univ** Untargeted Metabolomics of the Maternal Immune Activation Brain Model Pathogenicity	**David Degnan, PNNL** Evaluating retention index score assumptions refined existing metrics for GC-MS small molecule identification
1:25	**Vidya Suseela, Clemson Univ** Utilizing phytometabolome to visualize the parasitic and mutualistic	**Hannah Heath, Cal Poly San Luis Obispo** Metabolomics profiling in plasma distinguish metabolic alterations across pregnancy in	**Brady Anderson, Univ of Michigan** Improved Untargeted Metabolomics Compound Identification and Annotation by Using

	phenotypes of arbuscular mycorrhizal fungi	women with gestational diabetes: A case-control time-course analysis	Longer Gradients, Increased Sample Loading, and Iterative Acquisition
1:40 – 1:50	Break (10 minutes)		
1:50 – 3:00	**Interactive Forums**		
	ABRF Metabolomics Research Group		
	Metabolomic Epidemiology: Everything you ever wanted to know, but were afraid to ask		
	MANA NMR Group		
3:00 – 3:10	BREAK (10 minutes)		
3:10 – 4:05	**Poster Lightening Talks**		
4:05 – 4:55	**PLENARY:** *Co-assessing dietary biomarkers and host metabolism: lessons learned from multi-matrix metabolomics in infants, children and adults following food intervention*		Elizabeth Ryan
5:00 – 6:30	**Poster Session 2**		
6:30 – 8:00	**Early Career Network:** Roads less traveled: a look into successful non-traditional career paths in metabolomics		

MANA Day 3 – Thursday October 21

Time	Activity	Presenter(s)
10:00 – 11:00	**Corporate Events**	
	Bruker: Live demo of lipid profiling workflows using MetaboScope, Sven Meyer	
	Biocrates: Zooming into your favorite metabolic pathway: Combining broad and targeted metabolite profiles for a deeper understanding of the metabolome	
	Avanti: Towards high-throughput metabolic phenotyping of human populations: From single-step extraction to 'omic scale quantitative lipid profiling (Juliana Ivanisevic)	
	Sciex: Combining electron-activated and collision-induced dissociation for lipid structural characterization and improved metabolite identification (Uri Keshet) & Global lipidomic and lipid mediator profiling strategies using the high sensitivity SCIEX 7500 system (Paul Norris)	
	Cambridge Isotopes Laboratories: Utilizing 13C-labeled Biological Materials for Targeted and Untargeted Metabolomics	
11:00 – 11:50	**PLENARY:** *A Metabolomics Toolbox to Understand How Plants Grow*	Susan Murch
11:50 – 12:00	BREAK (10 minutes)	

12:00 – 12:45	**Early Career Award Lecture**	Robert Quinn
	Mark P. Styczynski Early Career Award in Computational Metabolomics	Alexandr Smirnov
12:45 – 1:35	**PLENARY:** *Metabolomics approaches to study diet in health and cancer*	Jason Locasale
1:35	**Awards, Closing Remarks**	



MANA 2021

Virtual Conference | Oct 18 - 21

Tuesday, October 19th

Oral Session: Food & Nutrition

In-vivo sampling of healthy human intestinal tract regions using a novel ingestible sampling device with comprehensive metabolomics

Jacob Folz[1], Dari Shalon[2], Oliver Fiehn[1]

[1]UC Davis, West Coast Metabolomics Center, USA
[2]Envivo Bio Inc., USA
jfolz@ucdavis.edu

Abstract

Current studies on the human GI tract only consider stool samples, representing the colon microbiome. However, ignoring the upper gut intestinal tract leaves us blind to understand major processes in food digestion and illnesses like Crohn's disease. Using a newly developed non-invasive ingestible sampling device, 15 subjects were sampled 17 times each between the proximal small intestine and ascending colon over two days. Using different forms of this device the beginning, middle, and end of the intestinal tract were targeted for sampling and samples remained sealed during the rest of intestinal transit. Samples were retrieved and analyzed using MS based metabolomics methods. Stool samples were also collected from the 15 volunteers. Bile acids and short chain fatty acids were quantified using targeted LC-MS/MS and GC-MS respectively. Non-targeted LC-MS/MS using reverse phased, and hydrophilic interaction liquid chromatography and GC-MS were used to further probe the metabolome of the human intestinal tract. All analyses were performed using a total volume of 20 microliters of intestinal tract luminal liquid. More than 1000 unique metabolites were confidently annotated based on accurate mass, retention time, and MS/MS spectral matching. Bile acid chemical classes followed expected trends based on known physiology and functions of the human intestinal tract. Other metabolites are considered as biological classes of endogenous human metabolites, food metabolites, and/or microbial metabolites. Spatial GI-tract variation and inter- / intra-subject variation of intestinal metabolites are considered. We here present new technology that has been developed to non-invasively sample the entirety of the human intestinal tract, and report novel observations of the metabolome of the human upper intestinal tract.

Funding Sources (if applicable):

Pesticide residue intake from fruits and vegetables and alterations in the serum metabolome of women undergoing infertility treatment

Robert Hood[1], Donghai Liang[2], Yu-Han Chiu[3], Helena Sandoval-Insausti[4], Jorge E Chavarro[3,4,5], Dean Jones[6], Russ Hauser[3,7], Audrey J Gaskins [1]

[1]Department of Epidemiology, Emory University, USA
[2]Gangarosa Department of Environmental Health, Emory University, USA
[3]Department of Epidemiology, Harvard University, USA
[4]Department of Nutrition, Harvard University, USA
[5]Channing Division of Network Medicine, Brigham and Women's Hospital and Harvard University, USA
[6]Division of Pulmonary, Allergy, & Critical Care Medicine, Emory University School of Medicine, USA
[7]Department of Environmental Health, Harvard University, USA
rhood2@emory.edu

Abstract

BACKGROUND. Pesticide exposure is linked to a myriad of negative effects; however, the mechanisms underlying these associations are less clear. We utilized metabolomics to describe the alterations in serum metabolome associated with high and low pesticide residue intake from fruits and vegetables (FVs), the most common route of exposure in humans. **METHODS.** This analysis included 171 women undergoing in vitro fertilization who completed a validated food frequency questionnaire and provided a serum sample during controlled ovarian stimulation (2007-2015). FVs were categorized as high or low-to-moderate pesticide residual using a validated method based on pesticide surveillance data from the USDA. We conducted untargeted metabolic profiling using liquid chromatography with high-resolution mass spectrometry and two chromatography columns. We used multivariable generalized linear models to identified metabolic features ($p<0.005$) associated with high and low-to-moderate pesticide residual FV intake, followed by enriched pathway analysis. **RESULTS.** We identified 50 and 109 significant features associated with high pesticide residual FV intake in the C18 negative and HILIC positive columns, respectively. Additionally, we identified 90 and 62 significant features associated with low-to-moderate pesticide residual FV intake in the two columns, respectively. Four metabolomic pathways were associated with intake of high pesticide residue FVs including those involved in energy, vitamin, and enzyme metabolism. 12 pathways were associated with intake of low-to-moderate pesticide residue FVs including cellular receptor, energy, intercellular signaling, lipid, vitamin, and xenobiotic metabolism. One energy pathway was associated with both high and low-to-moderate pesticide residue FVs. **CONCLUSIONS.** We identified limited overlap in the pathways associated with intake of high and low-to-moderate pesticide residue FVs, which supports findings of disparate health effects associated with these two exposures. The identified pathways suggest there is a balance between the dietary antioxidant intake associated with FVs intake and heightened oxidative stress as a result of dietary pesticide exposure.

Funding Sources (If applicable)**:**

This work was supported by the following grants from the NIEHS (P30-ES019776, R01-ES009718, R01-ES022955, P30-ES000002, and R00-ES026648) and NIDDK (P30DK046200). The funding sources had no involvement in the study design, collection, analysis, or interpretation of the data; in the writing of the report; and in the decision to submit the article for publication.

A metabolomics approach to iron chlorophyll derivative identification from kale leaf (*Brassica oleracea L. var. sabellica*) following moderate electric field (MEF) treatment

Siqiong Zhong[1], Jin Hong Mok[2], Ziqi Li[3], Sudhir Sastry[2], Rachel E Kopec[1,4]

[1]Human Nutrition Program, Department of Human Sciences, The Ohio State University, USA
[2]Food, Agricultural and Biological Engineering, College of Food, Agricultural, and Environmental Sciences, The Ohio State University, USA
[3]Department of Food Science and Technology, The Ohio State University, USA
[4]Foods for Health Discovery Theme, The Ohio State University, USA
zhong.198@osu.edu

Abstract

Iron bioavailability (i.e. the amount absorbed) from plant sources is low due to anti-nutrients found in leafy greens and the physical-chemical properties of inorganic iron, as compared to heme iron rich in red meat. We tested a series of novel food processing techniques (i.e. vacuum treatment, bath-immersion, and moderate electric field (MEF) to produce chlorophyll-bound iron within kale leaf (*Brassica oleracea L. var. sabellica*) – chemical form of iron that is structurally analogous to heme. A metabolomics approach was used to identify iron chlorophyll derivatives (ICDs) produced after each step of processing. Treated kale leaves were extracted using a biphasic method, with the petroleum ether fraction separated on a C30 column, and the polar (i.e. methanol/water/acetone) phase separated using C18 column. Analyses were made using ultra high liquid chromatography-diode array detection-quadrupole time-of-flight detection mass spectrometry (UHPLC-DAD-QTOF) with mass scans from 100-1700 *m/z* followed by iterative MS/MS fragmentation. Areas of chromatographic interest were narrowed by identifying regions of the UV-Vis spectra which contained analytes that absorbed light at 400 nm and 600 nm and produced a hypsochromic spectral shift relative native chlorophyll a. Putative structures anticipated following treatment were generated manually, and expected precursor isotopes predicted using the Isotope Distribution Calculator. Features within these regions were then compared, in addition to comparison with the precursor and fragmentation of chlorophyll a and the only available ICD chemical standard (i.e. iron chlorophyllin e4). Iron pheophytin a (*m/z* 925.5175), iron Fe-dehydro-pyropheophytin a (*m/z* 865.4990), and an iron chlorin species (*m/z* 616.1771) in aqueous phase were identified, and most abundant in extracts of leaves treated with moderate electric field treatment. These results demonstrate the utility of metabolomics in identifying unknown metabolites following novel food processing techniques. They also reveal the successful chelation of iron with a chlorophyll backbone. Future studies will determine if these novel ICD metabolites are a more bioavailable source of iron, relative to inorganic iron found in unprocessed kale, or heme-bound iron.

Funding Sources (if applicable):

This research was supported by OARDC via a SEEDS grant and also via Hatch #W4122.

1H-NMR and LC-MS untargeted metabolomic profiling of mouse colon in response to probiotic yogurt consumption

Haley Chatelaine[1], Kathy Tober[2], Michael T. Bailey[3-5], Zhongtang Yu[6], Emmanuel Hatzakis[7,8], Rachel E. Kopec [1,8]

[1]Human Nutrition Program, The Ohio State University, USA
[2]Quanta BioDesign, Ltd., OH, USA
[3]Department of Pediatrics, The Ohio State University College of Medicine, USA
[4]Center for Microbial Pathogenesis, Nationwide Children's Hospital, OH, USA
[5]Oral and GI Research Affinity Group, Nationwide Children's Hospital, OH, USA
[6]Department of Animal Sciences, The Ohio State University, USA
[7]Department of Food Science and Technology, The Ohio State University, USA
[8]Foods for Health and Metabolomics Discovery Theme, The Ohio State University, USA
haley.chatelaine@nih.gov

Abstract

Probiotic yogurt consumption is associated with a variety of beneficial gut health outcomes including improved gut barrier function, production of key nutrients, and immune regulation. However, metabolites that mediate host-microbiome interactions are largely unknown. We hypothesize that consumption of probiotic-containing yogurt during adolescence would result in distinct metabolic signatures driven by increased abundances of lactobacilli and bifidobacteria. A juvenile mouse model (3-week-old C57BL/6N, n = 32 male, 32 female) was employed and fed an AIN-93G diet for 3 weeks, followed by 3 weeks of daily gavage of probiotic yogurt (PY), heat-inactivated yogurt (HY), milk (M), or water (W). Animals were singly housed during the gavage phase. Polar and nonpolar colon extracts were analyzed using three ultra-high performance liquid chromatography-high resolution mass spectrometry methods (i.e. C18, HILIC, and C8 UHPLC-QTof) and ^{1}H NMR metabolomics. Pairwise comparisons were made between UHPLC-QTof metabolite profiles of probiotic yogurt and control diet groups (e.g., heat-inactivated yogurt, milk, and water controls). Probiotic yogurt consumption resulted in increased concentrations of tryptophan metabolites (kynurenine, hydroxy-tryptophan, indole-acetaldehyde), glutathione metabolites (glutathione, S-formylmethyl-glutathione), neurotransmitters (acetylcholine, methacholine, methoxy-hydroxyphenylglychol sulfate), and fat-soluble vitamin metabolite (gamma-carboxyethyl-hydroxychroman, 1-tetranorcholecalciferol) classes, relative to the control treatment groups. These metabolites belong to pathways that may be influenced by microbiome metabolism and have been associated with previously reported benefits of probiotics in both gastrointestinal and systemic health, including improved mood and cognition, xenobiotic metabolism, increased gut barrier function, and decreased inflammation. These results, therefore, provide novel hypotheses by which probiotic yogurt consumption may be influencing colon metabolism to confer the health benefits previously reported.

Funding Sources (if applicable):

This project was partially sponsored by the Dannon Yogurt, Probiotics, and Gut Microbiome Fellowship awarded to HC. Sample analyses were partially supported by NIH Award Number Grant P30 CA016058, OSU, and OSUCCC.

Dietary tomato phytochemicals impact the mouse liver transcriptome and metabolome.

Michael Dzakovich[1, 2], Jennifer M. Thomas-Ahner[3], Steven K. Clinton[3], David M. Francis[4], Jessica L. Cooperstone[1, 5]

[1]Horticulture and Crop Science / The Ohio State University, USA,
[2]Pediatric Nutrition / USDA-ARS Children's Nutrition Research Center, Baylor College of Medicine, USA
[3]Department of Internal Medicine / Division of Medical Oncology, The Ohio State University, USA,
[4]Horticulture and Crop Science / Ohio Agricultural Research and Development Center, The Ohio State University, USA
[5]Food Science and Technology / The Ohio State University, USA
michaelpdz@gmail.com

Abstract

Tomato consumption is associated with many health benefits including lowered risk for developing certain cancers. Absorbed tomato phytochemicals and their metabolites may mediate bioactivity and health outcomes. However, the effects of tomato consumption on cellular processes and the metabolomic landscape of mammalian liver is not well understood. We hypothesized that tomato consumption would differentially alter gene expression and metabolomic signatures in mouse liver. We further propose that tomato varieties with unique phytochemical profiles will produce specific signatures. C57BL/6 mice (n=12/group) were fed a macronutrient matched semi-purified AIN diet containing either 10% red tomato, 10% tangerine tomato, or no tomato powder for 6 weeks after weaning. RNA-Seq analyses revealed that tomato type and consumption, in general, had a modest effect on the transcriptional profiles of mouse livers; altering between 0.02 to 5.6% of total transcripts measured. Although expression profiles varied by diet, gene set enrichment analyses indicated that Phase I and II xenobiotic metabolism were modulated by both tomato varieties. Untargeted metabolomics analysis revealed that mice consuming diets containing tomatoes had altered chemical profiles in liver tissue. Among the features detected, 56-119 (2.2-4.8% of total features) were statistically significant between treatment groups. We confirmed the identity of two steroidal alkaloids (level 1) and identified 17 other Phase I and II metabolites (level 2), including many reported for the first time. Steroidal alkaloids strongly differentiated mice that consumed diets enriched with tomatoes from those that did not. Our findings indicate that tomato consumption can modestly impact transcriptional signatures within the liver due to the presence of phytochemicals and that steroidal alkaloids derived from tomatoes differentiate the liver metabolome of mice fed tomato-rich diets.

Funding Sources (if applicable):

Foods for Health, a focus area of the Discovery Themes Initiative at The Ohio State University.

Multi-platform fecal metabolomics and lipidomics reveals significant differences between vegan and omnivore diets

Sneha P. Couvillion[1], Meagan C. Burnet[1], Sean M. Colby[1], David W. Hoyt[2], Jessica L. Bade[1], Nathalie M. Munoz[2], Jennifer E. Kyle[1], Jesse B. Trejo[1], Kent J. Bloodsworth[1], Josie G. Eder[1], Sandra M. Da Silva[3], Paulina K. Piotrowski[3], Stephanie L. Servetas[3], Scott A. Jackson[3], Thomas O. Metz[1]

[1]Biological Sciences Division, Pacific Northwest National Laboratory, Richland, WA
[2]Environmental Molecular Sciences Laboratory, Pacific Northwest National Laboratory, Richland, WA
[3] Biosystems and Biomaterials Division, National Institute of Standards and Technology, Gaithersburg, Maryland, USA
Email: sneha.couvillion@pnnl.gov

Abstract

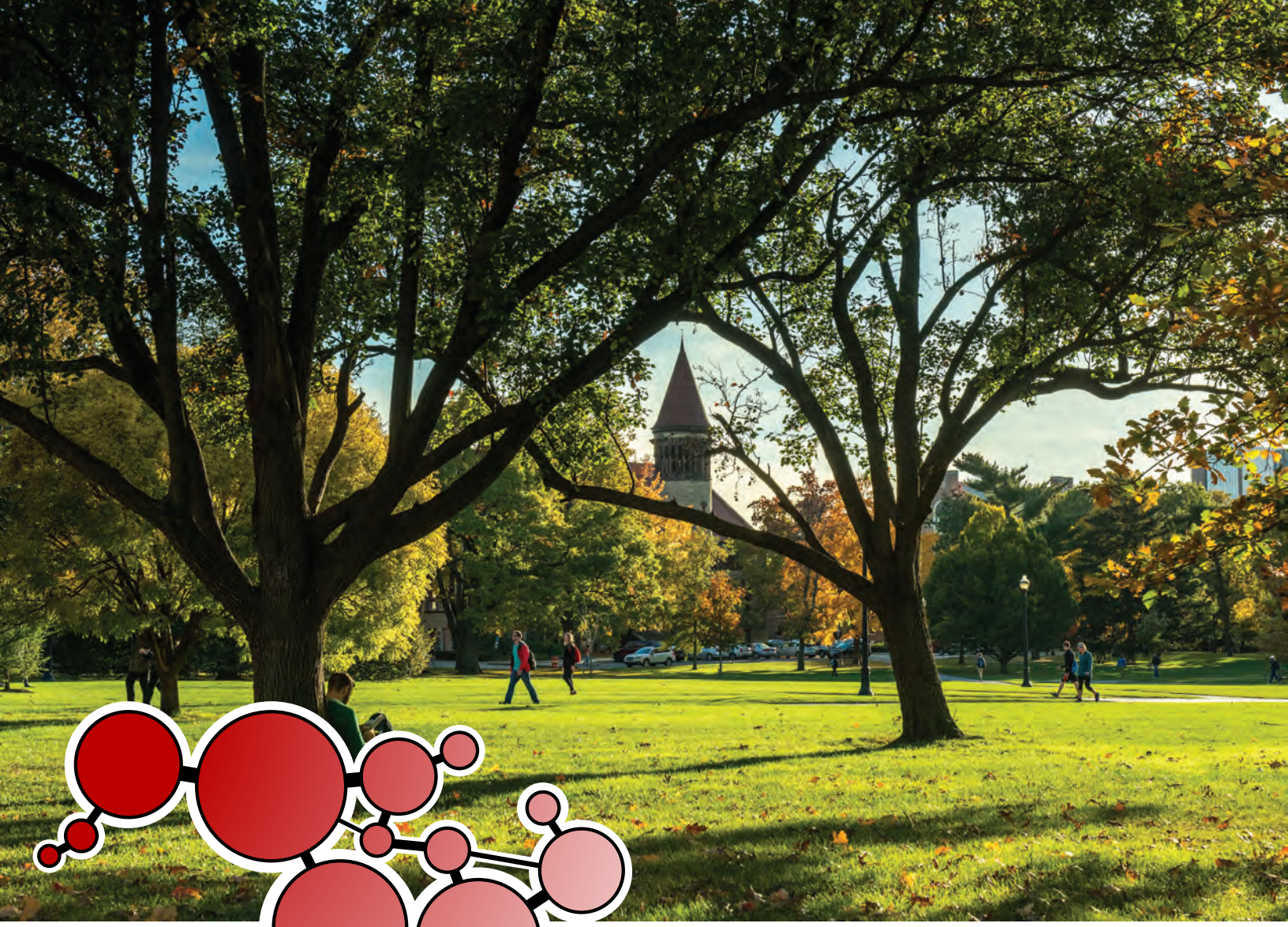
Metabolomics measurements on fecal samples is a powerful approach for characterizing the diverse array of small molecule metabolites that take part in the complex interplay between the microbiota, host, and environment. Given that metabolites, which range from polar small molecules to lipids, are chemically very diverse, no single analytical platform can provide complete coverage of the metabolome.

To maximize fecal metabolome and lipidome coverage for informing biological understanding, we performed untargeted measurements using four analytical platforms including gas chromatography-mass spectrometry (GC-MS), liquid chromatography-tandem mass spectrometry (LC-MS/MS), nuclear magnetic resonance spectroscopy (NMR) and a multi-dimensional platform combining ion mobility spectrometry (IMS) and liquid chromatography-tandem mass spectrometry (LC-IMS-MS/MS). The advantage of the LC-IMS-MS/MS platform is that it provides improved separation and dynamic range of detection and provides an additional dimension of structural information for high confidence identifications. Across all the 4 platforms, distinct differences were observed in the fecal metabolomes and lipidomes from vegan and omnivore subjects.

In this study, we report untargeted metabolomics and lipidomics analyses that were conducted on reference stool material that was received from National Institute of Standards and Technology (NIST) as part of the Whole Stool Interlaboratory Study. We evaluate the metabolome coverage between conventional platforms and the LC-IMS-MS/MS approach which utilized our standards-free metabolite annotation workflow. We also report interesting trends observed in fecal triglycerides, highlighting the value of fecal lipidomics analyses in gut microbiome studies.

Funding Sources:

Funding for Pacific Northwest Advanced Compound Identification Core (PNACIC) comes from the National Institutes of Health, Common Fund Metabolomics Program and the National Institute of Environmental Health Sciences. Untargeted metabolomics and lipidomics analyses were performed in the Environmental Molecular Sciences Laboratory, a national scientific user facility sponsored by the U.S. OBER and located at PNNL in Richland, Washington. PNNL is a multi-program national laboratory operated by Battelle for the DOE under Contract DE-AC05-76RLO 1830.



MANA 2021

Virtual Conference | Oct 18 - 21

Tuesday, October 19th

Oral Session: Computational Approaches

Robust and reproducible time-domain NMR metabolomics of hepatic tissues using CRAFT

Hayden Johnson[1], Chidambaram Ramanathan[2], Melissa Puppa[2], Marie van der Merwe[2], and Aaryani Tipirneni-Sajja[1]

[1]Department of Biomedical Engineering, University of Memphis, USA
[2]College of Health Sciences, University of Memphis, USA,
htjhnson@memphis.edu

Abstract

NMR-based metabolomics is conventionally performed in the frequency-domain which requires many user-dependent steps such as zero-filling, apodization, phase and baseline correction, and quantification by integration or deconvolution and hence, can introduce user bias and inter-operator variability in metabolite quantification. In this study, we demonstrate a Bayesian approach to time-domain NMR parameter estimation, CRAFT (Complete Reduction to Amplitude-Frequency Table), for accurate, time-efficient quantification of lipids and aqueous metabolites in rat tissue samples. Resonances used for quantification of metabolites were identified first by collecting spectra of reference standards and using online databases. The rats were subjects of a time-restricted feeding (TRF) study on one of four diets (chow ad libitum, high fat ad libitum [HF-AD], high fat morning TRF [HF-AM], and high fat evening TRF [HF-EV]), and hepatic tissues were harvested and profiled by NMR to evaluate the effects of diet as the liver is the main regulator of systemic metabolic homeostasis. As expected, rats on the HF-AD diet expressed a significantly greater amount of hepatic triglycerides, cholesterol, and fatty acids than those on the standard chow diet. Lower levels of these lipids were measured in both TRF groups compared to their ad libitum counterparts, with HF-AM exhibiting significantly lower levels of triglycerides and fatty acids compared to HF-AD. The HF-AD was associated with a decrease in glucogenic amino, and TRF attenuated some the high-fat diet induced effects of the ad libitum diet on aqueous metabolite levels as the two TRF groups expressed significantly higher levels of acetate and some amino acids. The metabolite profiles measured by NMR suggest that TRF induces positive metabolic effects. In conclusion, CRAFT can accurately identify and quantify metabolites in biological tissue samples and hence, can increase automation and reproducibility in NMR-based metabolomics.

Funding Sources (if applicable):

NSF support for use of the JEOL spectrometer provided by the Department of Chemistry of the University of Memphis under NSF-grant # 1531466.

CoreMS: Mass Spectrometry software framework and acquisition-time data analysis

Yuri E. Corilo[1], William R. Kew[1], Allison M. Thompson[1], Anastasiya Prymolenna[1], Anubhav[2], Kevin M. Fox[1], Lee Ann McCue[1].

[1]Environmental Molecular Sciences Division, Pacific Northwest National Laboratory, USA
[2]Biological Sciences Division, Pacific Northwest National Laboratory, USA
corilo@pnnl.gov

Abstract

Data handling and software development for modern mass spectrometry (MS) based metabolomics is an interdisciplinary endeavor requiring skills in computational science and a deep understanding of MS. To enable scientific software development to keep pace with fast improvements in MS technology, we have developed a Python software framework named CoreMS. The software data structures were designed with an intuitive, mass spectrometric hierarchical structure, thus allowing organized and easy access to the data and calculations. Furthermore, the framework provides a fundamental, high-level basis for working with all MS metabolomics data types, allowing custom workflows for data signal processing and metabolite annotation. We will demonstrate applications to GC-MS, LC-MS, and FT-ICR MS-based metabolomics data processing and analysis to showcase the framework's capabilities.

Targeting the SRFA analysis used as quality control acquisitions in our FT-ICR mass spectrometers, we will present a novel software architecture that automatically uploads the data to a dedicated computational analysis node and processes the data. The results are then instantly available in a web dashboard within minutes of data acquisition. The ability to generate real-time results will allow the mass spectrometer operator to intervene as required whenever an acquisition job behaves as an outlier of acceptable pre-defined quality control metrics. In addition to automated quality control analysis, we will show our effort to enable real-time data analysis for all the metabolomics data types.

Funding Sources (if applicable):

This research was supported by the Intramural program at EMSL (grid.436923.9), a DOE Office of Science User Facility sponsored by the Biological and Environmental Research program and operated under Contract No. DE-AC05-76RL01830."

Exploring improved graph neural networks with topic modeling and attention for spectra prediction

Xinmeng Li[1], Hao Zhu[1], Liping Liu[1], Soha Hassoun[1,2]

[1]Department of Computer Science, Tufts University, USA
[2]Department Chemical and Biological Engineering, Tufts University, USA
xinmeng.li@tufts.edu

Abstract

A key metabolomics challenge is annotating dark matter with chemical identities. As experimental exploration is costly and time-consuming, computational approaches offer an attractive alternative. We improve on our previously developed model that predicts spectra from molecular graphs (Zhu et al, https://arxiv.org/abs/2010.04661). The input to our model is a molecular graph, where atoms are represented as nodes, and bonds are represented as edges. The output is an array of MS intensities for specified m/z bins. The neural network model comprises multiple layers of graph neural networks (GNNs) followed by pooling.

In this work we further improve on our prior work. Our MS prediction model uses GNN to encode atom attributes with a variety of instruments setting information, e.g., collision energy level and precursor types as node features, and bond attributes as edge features, such as bond type. Additionally, we apply an additional attention layer on the last layer of MS prediction to learn relationships between MS peaks. Further, we improve the GNN-based MS prediction model using multi-task learning methods with LDA (Latent Dirichlet Allocation) labels (van Der Hooft et al, 2016) that identify common spectral motifs within our dataset.

We train and evaluate the model using the 2020 NIST LC-MS/MS dataset. All molecules with spectra in the training set are excluded from the test set. We show 10.63% average rank improvements (from 9.57 to 8.55 for a 100-molecules candidate set) over using a neural network model that utilizes ECFP fingerprint as input. With the LDA and attention relationship improvements, our model learns comprehensive latent graph embedding for molecules with the topic modeling features and attention among MS peaks, which further improves 10.14% on the average rank (from 8.55 to 7.68).

Funding Sources (if applicable):

R01GM132391 and R03OD030601

Applications of data-driven network analysis in metabolomics and lipidomics data

Gayatri R. Iyer[1], Janis Wigginton[2], William Duren[1,2], Jennifer L. LaBarre[3], Marci Brandenburg[1,4], Charles Burant[5], George Michailidis[2,6] and Alla Karnovsky[1]

[1]Department of Computational Medicine and Bioinformatics, University of Michigan Medical School, USA
[2]Michigan Regional Comprehensive Metabolomics Resource Core, Biomedical Research Core Facilities, University of Michigan Medical School, USA
[3]Department of Nutritional Sciences, University of Michigan School of Public Health, USA
[4]Taubman Health Sciences Library, University of Michigan, USA
[5]Department of Internal Medicine, University of Michigan Medical School, USA
[6]Department of Statistics, University of Florida, USA
griyer@umich.edu

Abstract

Over the past decade, mapping and visualizing experimentally measured metabolites in the context of known biochemical pathways has become ubiquitous. However, pathway mapping is restricted to named metabolites from well-annotated biochemical pathways. Realizing the limitations of knowledge-based approaches, we recently developed a novel bioinformatics tool, *Filigree* that can perform data-driven network analysis from metabolomics data (Iyer *et al.*, 2020. PMID: 33255384). *Filigree* can handle "real world" metabolomics data when the number of metabolites exceed the number of samples and when the experimental groups are highly imbalanced. Filigree can be used or the analysis of targeted and untargeted metabolomics and lipidomics data. To demonstrate its utility in gaining mechanistic insights into metabolic changes underlying disease, we analyzed metabolomics datasets from type 1 and type 2 diabetes. The *Filigree* workflow consists of three main steps: (1) building differential partial correlation network across two experimental conditions; (2) network clustering to identify highly interconnected metabolic modules; (3) testing the resulting modules for enrichment.
In addition to differential network analysis, metabolic modules identified by *Filigree* can also be tested for their association with other phenotypes of interest. Using this approach, we were able to identify modules of lipids in maternal and cord blood related to infant birth weight in the Michigan Mother-Infant Pairs (MMIP) cohort.
Data-driven metabolic modules can also be derived from single-group data. To illustrate this, we used our previously published DSPC method (Basu *et al.*, 2017, PMID: 28137712) to analyze metabolomics data from Amyotrophic Lateral Sclerosis (ALS) patients and tested the resulting metabolic modules for their association with BMI trajectory over a period of 10 years prior to disease diagnosis. We will discuss these, and other applications of data-driven network analysis tools for metabolomics and lipidomics data.

Funding Sources (if applicable):

This work is partially supported by grants NIH 1U01CA235487 (A.K., G.M.) and NIH 5R21AI223380 (G.M.) and 4R01GM11402905 (G.M.) and 5R01GM114029-05 (G.M.)

AccuCor2: isotope natural abundance correction for dual-isotope tracer experiments

Yujue Wang[1,2], Lance R. Parsons[3], Xiaoyang Su[1,2]

[1]Department of Medicine, Rutgers-Robert Wood Johnson Medical School, USA
[2]Metabolomics Shared Resource, Rutgers Cancer Institute of New Jersey, USA
[3]Lewis Sigler Institute for Integrative Genomics and Department of Chemistry, Princeton University, USA
yw429@rwjms.rutgers.edu

Abstract

Stable isotope labeling techniques have been widely applied in the field of metabolomics and proteomics. Before the measured mass spectral data can be used for quantitative analysis, it must be accurately corrected for isotope natural abundance and tracer isotopic impurity. Despite the increasing popularity of dual-isotope tracing strategy such as ^{13}C-^{15}N or ^{13}C-^{2}H, there are no accurate tools for correcting isotope natural abundance for such experiments in a resolution dependent manner. Here, we present AccuCor2 as an R-based tool to perform the correction for ^{13}C-^{15}N or ^{13}C-^{2}H labeling experiments. Our method uses a newly designed algorithm to construct the correction matrices that link labeling pattern and measured mass fractions, then use non-negative least-squares to solve the labeling patterns. Our results show that the dual-isotope experiments often require a mass resolution that is high enough to resolve ^{13}C and ^{15}N or ^{13}C and ^{2}H. Otherwise, the labeling pattern is not solvable. However, this mass resolution may not be sufficiently high to resolve other non-tracer elements such as oxygen or sulfur from the tracer elements. Therefore, we design AccuCor2 to perform the correction based on the actual mass resolution of the measurements. Using both simulated and experimental data, we show that AccuCor2 performs accurate and resolution dependent correction for dual-isotope tracer data.

Funding Sources (if applicable):

This research is supported, in part, by NIH grants P30CA072720-5923 (XS).

Automatic NMR spectral decomposition through computational fitting of time-domain signals

Yue Wu[1], Goncalo Gouveia[2], Sicong Zhang[2, 3], Krish Krishnamurthy[4], Frank Delaglio[5], Arthur S. Edison[1, 2, 3, 6]

[1] Institute of Bioinformatics, University of Georgia, USA
[2] Department of Biochemistry and Molecular Biology, University of Georgia, USA
[3] Complex Carbohydrate Research Center, University of Georgia, USA
[4] Chempacker LLC, USA
[5] Institute for Bioscience and Biotechnology Research, NIST, USA
[6] Department of Genetics, University of Georgia, USA
Yue.Wu@uga.edu

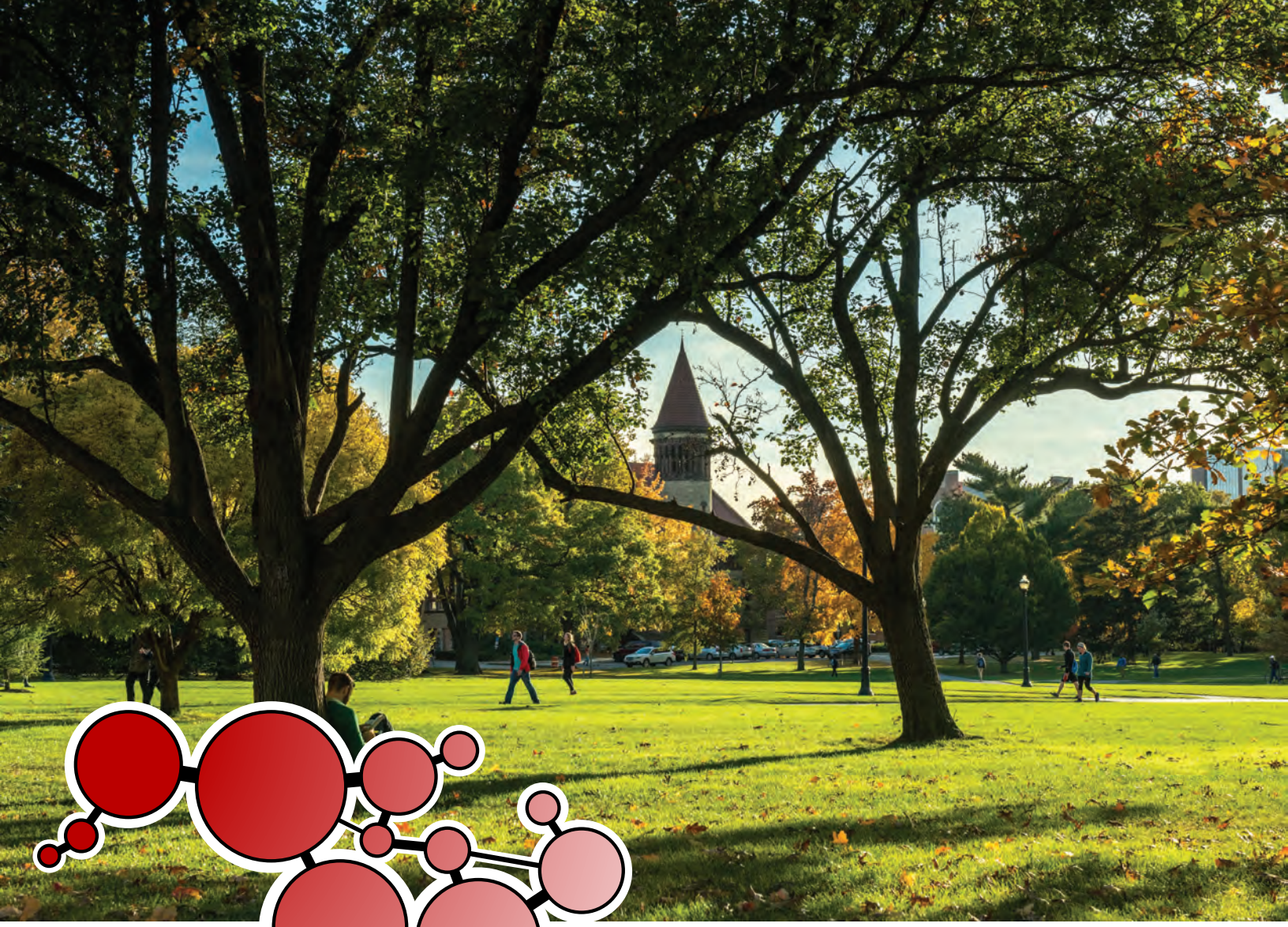
Abstract – **Maximum 300 words**

NMR enables efficient characterization of metabolism, measuring responses to genetic mutants and environmental perturbations or even *in vivo* metabolic dynamics. Data can be collected rapidly, but automation of analysis is a bottleneck, leaving metabolomics harder than transcriptomics in extensive quantification. This objective requires automating the current labor-intensive workflow of spectral annotation and quantification. Such automation is often obstructed by spectral overlap and distorted baselines. Spectral decomposition can tackle these problems by enabling direct observation of the overlapped peaks for annotation and quantification. However, existing approaches cannot provide reliable extensive metabolic quantification as they often depend on matching measured NMR signals, with experimental imperfections, to spectra with ideal line shapes.

We implemented a general solution for time-domain spectral decomposition. Fitting in the time domain makes it easier to deal with baseline artifacts, since baseline distortion that spans the spectrum is limited to a small number of points at the start of the corresponding time domain. The signal was fitted by a sum of damped sine waves to ensure Lorentzian spectral peaks and parameters were estimated by the Metropolis method. Frequency-based subsampling was also used to reduce computational complexity and enable parallelization. Our decomposition approach was tested with simulated and experimental datasets, including 146 NMR spectra of different *Caenorhabditis elegans* mutant strains. In each spectrum, around 800 features were extracted. Besides providing accurate peak quantification, we also proposed annotations by correlation network clusters of those decomposed features in 1D spectra. Annotations will be validated with spectra of HPLC fractions of the same material. Hence, by our decomposition approach, NMR spectra can be viewed through an automatically generated feature list with proposed annotations, just as in other omics. Our approach can be expanded to higher dimensionality and can accommodate non-uniformly sampled data.

Funding Sources (if applicable):

NSF 2041546
NIH 5U2CES030167-04



MANA 2021

Virtual Conference | Oct 18 - 21

Tuesday, October 19th

Oral Session: Biomedical 1

Multiomic analyses of sphingolipid pathway identifies potential drugs for Alzheimer's disease

Priyanka Baloni[1]*, Matthias Arnold[2,6]*, Herman Moreno[3]*, Kwangsik Nho[4]*, Luna Buitrago[3], Kevin Huynh[5], Barbara Brauner[2], Gregory Louie[6], Alexandra Kueider-Paisley[6], Karsten Suhre[7], Andrew J. Saykin[4], Kim Ekroos[8], Peter J. Meikle[5], Leroy Hood[1], Nathan D. Price[1], The Alzheimer's disease Metabolomics Consortium, P. Murali Doraiswamy[6], Cory C. Funk[1], Gabi Kastenmüller[2], Rebecca Baillie[9], Xianlin Han[10‡] and Rima Kaddurah-Daouk[6, 11,12‡]

1Institute for Systems Biology, WA, USA
2 Institute of Computational Biology, Helmholtz Zentrum München - German Research Center for Environmental Health, DE
3 Department of Neurology/Pharmacology, SUNY Downstate Medical Center, USA
4 Indiana Alzheimer Disease Center and Department of Radiology and Imaging Sciences, Indiana University School of Medicine, Indianapolis, IN, USA
5 Metabolomics Laboratory, Baker Heart and Diabetes Institute, Melbourne, AU
6 Department of Psychiatry and Behavioral Sciences, Duke University School of Medicine, USA
7 Department of Physiology and Biophysics, Weill Cornell Medicine-Qatar, Education City, QA
8 Lipidomics Consulting Ltd., Esbo, FI
9 Rosa & Co LLC, CA, USA
10 University of Texas Health Science Center at San Antonio, USA
11 Department of Medicine, Duke University, USA
12 Duke Institute of Brain Sciences, Duke University, USA
pbaloni@isbscience.org

Alzheimer's disease (AD) is the leading cause of dementia and neurodegeneration in AD has been correlated with metabolic dysfunction. Ceramides and sphingomyelin are important metabolites in sphingolipid pathway that have been implicated in AD. Sphingomyelins (SM) have been shown to be disrupted in AD and in other neurodegenerative diseases. Ceramides are known to be associated with Aβ production and inflammation. The detail mechanism of ceramide/sphingomyelin dysregulation and its impact on AD pathogenesis is not fully understood. In this study we used post-mortem brain transcriptome data of 1000 AD and cognitively normal individuals from multiple cohorts and identified differentially expressed genes in SM pathway. Using the metabolic networks of brain regions, we carried out metabolic flux analysis to identify reactions and genes that are altered in SM pathway. We performed multimodal neuroimaging analysis and identified genetic variants linked to genes in SM pathway and associated with AD pathogenesis. We expanded our study by analyzing lipidomic data from ADNI participants and correlated it with the genetic association studies to identify SM species that are linked with AD pathogenesis. The multi-omics analysis suggested increase in ceramide and depletion of sphingosine-1-phosphate (S1P) that alters sphingolipid homeostasis in AD. We tested our hypothesis by modulating S1P activity in APP/PS1 mice by targeting the receptor using fingolimod, an FDA-approved drug for multiple sclerosis. The experimental analysis showed that altering S1P signaling rescues cognitive deficits in the treated mice. The study also identified fingolimod and other modulators of S1P metabolism might be potent drug repositioning candidates for AD. Our study used transcriptomics, metabolomics, lipidomics, genetic associations, constraint-based modeling and neuroimaging data to inform the key regulatory steps in SM pathway and potential drugs that were experimentally validated in animal models. The power of big data and complementary multiomics approaches raises the possibility of repositioning existing drugs for AD.

Funding Sources (if applicable):

Data collection and sharing for this project was funded by the Alzheimer's Disease Neuroimaging Initiative (ADNI) (National Institutes of Health Grant U01 AG024904) and DOD ADNI (Department of Defense award number W81XWH-12-2-0012). ADNI is funded by the National Institute on Aging, the National Institute of Biomedical Imaging and Bioengineering, and through generous contributions from the following: AbbVie, Alzheimer's Association; Alzheimer's Drug Discovery Foundation; Araclon Biotech; BioClinica, Inc.; Biogen; Bristol-Myers Squibb Company; CereSpir, Inc.; Cogstate; Eisai Inc.; Elan Pharmaceuticals, Inc.; Eli Lilly and Company; EuroImmun; F. Hoffmann-La Roche Ltd and its affiliated company Genentech, Inc.; Fujirebio; GE Healthcare; IXICO Ltd.; Janssen Alzheimer Immunotherapy Research & Development, LLC.; Johnson & Johnson Pharmaceutical Research & Development LLC.; Lumosity; Lundbeck; Merck & Co., Inc.; Meso Scale Diagnostics, LLC.; NeuroRx Research; Neurotrack Technologies; Novartis Pharmaceuticals Corporation; Pfizer Inc.; Piramal Imaging; Servier; Takeda Pharmaceutical Company; and Transition Therapeutics. The Canadian Institutes of Health Research is providing funds to support ADNI clinical sites in Canada. Private sector contributions are facilitated by the Foundation for the National Institutes of Health (www.fnih.org). The grantee organization is the Northern California Institute for Research and Education, and the study is coordinated by the Alzheimer's Therapeutic Research Institute at the University of Southern California. ADNI data are disseminated by the Laboratory for Neuro Imaging at the University of Southern California.

The Religious Orders and the Rush Memory and Aging studies were supported by the National Institute on Aging grants P30AG10161, R01AG15819, R01AG17917, U01AG46152, and U01AG61356. National Institute on Aging (NIA) supported this work (R01 AG059093). NIA also supported the Alzheimer Disease Metabolomics Consortium which is a part of NIA's national initiatives AMP-AD and M²OVE-AD (R01 AG046171, RF1 AG051550, 3U01 AG061359-02S1, and 3U01 AG024904-09S4). Additionally, M.A., R.K.D., and G.K. are supported by NIA grants RF1 AG058942 and R01 AG057452. M.A. and G.K. are also supported by funding from Qatar National Research Fund NPRP8-061-3-011. K.N. is supported by NIH grants NLM R01 LM012535 and NIA R03 AG054936. X.H. is supported by NIA grant RF1 AG061872. P.B. acknowledges the support of 5U01 AG061359-02 and 5U01 AG061359-03 from NIA. C.F. acknowledges the support of R01AG062514, U01AG046139 from NIA.

Unique metabolite and pathway differences between planktonic and biofilm states in *Pseudomonas aeruginosa* by NMR-based metabolomics

Abigail Leggett[1,2,3], Devin Sindeldecker[2], Da-Wei Li[4], Lei Bruschweiler-Li[4], Paul Stoodley[2], and Rafael Brüschweiler[3,4,5]

[1]Ohio State Biochemistry Program, The Ohio State University, USA
[2]Department of Microbial Infection and Immunity, The Ohio State University, USA
[3]Department of Chemistry and Biochemistry, The Ohio State University, USA
[4]Campus Chemical Instrument Center, The Ohio State University, USA
[5]Department of Biological Chemistry and Pharmacology, The Ohio State University, USA
leggett.49@osu.edu

Abstract

P. aeruginosa is an opportunistic human pathogen that causes acute and chronic infections. Due to its threat to human health, in 2017 the World Health Organization rated *P. aeruginosa* as a priority pathogen needing research attention. It readily forms biofilms in diverse environments, in which the cells become embedded in a gel-like matrix of self-produced extracellular polymeric substances such as polysaccharides, proteins, and DNA. Biofilms are difficult to eradicate because of their resistance to most antibiotics, greatly contributing to the persistence of infection. Therefore, there is a critical need for new approaches to accurately regulate and prevent biofilm formation. Biofilm development results from multiple changes in phenotype, however, the underlying biological mechanisms that allow the transition from the planktonic free-floating state to attached biofilm communities are not fully understood. Metabolomics provides a global analysis of metabolites, giving an unbiased view of cellular activity. We hypothesize that the metabolic profiles of cells in the planktonic and biofilm states will reveal differences in pathways linked to coordinating the change in phenotype, which could increase the understanding of mechanisms required for biofilm formation. We utilize multidimensional NMR, which has the ability to reproducibly detect, quantify, and reveal molecular structural information of all abundant known/unknown metabolites in complex mixtures in a single set of measurements. Our NMR data analysis including metabolite identification, quantification, and uni-/multi-variate statistical analysis is completed using our COLMAR webservers. We report newly discovered metabolite differences between planktonic and biofilm states. Of the 66 metabolites identified so far, 24 have a significant difference ($p<0.01$ and fold change >2) between planktonic and biofilm sample cohorts (n=9). Several of these metabolites are specific carbohydrates and metabolites from amino acid degradation pathways. Our results point to pathways or enzymes that could potentially be targeted to slow or prevent biofilm formation.

Funding Sources (if applicable):

Ohio State Biochemistry Program
National Institutes of Health (NIH)

Steroid metabolite profiles at age 1 are indicative of immune-related outcomes in children through age 6

Nicole Prince[1], Mengna Huang[1] , Rachel S. Kelly[1], Min Kim[2], Gözde Gürdeniz[2], Bo L. Chawes[2], Jessica Lasky-Su[1]

[1]Channing Division of Network Medicine, Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, USA
[2]Copenhagen Prospective Studies on Asthma in Childhood (COPSAC), Herlev and Gentofte Hospital, University of Copenhagen, DK
reapr@channing.harvard.edu

Abstract

Immune development during early childhood is a complex process governed by a range of genetic and environmental influences, with lasting impacts on long term health. The first year of life, in particular, is regarded as a critical time for immune development that can shape innate and adaptive immunity. Considering the high rates of morbidity and mortality in children as a result of increased susceptibility to infections, better understanding of the early influences that shape immune response is critically important. Towards this goal, this study investigated the associations of steroid metabolites with cumulative infection counts in two cohorts of children: the Vitamin D Antenatal Asthma Reduction Trial (VDAART, N=396) and the Copenhagen Prospective Studies on Asthma in Childhood (COPSAC, N=433). Untargeted metabolite data were collected in plasma across five infant and early childhood timepoints to investigate the influence of steroid metabolite profiles over time. In the VDAART age 1 samples, 18 steroid metabolites in the androgenic, corticosteroid, pregnenolone, and progestin sub-pathways were inversely associated with cumulative infections through age 6 (p-values ranged from 3.30×10^{-13} to 2.61×10^{-4}). At age three, nine steroid metabolites were significantly associated with cumulative infections ($p=3.51 \times 10^{-4}$ to 4.84×10^{-2}), and at age six, eight were significant associated ($p=9.95 \times 10^{-8}$ to 4.84×10^{-2}). In COPSAC, we were able to validate the associations between early life steroid levels and cumulative infections through age 3 in plasma samples collected at six months and 18 months ($p= 1.01 \times 10^{-3}$ to 1.37×10^{-2}). These data suggested an important role for steroids in early immune development and that reduced steroidogenesis in infancy and early childhood is associated with higher infection proneness, representing a novel investigation into the impact of steroids on overall immune sufficiency. Further, these results suggested that age 1 is a critical time point for immune trajectories, which may hold clinical relevance for intervention strategies.

Funding Sources (if applicable):

This work was funded by NIH NHLBI R01HL141826. NP is supported by NIH T32 HL007427.

Integrated multi-omics analysis of thyroid cancer reveals key molecular pathways involved in tumor formation and metastasis

Si Wu[1]; Akshay Sanghi[1]; Lisa Ann Orloff[1]; Maya M. Kasowski[1]; Michael Snyder[1]

[1]Stanford University, USA
siwu@stanford.edu

Abstract

Thyroid cancer is one of the most common endocrine cancers with a continuously increasing incidence worldwide. In this study we collected 27 matched normal thyroid glands, 31 thyroid primary tumors, and 31 lymph node metastasis samples from 36 thyroid cancer patients, and performed a deep multi-omics profiling experiment, including WGS, ATAC-seq, RNA-seq, proteomics, metabolomics, and lipidomics. We observed dramatic molecular differences between primary tumor/metastasis and normal samples, whereas the differences between primary tumor and metastasis samples were subtle. Transcriptomic, proteomic, metabolomic and lipidomic analyses revealed a dynamic choreography of molecular and cellular events that present three different changing patterns: cancer progression, tumor formation and tumor metastasis. These involve processes such as cell cycle and cell proliferation, cancer-related signaling pathways, immune response, metabolic reprogramming (including Warburg effects, dysregulated energy metabolism, carbohydrates, nucleotides, amino acids, and lipids), decreased thyroid hormone biosynthesis. Intriguingly, we found that the AhR signaling pathway was one of the top significantly activated pathways at both gene expression and protein levels, playing crucial pro-tumor roles by immunosuppressive function. We also detected the significant increase of tryptophan catabolite kynurenine, the endogenous tumor-promoting ligand of AhR, from our metabolomics data. Moreover, we observed significantly decreased proportions of CD8 T cells in the tumor and metastasis samples, whereas significant decrease and increase for macrophage M1 (anti-tumor) and M2 (pro-tumor), respectively. This multi-omics integration example indicates that AhR signaling pathway may be a novel drug targets of thyroid cancer, manifested by the fact that the IDO1 inhibitor, which blocks the produce of ligand kynurenine and further block AhR signaling, is in clinical trials. Overall, our multi-omics integrative results reveal key molecular events during thyroid cancer formation and metastasis and its potential application in discovery of novel drug targets in thyroid cancer.

Funding Sources (if applicable):

Metabolomics within-person stability over 10 years among women in two large datasets

Oana A. Zeleznik[1], Clemens Wittenbacher[2], Amy Deik[3], Sarah Jeanfavre[3], Julian Avila-Pacheco[3], Bernard Rosner[1], Kathryn M. Rexrode[4], Clary B. Clish[3], Frank B. Hu[2], A. Heather Eliassen[1,5]

[1]Channing Division of Network Medicine, Brigham and Women's Hospital and Harvard Medical School, USA
[2]Department of Nutrition, Harvard T.H. Chan School of Public Health, USA
[3]Broad Institute of Massachusetts Institute of Technology and Harvard, USA
[4]Division of Women's Health, Brigham and Women's Hospital and Harvard Medical School, USA
[5]Department of Epidemiology, Harvard T.H. Chan School of Public Health, USA
nhotz@channing.harvard.edu

Abstract

Background: In epidemiological studies, samples are often collected long before disease onset or outcome assessment. Understanding the long-term stability of biomarkers measured in these samples is crucial. We estimated within-person stability over 10 years of lipid and lipid-related, and polar metabolites within the Nurses' Health Study (NHS).

Methods: We quantified plasma metabolomics using liquid chromatography mass spectrometry at the Broad Institute. The primary dataset included samples from 1880 women, 1184 of which provided two samples 10 years apart, 315 known and 6708 unknown metabolite features. The secondary dataset included samples from 1456 women, 488 of which donated two samples 10 years apart, 218 known and 3434 unknown metabolite features. We estimated within-person stability over 10 years by calculating intra-class correlation (ICC) using liner mixed models with participant IDs as a random effect.

Results: The median ICC was 0.43 (1st quartile (Q1): 0.35; 3rd quartile (Q3): 0.50) among known metabolites and 0.41 (Q1: 0.34; Q3: 0.47) among unknown metabolite features. The most stable metabolite classes were nucleosides, nucleotides, and analogues, phosphatidylcholine plasmalogens, and diglycerides. ICCs estimated among all women (median ICC: 0.43, Q1: 0.35; Q3: 0.50) tended to be lower than those estimated among fasting women (median ICC: 0.44, Q1: 0.37; Q3: 0.52) and women with a stable BMI (median ICC: 0.43, Q1: 0.36; Q3: 0.51). Results in the secondary dataset, which included polar metabolites and fasting women, were similar to corresponding results in the primary dataset.

Conclusion: Lipids and lipid-related, and polar metabolites show reasonable within-person stability over 10 years. Metabolomics within-person stability over 10 years varies by metabolite class, fasting status and with change in BMI. Similar results were observed in secondary analyses in which we performed a partial replication of polar metabolites among fasting women.

Funding Sources (If applicable):

National Cancer Institute

A metabolome atlas of the aging mouse brain

Jun Ding[1,2], Jian Ji[3], Zachary Rabow[1], Tong Shen[1], Jacob Folz[1], Christopher R. Brydges[1], Sili Fan[1], Xinchen Lu[1], Sajjan Mehta[1], Megan R. Showalter[1], Ying Zhang[1], Renee Araiza[4], Lynette R Bower[4], K. C. Kent Lloyd[4], Oliver Fiehn[1]

[1] West Coast Metabolomics Center, University of California-Davis, USA
[2] Department of Chemistry, Wuhan University, PR China
[3] State Key Laboratory of Food Science and Technology, Jiangnan University, PR China
[4] Mouse Biology Program, University of California-Davis, USA
ofiehn@ucdavis.edu

Abstract

The mammalian brain relies on neurochemistry to fulfill its functions. Yet, the complexity of the brain metabolome and its changes during diseases or aging remains poorly understood.

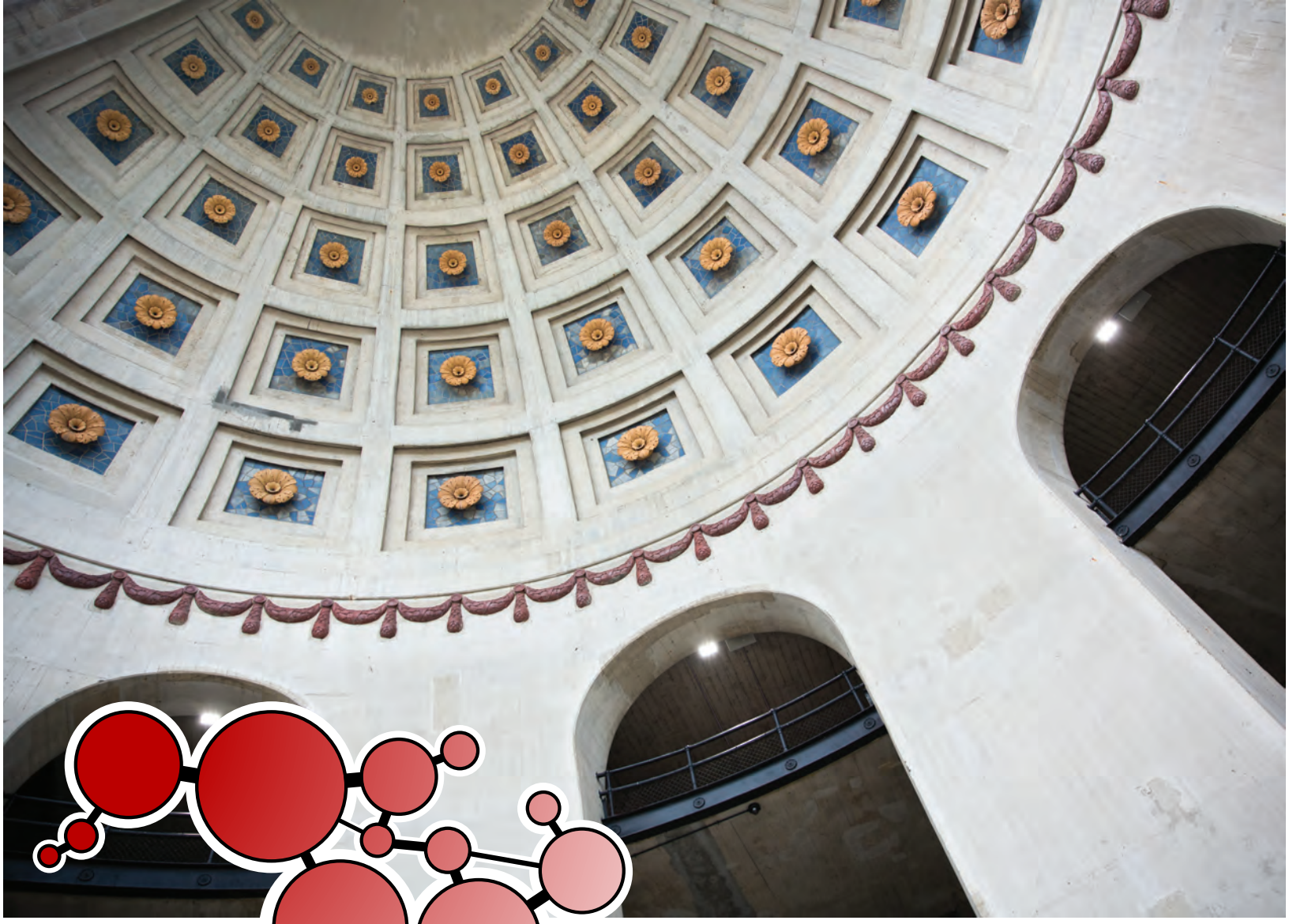
To start bridging this gap, we generated a metabolome atlas of the aging wildtype male and female mouse brain from 10 anatomical regions spanning from adolescence to old age. We combined data from three chromatography-based mass spectrometry assays and structurally annotated 1,547 metabolites to reveal the underlying architecture of aging-induced changes in the brain metabolome.

Overall differences between sexes were minimal. We found 99% of all metabolites to significantly differ between brain regions in at least one age group. We also discovered that 97% of the metabolome showed significant changes with respect to age groups. For example, we identified a shift in sphingolipid patterns during aging that is related to myelin remodeling in the transition from adolescent to aging brains. This shift was accompanied by large changes in overall signature in a range of other metabolic pathways. We found clear metabolic similarities in brain regions that were functionally related such as brain stem, cerebrum and cerebellum. In cerebrum, metabolic correlation patterns got markedly weaker in the transition from adolescent to adulthood, whereas the overall correlation patterns between all regions reflected a decreased brain segregation at old age. We were also able to map metabolic changes to gene and protein brain atlases to link molecular changes to metabolic brain phenotypes.

Metabolic profiles can be investigated via https://mouse.atlas.metabolomics.us/ .
This new resource enables brain researchers to link new metabolomic studies to a foundation data set

Funding Sources (If applicable):

This work was funded by NIH U2C ES030158 and U19 AG023122 (to O.F.),
KOMP2-Phase 2 Production and Phenotyping by the DTCC Consortium (NIH UM1 OD023221, to K.C.L.),
and a fellowship from the Postdoctoral International Exchange Program of China Postdoctoral Science Foundation to J.D.



MANA 2021

Virtual Conference | Oct 18 - 21

Wednesday, October 20th

Oral Session: Agriculture, Ecology & the Environment

Root exudation of secondary metabolites by three tomato varieties in response abiotic and biotic stressors

Oliver Baars[1], Vineeta Rai[1], Althea Loucanides[1]

[1]Department of Entomology and Plant Pathology, North Carolina State University, USA
obaars@ncsu.edu

Abstract

In this study, we report (1) how tomato roots shift their secondary metabolite exudation profiles to respond to specific abiotic and biotic stress scenarios, (2) how secreted exudates contrast with root tissue metabolite concentrations and (3) how different secreted metabolites are between three different tomato varieties. The stressors consisted of resource limitation (iron or phosphate) and infection with a tomato-field-isolated enterobacterium. The tomato varieties consisted of two commercial lines (*Solanum lycopersicum* 'Heinz 1706' and 'Hawaii line 7996') and a wild tomato relative (*S. pimpinellifolium*).

Plants were grown in hydroponic cultures with chemically defined mineral media. Root exudates were desalted by solid-phase-extraction (Oasis HLB cartridges). To compare exuded metabolites with intracellular metabolites, root tissue of iron limited plants was extracted using methanol. All samples were analyzed by high-resolution LC-MS/MS (Orbitrap ID-X) in combination with LC-UV/vis and LC-Charged Aerosol Detection. Additional analyses were performed to characterize nutrient limitation, including tissue metal analyses by ICP-MS and approximating measurements of phenolics and reducing activity (Folin–Ciocalteu reagent assay, FRAP assay).

After combining positive and negative ion modes and filtering to remove adducts and other co-eluting features, we detected 2,176 features that were robustly present in all replicates of at least one condition. Several secondary metabolites were constitutively secreted by all plants, notably the glycosteroid tomatine at high concentrations. In addition, there were stress and variety-specific exudates, in particular a variety of phenylpropanoid pathway products. The results presented in this study show how exudation varies qualitatively and quantitatively between plant varieties, how much plants control their exudation patterns in response to specific stressors, and which secondary metabolites are likely to shape the rhizosphere microbiome of tomato.

Funding Sources (if applicable):

NC Biotechnology Center Flash Grant to OB (2020-FLG-3857)
NIFA Hatch Grant to OB (NC02756)

High spatial resolution laser ablation electrospray ionization mass spectrometry for target single cell analysis and imaging application

Michael J. Taylor[1], Andrey Liyu[1], Akos Vertes[2], Christopher R. Anderton[1]

[1]Earth and Biological Sciences Directorate, Pacific Northwest National Laboratory, USA
[2]Department of Chemistry, The George Washington University, USA
Christopher.Anderton@pnnl.gov

Abstract

Spatially resolved mass spectrometry approaches, including mass spectrometry imaging (MSI), have the ability to reveal the metabolome within anatomical compartments of tissues and across populations of individual cells. Laser ablation electrospray ionization mass spectrometry (LAESI-MS) is an emerging method that has shown notable potential in the field of spatial metabolomics. This is in part due to LAESI-MS being an ambient ionization method that requires minimal sample preparation and uses (endogenous) water for *in situ* analysis. Recently, we reported the integration of a microscope into the optical train of our conventional LAESI source to allow for visually informed ambient in situ single cell analysis and MSI with improved lateral resolution. When we coupled this 'LAESI microscope' to a drift-tube ion mobility mass spectrometer, we were able to separate isobaric species and allow for the determination of ion collision cross sections, in conjunction with accurate mass measurements, for ambient single cell metabolomics analysis. We also coupled this LAESI microscope to a Fourier transform mass spectrometer to obtain higher throughput and more sensitive analysis of single *Allium Cepa* epidermal cells. Also under this configuration, we were able to image native plant tissue at the highest spatial resolution (40 µm) reported to date. This enabled us to detect specific metabolites localized in different anatomical regions of *Fittonia argyroneura* leaves. We are currently working on including dual channel optical imaging in our source to permit multiwavelength microscopy (e.g., brightfield plus fluorescence), to increase our optically targeted spatially resolved mass spectrometry capability.

Funding Sources (if applicable):

The influence of caging on the dreissenid mussel metabolome

Amanda Bayless[1], Eléna Legrand[1,2], Annie Jacob[3], Erik Davenport[4], Ed Johnson[4], Tracey Schock[1]

[1]National Institute of Standards and Technology, Chemical Sciences Division, USA
[2]Faculty of Agricultural and Environmental Sciences, McGill University, CA
[3]CSS Inc., VA, USA
[4]NOAA National Center for Coastal Ocean Science, USA
amanda.bayless@nist.gov

Abstract

NMR metabolomics has been successfully applied to examine changes in the metabolome of dreissenid mussels with exposure to different pollutant scenarios in the environment. This advancement is promising for mussel biomonitoring studies as it provides in-depth information on the organism's response to chemical stressors. For deployment at sites of interest mussels are often caged to enable ease of collection at appropriate time points. Caged mussels can be transplanted from control sites to polluted sites, providing a controlled response for acute exposures. The same population can be sampled multiple times throughout the study period, controlling for reproductive status and age of the mussels. In a previous study, we observed a metabolic difference between *in situ* vs caged mussels at a single location. Here, we systematically evaluate the effect of caging on the mussel metabolome to determine if this handling technique might confound metabolomic comparative studies. *In situ* dreissenid mussels at two different sites (LMMB-04 and LMMB-05) in the Milwaukee Harbor, USA were placed in cages in June 2018. Four weeks later (July 2018), both caged and *in situ* mussels were collected from the two sites. Mussel metabolomes were examined using NMR spectroscopy with both sites showing subtle metabolic effects of caging. However, when examining both the sampling site and the handling technique, the sampling site accounted for the distinction in the data set. The metabolic effects caused by caging mussels were less influential than the environment of the site, thus verifying that the method of caging can be utilized on mussels for biomonitoring metabolomics in areas of interest.

Funding Sources (if applicable):

Discovery of indole-tropane hybrid alkaloids from *Datura stramonium*.

Maris A. Cinelli[1,3], Josh Grabar[2], Hannah M. Parks[1,2], Cornelius S. Barry[2], and A. Daniel Jones[1]

[1]Department of Biochemistry and Molecular Biology, Michigan State University, USA
[2]Department of Horticulture, Michigan State University, USA
[3]Current address: Department of Chemistry, Northern Michigan University, USA
macinell@nmu.edu

Plants in the genus *Datura* (*Solanaceae*) produce a wide array of tropane alkaloids, including the well-known medicinal compounds hyoscyamine (atropine), and scopolamine. Because of the presence of these alkaloids, *Datura* species have been used medicinally for centuries, and finding new alkaloids in this genus could have implications in drug, pesticide, or novel gene discovery. During untargeted LC-ESIMS-based metabolite profiling of *Datura stramonium* (a widespread American *Datura* species also known as Jimsonweed), we discovered a unique class of tropane alkaloids with odd nominal masses for MH^+, suggesting (along with their MS/MS spectral features) the presence of an extra nitrogen in the acyl portion. The MS/MS spectra across this class of alkaloids are consistent with tropane esters of indole-3-lactic acid. Although numerous compounds containing indole-like features were detected, the two most abundant of these alkaloids, which we named *indolelactoyltropine* (*m/z* 329) and *O-acetyl-indolelactoyltropine (m/z* 371) were isolated from *D. stramonium* root extract by semi-preparative HPLC (using biphenyl-phase and C_{18} columns). Structural analysis by NMR confirmed the hybrid tropine/indole-3-lactate core. Acyltropane alkaloids containing nitrogen in their ester substituents are extremely uncommon in the *Solanaceae*, *Erythroxylaceae*, and *Convolvulaceae* families, and this is the first report of *any* tropane alkaloid containing an indole. Because of the presence of both the tropane and indole functionalities, the *indolelactoyltropine* alkaloids may have interesting psychoactive activity, a hypothesis which merits future study.

Funding sources (If applicable):

NSF IOS-1546617

Mapping the cellular physiology of glyphosate resistance in Palmer amaranth using global metabolomic approaches

Pawanjit Kaur Sandhu[1], Elizabeth Leonard[1], Vijay Nandula[2], Nishanth Tharayil[1]

[1]Department of Plant and Environmental Sciences, Clemson University, USA
[2]National Institute of Food and Agriculture, United States Department of Agriculture, USA
psandhu@clemson.edu

Abstract

Competition from weeds costs 33 billion US$ in crop production annually. Palmer amaranth is a troublesome weed in row crop production systems of southeastern US, causing yield losses of 50-90%. Palmer amaranth dominates agricultural systems primarily due to its ability to escape glyphosate, the most widely used herbicide globally. The herbicidal effect of glyphosate is due to inhibition of the 5-Enolpyruvylshikimate-3-phosphate synthase (EPSPS) enzyme that prevents the biosynthesis of aromatic amino acids (AAA). Even though the major mechanism of glyphosate resistance in Palmer amaranth is EPSPS gene amplification, there is significant uncertainty in the cellular-level toxicity induced by glyphosate and potential evasive mechanisms prevalent in resistant populations (biotypes) of Palmer amaranth. Across five populations of Palmer amaranth that are resistant (GR) and susceptible (GS) to glyphosate, we employed untargeted metabolomics approaches to elucidate the: i) commonality in native cellular metabolism in the absence of glyphosate, and ii) differential glyphosate-induced stress responses.

We captured the primary and secondary metabolite profile of the control and glyphosate-treated plants forty-four hours after glyphosate application using GC-MS and LC-MS/MS approaches. Glyphosate induced metabolic perturbations in both the GR- and GS-biotypes. The primary metabolism was more significantly affected in GS-biotypes, while GR-biotypes displayed a more significant increase in the abundance of secondary metabolites (phenylpropanoids and terpenoids). Even in the absence of glyphosate, the metabolite pool of GR-biotypes was different from GS-biotypes. The GR-biotypes had an innately higher abundance of non-aromatic amino acids, sugars, and organic acids (primary metabolites) while GS-biotypes showed a higher abundance of hydroxycinnamic acid amides (secondary metabolites). Despite the increased abundance of EPSPS enzyme, the abundance of downstream metabolites – AAA and phenylpropanoids – was not higher in GR-biotypes in the control treatment. Overall, GR-biotypes exhibited a natively higher primary metabolism which was less affected by glyphosate than GS-biotypes. The secondary metabolism, although natively higher in GS-biotypes, was more upregulated in GR-biotypes.

Funding Sources (if applicable):

Utilizing phytometabolome to visualize the parasitic and mutualistic phenotypes of arbuscular mycorrhizal fungi

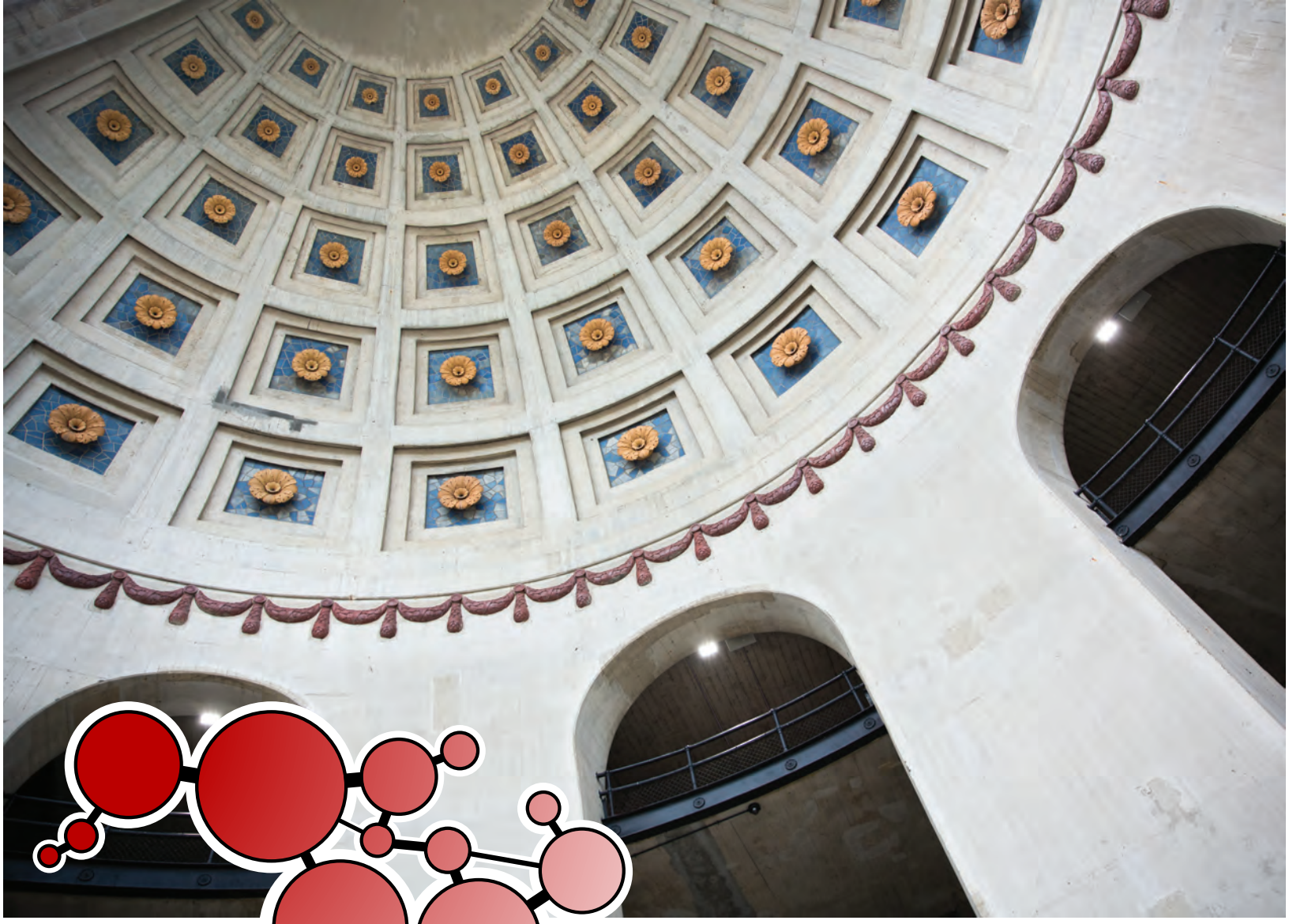
Sukhmanpreet Kaur[1], Vidya Suseela[1]

[1]Department of Plant and Environmental Sciences, Clemson University, Clemson, South Carolina, USA
vsuseel@clemson.edu

Abstract

The association of plant roots with arbuscular mycorrhizal fungi (AMF) is the most ancient and widespread symbiosis in terrestrial ecosystems helping plants to obtain their phosphorus requirements in return for plant photosynthates. Plant-AMF symbiosis exhibits both mutualistic or parasitic phenotypes resulting in increased growth benefits or plant growth depression, respectively. However, we lack an understanding of the cellular-level mechanisms that underlie these phenotypes. Understanding the dynamics of the phytometabolome under a mutualistic or parasitic AMF phenotype can provide a better molecular insight into the differential outcome of the plant-AMF symbiosis. Utilizing GC-MS and LC-MS/MS (Orbitrap Mass spectrometer), we obtained the phytometabolome of two sorghum genotypes supplied with sparingly soluble iron phosphate and subjected to AMF treatments such as *Rhizophagus irregularis*, *Gigaspora gigantea*, a mixture of both AMF species and a non-AMF control. *Rhizophagus irregularis* exhibited a mutualistic phenotype with increased plant biomass and phosphorus uptake. However, G. gigantea exhibited a parasitic phenotype that led to plant growth depression. In the MIX treatment, the mutualistic symbiosis prevailed as both sorghum genotypes potentially rewarded *R. irregularis* thus outcompeting *G. gigantea*. The primary and secondary plant metabolome mirrored the symbiotic phenotype of the AMF. The symbiosis with *R. irregularis* that exhibited a mutualistic phenotype led to facilitatory metabolic responses including the upregulation of organic acids necessary for phosphorus uptake and secondary metabolites including several flavonoids critical for a functional symbiosis. However, the symbiosis with *G. gigantea* resulted in inhibitory plant metabolic responses including the higher abundance of amino acids and an oxime with antifungal properties, indicating that the plant perceived *G. gigantea* as a fungal invader. These findings suggest that the differential outcome of plant-AMF symbiosis could be regulated by or reflected in changes in the plant phytometabolome that arises from the interaction of the plant species with the specific AMF species.

Funding Sources (if applicable):



MANA 2021

Virtual Conference | Oct 18 - 21

Wednesday, October 20th

Oral Session: Biomedical 2

Chemical cartography-based metabolomics to guide rational drug development

Ekram Hossain[1,2], Danya A. Dean[1,2], Chaoyi Wu[1,2], Sharon Lostracco-Johnson[3], Diane Thomas[3], Shelley S. Kane[1,2], Adwaita R. Parab[2,4], Karina Flores[2,5], Mitchelle Katemauswa[1,2], Camil Gosmanov[1,2], Danyang Li[6], Laura-Isobel McCall[1,2,4]

[1]Department of Chemistry and Biochemistry, University of Oklahoma, USA
[2]Laboratories of Molecular Anthropology and Microbiome Research, University of Oklahoma, USA
[3]Skaggs School of Pharmacy and Pharmaceutical Sciences, University of California San Diego, USA
[4]Department of Microbiology and Plant Biology, University of Oklahoma, USA
[5]Department of Biology, University of Oklahoma, USA
[6]College of Chemistry, Beijing Normal University, CN
lmccall@ou.edu

Abstract

The world is currently facing an antimicrobial crisis on all fronts, from newly-emerged diseases such as COVID-19 for which limited treatments are available, to the development of antimicrobial resistance leading to the loss of efficacy of existing treatment regimens. Most anti-infectious drug development has focused on developing agents that kill the pathogen, although these may be particularly prone to the emergence of drug resistance. However, disease symptoms actually emerge from the conjunction of pathogen-derived effects and host mechanisms associated with pathogen clearance. Approaches to mitigate pathogen-induced damage (pro-tolerance mechanisms) can prevent severe symptoms or even mortality, even in the face of high pathogen load. Pro-tolerance mechanisms have however been under-studied. Spatial metabolomic approaches ("chemical cartography") present unique opportunities to identify metabolic pathways associated with localized pathogen colonization and tissue damage, to guide the development of pro-tolerance metabolic modulators. As a proof-of-concept, we applied this approach to infection with *Trypanosoma cruzi* parasites, causative agents of Chagas disease. Current antiparasitic agents that kill *T. cruzi* are unable to improve severe disease symptoms, indicating a need for a pro-tolerance approach in this disease context. Chemical cartography of *T. cruzi* infection revealed localized metabolic perturbation at sites that match with Chagas disease symptoms. Acylcarnitine metabolism was locally perturbed at tissue sites of Chagas disease across *T. cruzi* strains and infection timepoints. Based on these findings, we developed a carnitine-based treatment regimen for Chagas disease. This treatment abrogated acute-stage disease-associated mortality, via a mechanism that involved restoration of cardiovascular metabolism, leading to improved cardiac functions. Overall, these results demonstrate a new paradigm using metabolomics to guide the development of novel treatment approaches for Chagas disease, with broad applicability.

Funding Sources (if applicable):

Start-up funds from the University of Oklahoma and NIH R21AI148886.

Metabolomics integration with gene expression profiling elucidates IL4I1 as modulator of ibrutinib resistance in ABC-diffuse large B cell lymphoma

Choueiry, Fouad[1*], Singh, Satishkumar [2,4*], Sircar, Anuvrat [2,4], Laliotis, Georgios [3,4], Sun, Xiaowei[1], Chavdoula, Evangelia [3,4], Zhang, Shiqi[1], Helmig-Mason, JoBeth[2], Hart, Amber[2], Narendranath Epperla [2,4], Tsichlis, Philip [3,4], Baiocchi, Robert[2,4], Alinari, Lapo[2,4], Zhu, Jiangjiang[1,4], and Sehgal, Lalit[2,4]

[1] Department of Human Sciences, The Ohio State University, USA
[2] Division of Hematology, Department of Internal Medicine, The Ohio State University, USA
[3] The Ohio State University, Department of Cancer Biology and Genetics, USA
[4] James Comprehensive Cancer Center, The Ohio State University, USA
Choueiry.2@osu.edu

Abstract

Introduction: Diffuse large B-cell lymphoma (DLBCL) is the most prevalent form of non-Hodgkin lymphoma. They rely on Bruton's tyrosine kinase (BTK) mediated B-cell receptor signaling for survival and disease progression. However, non-Hodgkin lymphoma is often resistant to BTK inhibitors or soon acquires resistance after drug exposure. Upon reaching drug tolerance, cells displayed faster proliferation rates suggesting increased metabolic activity. Here, we explored the development of this resistant phenotype via multi-omics analysis encompassing high resolution mass spectrometry and DNA microarray to expose deregulated metabolic pathways driving drug resistance.

METHODS: DLBCL cell lines HBL-1 and TMD8 were cultured in perpetual presence of ibrutinib to induce drug tolerance. Total RNA was isolated from these cells for microarray analyses while polar metabolites were extracted for untargeted metabolomics profiling. Data were integrated to uncover metabolic pathways altered at both the metabolic and transcriptional levels. mRNA expression of gene targets was validated using rt-PCR. BTK mutants of the cells were generated to observe a reversal of metabolic profile.

RESULTS: Multi-omics integration revealed alanine, aspartate and glutamate as well as cysteine and methionine pathways to be deregulated at both the metabolic and transcriptional level in the drug resistant cells. Integration of the genes and metabolites pertaining to those pathways revealed an L-amino acid oxidase at the crosstalk of both metabolic pathways. Data uncovered a switch towards oxidative phosphorylation from a glycolytic state in these cells. Deregulated genes were verified via rt-PCR to confirm expression. Wild-type BTK expression restored IL4I1 in resistant tumors.

CONCLUSIONS: Significantly deregulated pathways from both datasets of drug-resistant cell lines were integrated. We found IL4I1 to be at the junction of both pathways. Aberrant expression of IL4I1 is accompanied with a shift towards oxidative phosphorylation in these cancers, justifying the observed TCA anaplerosis and providing a mechanism of increased metabolic activity for survival.

Funding Sources (if applicable):

Research reported in this publication was supported by the National Institute of General Medical Sciences of the National Institutes of Health under Award Number R35GM133510 awarded to JZ and the Ohio State university startup funding to LS. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

Ultrahigh resolution imaging mass spectrometry reveals lipidome alterations in early-stage ovarian cancer

Xin Ma[1], Sylvia R. Yun[2], Andro Botros[2], Olga Kim[2], Eunyoung Park[2], Jaeyeon Kim[2], Facundo M. Fernández[1]

[1]Department of Chemistry and Biochemistry, Georgia Institute of Technology, USA
[2]Indiana University School of Medicine, USA
xin.ma@chemistry.gatech.edu

Abstract

Ovarian cancer is one of the deadliest cancers among women. Diagnosis of the cancer at an early stage remains challenging because of the poor understanding of disease pathogenesis and limited usefulness of the available diagnostic tools. In this work, we applied ultrahigh resolution FTICR MALDI mass spectrometry imaging to study the spatial distribution of lipids directly on reproductive system tissue sections of a triple-mutant (TKO) mouse model of the disease. Several differential fatty acids and lipids were detected and selected to build PCA models of cancerous vs. healthy fallopian tubes. Results were compared with longitudinal LC-MS serum lipidomic experiments performed on such animals to understand the origin of lipidome alterations that could be useful as biomarker panels.

MALDI images were collected at a spatial resolution of 50 µm to balance image fidelity and sample throughput. A tissue section that showed an advanced tumor in one side of the fallopian tube connecting with a healthy ovary/fallopian tube was studied. For each replicate experiment, approximately 150 different fatty acids and lipids were annotated with a false discovery rate < 10% using METASPACE and a mass error tolerance of 1 ppm. Among all annotated ions, 25 of them were selected based on their spatial distributions and LC-MS serum studies for discriminating between tumor and healthy tissue regions. Among the ions of interest, several of them, including arachidonic acid and PE(O-18:0/20:4), were found to be more abundant in the high-grade serous carcinoma region, whereas lipids such as Cer(34:1) and SM(d33:1) were accumulated in the heterogeneous necrotic region of the tumor. Sub-areas of the tumor will be characterized by H&E staining to better understand tumor structure. The 25-ion PCA models were successful in differentiating tumor region.

This is the first ultrahigh resolution MS imaging study of reproductive tissues from an animal model of ovarian cancer.

Funding Sources (if applicable):

We acknowledge support by NIH 1U2CES030167-01, the CMaT NSF Research Center (EEC-1648035), 1R01CA218664-01, R01NS101909-01, the NIH MoTrPAC Consortium (1U24DK112341), and NSF MRI CHE-1726528.

Metabo-endotypes, multi-omic endotypes and precision medicine: an example from asthma

Rachel S. Kelly[1], Kevin M. Mendez[1], Mengna Huang[1], Brian D Hobbs [1], Clary B. Clish[2], Robert Gerszten[3], Michael H. Cho [1], Craig E. Wheelock[4], Michael J. McGeachie[1], Anshul Tiwari[1], Su H. Chu[1], Juan C. Celedón[5], Scott T. Weiss[1], Jessica Lasky-Su [1]

[1]Channing Division of Network Medicine, Brigham and Women's Hospital and Harvard Medical School, USA
[2]The Broad Institute of MIT and Harvard, USA
[3]Department of Medicine, Beth Israel Deaconess Medical Center, USA
[4]Division of Physiological Chemistry, Department of Medical Biochemistry and Biophysics, Karolinska Institute, SE
[5]Division of Pediatric Pulmonary Medicine, UPMC Children's Hospital of Pittsburgh, University of Pittsburgh, USA

Abstract

Asthma is a heterogenous condition that remains poorly understood. Classification of asthma has historically relied largely on symptoms and clinical manifestation. However, the outward presentation of a disease is not necessarily representative of its underlying mechanisms. Therefore, subgrouping individuals based on phenotype can lead to misclassification and suboptimal therapeutic management in certain groups. Instead, it is likely to be more informative to look upstream on the central biological dogma; to classify individuals by the functional or pathobiological mechanisms of their disease and tailor therapies appropriately.

Metabolomics provides a particularly compelling approach to the derivation of such disease endotypes (ie. subtypes defined by mechanism). We have previously demonstrated that we can derive and validate metabolomic driven endotypes, or "metabo-endotypes", of asthma that differ in clinically relevant asthma phenotypes. However, disease dysregulation often occurs at multiple levels and therefore the most accurate endotypes may be defined by incorporating multiple levels of omic data.

In this study we integrated untargeted plasma metabolomic data with serum microRNA counts in 1151 children with asthma using Similarity Network Fusion to derive four multi-omic endotypes of asthma. Comparison of the multi-omic versus metabo-endotypes determined that individuals tended to cluster very similarly when based on their integrated metabolomic-microRNA profile and when based on their metabolomic profile. We again observed a significant difference in asthma phenotypes across the multi-omic endotypes including lung function (FEV_1/FVC ratio, p-ANOVA=5.02×10^{-6}). Interrogation of the key drivers of the multi-omic endotypes demonstrated that the metabolome has a bigger influence on the formation of the endotypes than the microRNAome. However, the key metabolite and microRNA drivers provided complementary information to support the hypothesis that dysregulated pulmonary surfactant homeostasis explains the clinical differences observed between the endotypes.

These findings suggest that multi-omic endotypes can both help improve disease classification whilst informing on underlying biology.

Funding Sources (if applicable):

K01HL146980 (*NHLBI*); Trans-Omics in Precision Medicine (TOPMed) program (*NHLBI*)

Untargeted metabolomics of the maternal immune activation brain model pathogenicity

Boryana, Petrova[1,2], Jin Cui[2] , Andrew Culhane[1] , Tiara Lacey[1] , Maria Lehtinen,[1,2] , Naama Kanarek[1,2,3]

[1] Boston Children's Hospital, USA
[2]Harvard Medical School, USA
[3]Broad Institute of Harvard and Massachusetts Institute of Technology, USA
Boryana.Petrova@childrens.harvard.edu

Abstract

Maternal immune activation (MIA) by viral, bacterial, or parasitic infection, can impact the fetus, resulting in adverse central nervous system (CNS) phenotypes and neurological disorders. A MIA model in pregnant mice displays measurable neurochemical and anatomic changes in the brain that correspond to those found in pregnant women and enables mechanistic studies that can illuminate the MIA-induced pathology in humans. We recently discovered that the embryonic choroid plexus (ChP) provides a conduit for peripheral inflammation to enter the developing brain as reflected by an abnormal CSF cytokine signature. We hypothesize that there are additional changes in CSF composition following inflammation besides cytokines, and that the CSF metabolome is a key factor in inflammation-induced neuropathology. We propose to test whether MIA-induced metabolic changes in the developing CSF play a role in the ensuing neuropathology and by that provide better mechanistic understanding of inflammation-induced neuropathology in the developing brain.

Our study aims to characterize changes in mouse embryonic CSF upon maternal immune activation using the quantitative power of LC-MS. We employed untargeted metabolomics in two ways – first to build an in-depth characterization of the embryonic CSF and second, to profile MIA-induced metabolic changes. Our untargeted metabolomics informed a follow-up targeted metabolomics analysis revealing a significant elevation of the related metabolites kynurenine and kynurenic acid. The kynurenine pathway is associated with several neuropathologies, and its elevation following inflammation is in accordance with previous findings in adult CSF. Currently, we interrogate the functional role of kynurenine pathway in MIA and association with embryonic developmental impairment. Our in-depth characterization of the embryonic CSF will serve the broader community and reveal causative abnormalities in CSF composition, provide biomarkers of MIA-induced brain damage, and inform future therapies.

Funding Sources (if applicable):

Boston Children's Hospital Investigator Award

Metabolomic profiling in plasma distinguish metabolic alterations across pregnancy in women with gestational diabetes: A case-control time-course analysis

Hannah Heath[1], Rodrigo Rosario[1], Lauren E. McMichael[1], Catherine Johnson[1], Rob Fanter[2,3], Noemi Alarcon[4,5], Adilene Quintana-Diaz[5,6], Kari Pilolla[1,5], Andrew Schaffner[5,7], Elissa Jelalian[6], Alex Brito[8,9], Rena R. Wing[6] , Suzanne Phelan S[4,5], Michael R. La Frano[1,3,5#]

[1]Department of Food Science and Nutrition, California Polytechnic State University, USA
[2]College of Agriculture, Food and Environmental Sciences, California Polytechnic State University, USA
[3]Cal Poly Metabolomics Service Center, California Polytechnic State University, USA
[4]Department of Kinesiology and Public Health, California Polytechnic State University, USA
[5]Center for Health Research, California Polytechnic State University, USA
[6]Brown University, USA
[7]Department of Statistics, California Polytechnic State University, USA
[8]Laboratory of Pharmacokinetics and Metabolomic Analysis. Institute of Translational Medicine and Biotechnology. I.M. Sechenov First State Moscow Medical University, RU
[9]World-Class Research Center "Digital Biodesign and Personalized Healthcare", I.M. Sechenov First Moscow State Medical University, RU
haheath@calpoly.edu

Abstract

Introduction: Gestational Diabetes Mellitus (GDM) results in complications affecting both mother and child. The implementation of metabolomics to assess metabolite alterations across pregnancy provides an opportunity to better understand its etiology and pathophysiology.

Objective: Metabolomics analysis was conducted on first (10-16 weeks gestation) and third (28 -35 weeks) trimester samples to assess changes associated with GDM.

Methods: Forty pregnant women with overweight/obesity from a multi-site clinical trial of a lifestyle intervention were included. Participants who developed GDM (n=20; GDM group) were matched on age, body mass index, ethnicity, and treatment group with those who did not develop GDM (n=20; Non-GDM group). Plasma samples collected at first and third trimester were analyzed with UPLC-MS using primary metabolomics, aminomics, and lipidomics assays. Cardiovascular disease markers, dietary recalls, and physical activity metrics were also assessed.

Results:

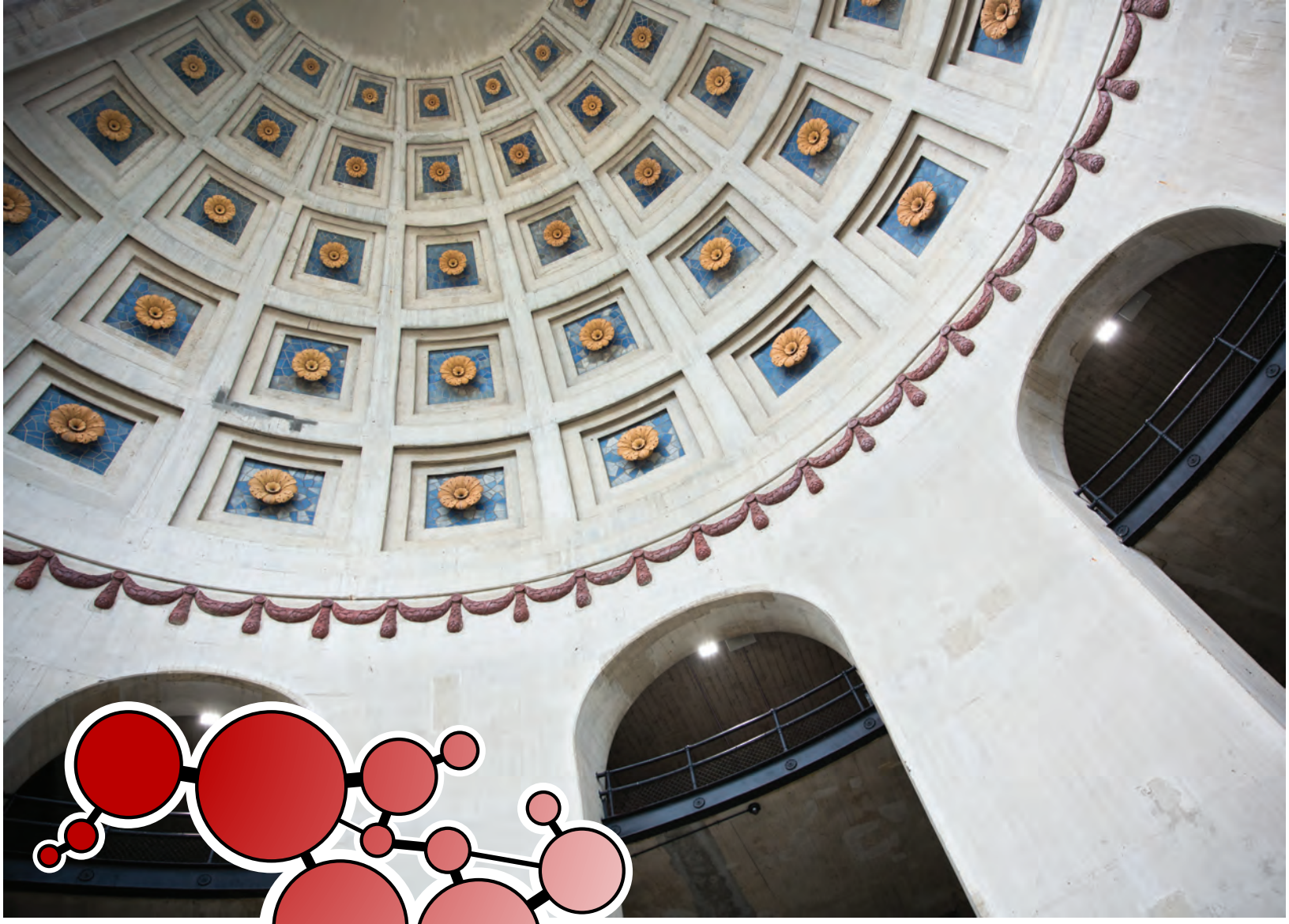
There was a significant group X time interaction in four medium-chain acylcarnitines, lauroyl-, octanoyl-, decanoyl-, and decenoyl-carnitine, as they decreased over the course of pregnancy in the GDM versus Non-GDM group ($p<0.05$). Inosine monophosphate and hypoxanthine ($p<0.04$) were elevated in GDM patients. In both groups over time, bile acids and sorbitol increased while short-, medium- and long-chain acylcarnitines, C24:0 sphingomyelin, and alpha-hydroxybutyrate decreased ($p<0.05$).

Conclusion:

The present study highlights the first and third trimester metabolic alterations that occur with the development of GDM, including metabolites involved in fatty acid oxidation and purine degradation. Although these findings provide some insight into the etiology and pathophysiology of GDM, they require validation in a more diverse, larger population.

Funding Sources:

NIH National Heart, Lung, and Blood Institute (NHLBI; HL114377), California State University Agricultural Research Institute, 2020 and 2021 Doris Howell Foundation – California State University Program for Education and Research in Biotechnology (CSUPERB) Research Scholar Awards, Harvey and Maud Sorensen Foundation Summer Undergraduate Research Program, and the Cal Poly College of Agriculture Food and Environmental Sciences (CAFES) Summer Undergraduate Research Program (SURP). AB was supported by the Ministry of Science and Higher Education of the Russian Federation within the framework of state support for the creation and development of World-Class Research Centers “Digital biodesign and personalized healthcare №075-15-2020-926”.



MANA 2021

Virtual Conference | Oct 18 - 21

Wednesday, October 20th

Oral Session: Metabolite ID

NP-MRD: the world's largest NMR database for natural products

Zinat Sayeeda[1,2], Zachary Bunduski [2], Manoj Rout[1], Brian Lee[1], Mark Brejanskii[1], AnChi Guo[1],Vasuk Gautam[1,] Harrison Peters[2], Raynoard Dizon[2] ,Amy Jystad[3], Jeffrey Van santen[4], Rajarshi Ghosh[5,6], Ryan S. Renslow[3], Roger Linington[4], Lloyd Sumner[5], John R. Cort[3], David S. Wishart[1,2]

[1]Department of Biological Sciences and
[2]Computing Sciences, Faculty of Science, University of Alberta, CA
[3] Biological Sciences Division, Pacific Northwest National Laboratory, Richland, USA
[4] Department of Chemistry, Simon Fraser University, CA
[5] Division of Biochemistry and [6]MU Metabolomics Center, University of Missouri, USA

Abstract

NP-MRD or the Natural Products Magnetic Resonance Database is a new, open-access resource developed in collaboration with NIH's National Center for Complementary and Integrative Health (NCCIH). It was officially launched in the second quarter of 2021. The NP-MRD is designed to contain all available NMR data on all known natural products, including 1D and 2D NMR spectra, chemical shift assignments and chemical structures. The NP-MRD is intended to assist with the identification, characterization and dereplication of natural products and to complement other natural product databases such the GNPS mass spectrometry database. The NP-MRD uses a very broad definition of natural products and welcomes the submission of any compounds produced or synthesized by animals, plants, marine organisms or microbes. This includes both primary and secondary metabolites. In this regard, the NP-MRD will soon house the largest collection of metabolite NMR spectra in the world and should be particularly useful to the metabolomics community. The NP-MRD has been designed to be a freely available cloud-based, FAIR electronic database. It accepts NMR data (spectra and assignments) and associated metadata from newly undertaken natural product studies. It also accepts, converts and stores all major NMR vendor formats and several common NMR data exchange formats from nearly all standard NMR experiments. Data deposition for NP-MRD has been designed to be fast and easy using the NPN-Dep system. Furthermore, all deposited structures and spectral files are run through a series of validation checks to inform submitters of any potential problems. A number of utilities are also available through NP-MRD to assist users with NMR spectral assignments and NMR-based structure elucidation. Currently NP-MRD has >10,000 structures with experimental NMR data and >200,000 structures with accurately predicted NMR data.

Funding Sources (if applicable):

The National Centre for Complementary and Integrative Health (NCCIH)
The Office of Dietary Supplements (ODS)
The National Institutes of Health (NIH)

Drift time shift modeling in IMS-MS/MS fragmentation matching

Jessica Bade[1], Sean Colby[2], Aivett Bilbao[3], Yehia M. Ibrahim[4], Richard D. Smith[5], Athena Schempoes[6], Kent Bloodsworth[7], Ryan Renslow[8], Thomas Metz[9]

[1, 2, 9] Earth and Biological Sciences, Pacific Northwest National Laboratory, USA,
[3-7, 9]Environmental and Molecular Sciences Laboratory, Pacific Northwest National Laboratory, USA,
jessica.bade@pnnl.gov

Abstract

In ion mobility spectrometry (IMS), the drift time (DT) is a measurement of the mobility of a molecule in the gas phase while collisions with a neutral buffer gas separates it from other analytes. The DT reflects the ion's collision cross sections (CCS), a property that represents the three-dimensional structure of the corresponding ion. In drift tube-IMS, collision-induced dissociation can be activated by applying high collision energy (CE) voltages after the mobility separation. Low and High CEs can be continuously alternated to collect precursor and fragment ion spectra within a single run. Fragment ions are then assigned to their corresponding precursor based on their DT. A DT "shift" is a delay relative to the precursor ion that has been observed in fragments due to passing the collision cell. Currently available software assumes a windowed approach to assigning fragment ions within a tolerance of the precursor m/z drift time. Real fragments of the precursor can be found within this window, but it is unknown if this approach is exhaustive enough to effectively separate enough true positive assignments from false positive ones. An expanded version of this approach has been observed, which combines applying an artificial shift (e.g. 12 ms) to fragment DTs and searching within a second window tolerance. Although it could be more effective, it still does not address the issue that fragmentation shift phenomenon is suspected to be a function of the ratio between precursor m/z and fragment m/z, with even more diversity across voltages and modes. Here we explore and discuss the complex relationship between fragment and precursor ions in data sets comprised of known, low complexity (non-overlapping DT distributions) samples run on IMS-MS/MS, positive and negative modes, at 5 CE voltages with 3 replicates. We have modeled the relationship between precursor and fragment ions across modes, voltages, and m/z. The results show that a more robust approach needs to be applied to properly capture real fragments and simultaneously filter false fragments. A future implementation of this work is incorporation of a dynamic drift time shift prediction applied to real-time, high-throughput IMS-MS/MS data processing for higher confidence in fragment assignment with data independent acquisition experiments.

Funding Sources (if applicable):

Common Fund Metabolomics Program Phase II, PNACIC Grant: NIH CIDC U2CES030170

Building a fraction library for metabolomics

Goncalo J. Gouveia[1,5], Brianna M. Garcia[2,5], Amanda O. Shaver[3,5], Sicong Zhang[1,5], Yue Wu[4,5], Ricardo M. Borges[6], John Glushka[5], Franklin E. Leach 3rd [2,5], Arthur S. Edison[1,3,4,5]

[1] Department of Biochemistry and Molecular Biology, University of Georgia, USA
[2] Department of Chemistry, University of Georgia, USA
[3] Department of Genetics, University of Georgia, USA.
[4] Institute of Bioinformatics, University of Georgia, USA
[5] Complex Carbohydrate Research Center, University of Georgia, USA
[6] Walter Mors Institute of Research on Natural Products - Federal University of Rio de Janeiro, BR
goncalog@uga.edu

Abstract

Fractionation methods to purify and concentrate extracts containing a compound of interest have historically been used to aid the chemical structure elucidation process. However, applying this methodology to untargeted metabolomics is a considerable challenge due to the number of metabolites under investigation and limited sample availability. Biologically relevant reference materials can facilitate this process, as ample quantities of material can be fractionated, thus reducing spectral overlap and concentrating metabolites.

Here we present an approach to couple semi-preparative HPLC (high-performance liquid chromatography) fractionation and untargeted metabolomics to create a metabolomics fraction library. The elutants of semi-preparative reverse phase and HILIC (hydrophilic interaction chromatography) separations were collected at 60- and 30-second intervals respectively. The process was repeated for each chromatography and replicate fractions combined. A small sub-sample of each fraction was then analyzed by LC-MS/MS (liquid chromatography – mass spectrometry) and the majority used for NMR (nuclear magnetic resonance) spectroscopy.

We have applied this approach to a *Caenorhabditis elega*ns reference material and generated a library comprised of complementary data for structural elucidation of unknown metabolites. LC-MS/MS measurements provided data to obtain elemental formulas and fragmentation patterns of metabolites of interest, as well as spectral similarity networks from GNPS (global natural products social network).

NMR experiments (HSQC, TOCSY, DQF-COSY, HMBC, Jres and 1D ^{1}H) were acquired to generate data that can define the molecular components of a metabolite of interest and their structural arrangement. Furthermore, a statistical correlation network from deconvoluted 1D ^{1}H NMR spectra allowed to define clusters of features that represent single metabolites, aiding and offsetting some of 2D NMR limitations.

Future work could leverage other experimental measurements and molecular computations (QM, machine learning, *etc.*) to relate the experimental components of this platform for the structural elucidation of unknown metabolites and improve metabolite annotation.

Funding Sources (if applicable):

NIH Grant #: 5U2CES030167-02

Experimental approaches for confident annotation of ammonium adducts in LC-HRMS metabolomics data

Wenyun Lu[1], Lin Wang[1], Xi Xing[1], Li Chen[1], Joshua D. Rabinowitz[1]

[1]Lewis-Sigler Institute for Integrative Genomics and Department of Chemistry, Princeton University, USA
wlu@princeton.edu

Abstract

Adduct formation is a common phenomenon in LC-ESI-MS based metabolomics. Some adducts can be annotated based on Δm/z and RT match, such as sodium adduct with a unique Δm/z of 21.9820. Others are more complicated such as in the case of ammonium adducts where two peaks with a Δm/z of 17.0265 can result from adduct formation, in-source fragmentation, or actual distinct metabolites. We demonstrated that there are two ways to deal with this problem, using the powerful tool of stable isotope labeling. The first approach is suitable for microbial species where they can grow in unlabeled, ^{13}C-, ^{15}N-, and dual $^{13}C/^{15}N$-labeled media. Biological peaks can be differentiated from nonbiological peaks based on whether the peaks exhibit mass shifts between unlabeled and labeled samples. Moreover, the mass shifts can be used to derive metabolite C and N atom counts, and the carbon or nitrogen atoms coming from adducting remaining unlabeled. Metabolite and adduct peaks share the same C and N counts, even though their masses are different, facilitating annotation. The second approach is suitable for sample that cannot get labeled. In this case we can modify the LC buffer be replacing $^{14}NH_3$ by $^{15}NH_3$. The ammonium adduct now should show a mass shift of 0.997 from the unlabeled buffer, that corresponds to the mass difference between ^{14}N and ^{15}N. Applying these methods to yeast extract shows that two approaches give comparable result and allows for more confident annotation for ammonia adducts. These approaches can be extended to other adducts such as acetate and formate adducts, facilitating untargeted metabolomics data analysis.

Funding Sources (if applicable):

NIH R50CA211437

Evaluating retention index score assumptions refined existing metrics for GC-MS small molecule identification

David Degnan[1], Lisa Bramer[2], Javier Flores[1], Allison Thompson[3], Vanessa Paurus[3], Isaac Attah[3], Yuri Corilo[3], Chaevien Clendinen[3]

[1]Computational Analytics Division, Pacific Northwest National Laboratory, USA
[2]Biological Sciences Division, Pacific Northwest National Laboratory, USA
[3]Environmental Molecular Sciences Division, Pacific Northwest National Laboratory, USA
david.degnan@pnnl.gov

Abstract

In GC-MS small molecule untargeted metabolomics, spectral data (e.g., *m/*z, relative abundance, retention time) are matched to metabolite libraries and scored with similarity metrics. Since retention times are known to drift due to experimental parameters such as column temperature and age, retention indices (RIs) are used to normalize retention time to co-eluting standards. RIs are advantageous due to their independence from experimental parameters and are thus comparable across MS runs. However, existing RI scores may be unreliable due to uninvestigated assumptions, such as RI scores following a normal distribution. Here, we examine whether the assumptions of existing RI scoring methods are valid and propose ways to improve the scores by clustering the distributions of the difference between library and query RIs.

We compiled 492 datasets collected on the Agilent Single Quad GC of varying complexity and sample types (*e.g.,* amino acid mixtures, human urine, three fungal species). Our reference library contained 1,284 spectra. We divided our query library into test and validation datasets comprised of 72,899 and 22,322 spectra, respectively. CoreMS software was used to match query and library spectra with a generous RI window of 30 to avoid unintentional removal of valid compound identifications. All matches were hand-verified as true matches, and only metabolites with at least 30 verified identifications were included in downstream analysis. Our results show that the RI score assumptions of normality, consistent variance across metabolites, and a mean error centered at 0 are not valid. We demonstrate that these distributions can be clustered with the Wasserstein Distance and use this information to propose a refined RI score based on groupings of metabolites.

Funding Sources (If applicable):

PNNL Lab Directed Research & Development – m/q Initiative

Improved untargeted metabolomics compound identification and annotation by using longer gradients, increased sample loading, and iterative acquisition

Brady G. Anderson[1,2], Alexander Raskind[2-3], Hani Habra[3], Robert T. Kennedy[1,2,4], Charles R. Evans[2,3,5]

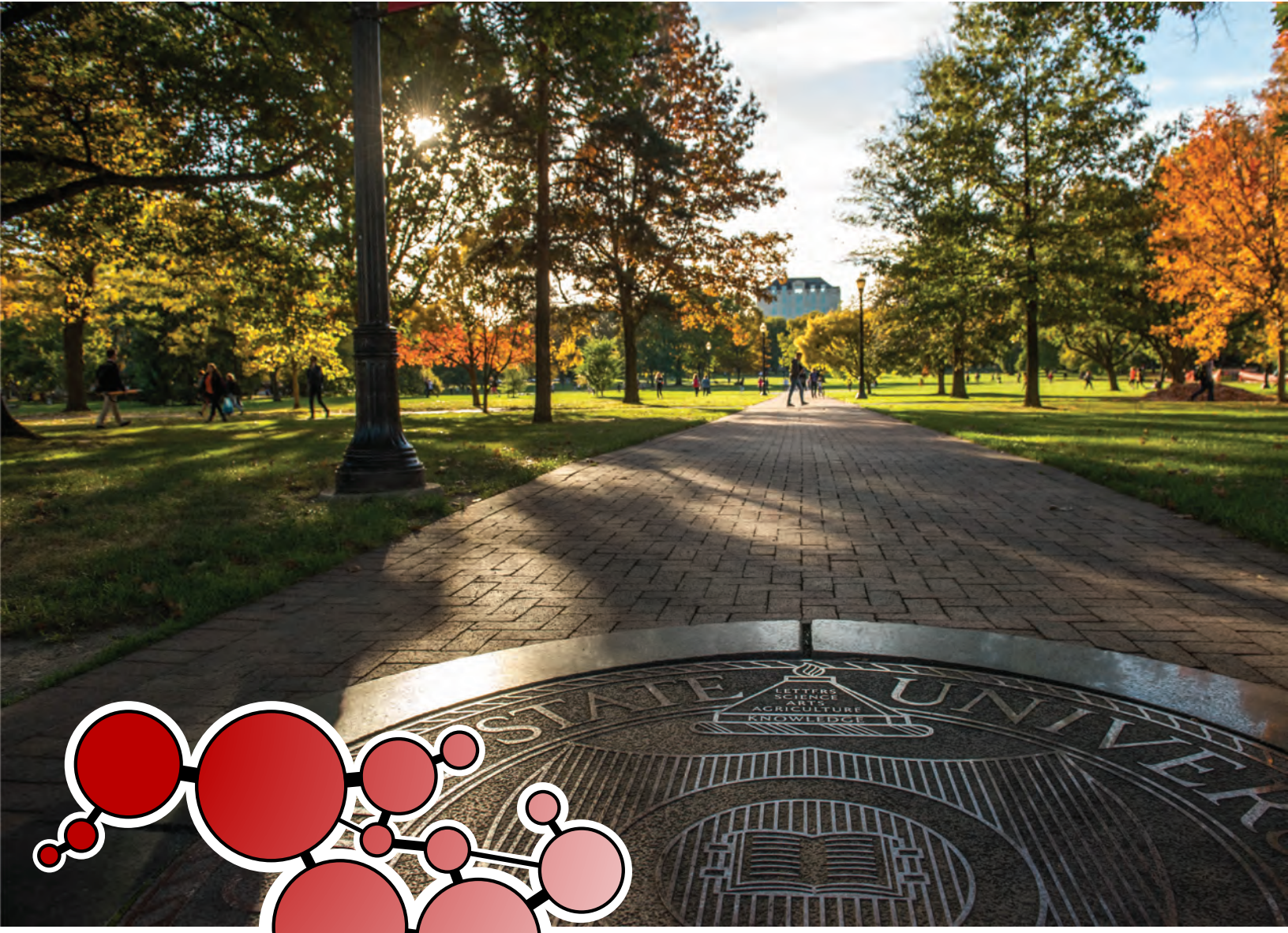
[1]Department of Chemistry, University of Michigan, USA
[2]Biomedical Research Core Facilities Metabolomics Core, University of Michigan, USA
[3]Department of Computational Medicine and Bioinformatics, University of Michigan, USA
[4]Department of Pharmacology, University of Michigan, USA
[5]Department of Internal Medicine, University of Michigan, USA
anderbra@umich.edu

Abstract

Detecting and identifying as many endogenous and exogenous metabolites as possible in untargeted metabolomics data is a crucial step toward enhancing our understanding of biological systems. Unfortunately, most detectable features in a typical dataset remain unidentified. Instrumentation including liquid chromatography-tandem mass spectrometry (LC-MS/MS) produce spectra that can be searched against databases to help identify or classify these unknowns, but many of these features are too low of abundance or generate too few fragment ions to be identified. We explored modifications of LC-MS/MS chromatography conditions which include extended gradient lengths, increased sample loading, and rolling precursor ion exclusion for reversed phase (RPLC) and hydrophilic interaction liquid chromatography (HILIC). These modifications substantially improved compound identification performance in human plasma samples. Spectral match score, spectral entropy, and total intensity thresholds were set at reasonable thresholds based on a manual review of spectral matches from the HILIC dataset. Compared to typical LC-MS/MS conditions, methods adapted for compound identification increased the total number of unique metabolites that could be confidently matched to a spectral database from 170 to 1,829. Following alignment using *metabCombiner, a* software tool for alignment of disparate LC-MS data, nearly 70% of newly identified features from the modified conditions could be confidently mapped to conventional LC-MS run conditions where more routine and quantitative profiling can be performed. Finally, remaining unidentified MS/MS spectra were subjected to a localized machine learning model to discern features that shared several spectral characteristics with successfully identified metabolites. These represent higher-priority targets for follow up analysis and are predicted to have a greater likelihood of being identified by orthogonal approaches or as databases improve. Overall, a strategy to annotate untargeted metabolomics data more deeply was achieved with a modest additional investment of time and sample.

Funding Sources (if applicable):

National Institutes of Health (NIH) Common Fund Metabolomics program, grant U2CeS030164 (C.R.E. and Alexey Nesvizhskii) and NIDDK grant R01dK101473-01A1 (R.T.K).



MANA 2021

Virtual Conference | Oct 18 - 21

Poster Abstracts

Session listing followed by abstracts in numerical order

Poster Number	Presenter	Title
Advancements in Software (Tuesday October 19th)		
25	Sean Colby	DEIMoS: an open-source tool for processing high-dimensional mass spectrometry data ñ updates and improvements
98	Jerry Chen	Trace Cloud: An Online Resource for Detecting Low-Intensity Signal Peaks in CE/LC/GC/-MS Data Using Neural Networks
103	Ciara Conway	Development of a Knowledgebase of Environmentally Relevant Compounds for Exposomics
106	Rasmus Erlemann	Application of the goodness-of-fit test to prioritizing unknown metabolites
153	Aleksandr Smirnov	ADAP-KDB Spectral Knowledgebase: an online resource for searching and prioritizing untargeted metabolomics data
Biomedical 1 (Tuesday October 19th)		
22	Su H Chu	A longitudinal investigation of the metabolomic architecture of ADHD: developmental metabolomics in utero through early childhood
64	Maribel Okiye	Game of Microbes: Natural Products as Weapons for Microbial Regulation in the Oral Cavity
82	Sarah Williams	Quantifying amine metabolites in single cells by integrating isobaric labeling, nanoPOTS sample preparation, and LC-MS
91	Joann Arce	Bacille Calmette-Guérin vaccine reprograms human neonatal lipid metabolism in vitro and in vivo
92	Djawed Bennouna	The Impact of Placebo or Multivitamin Supplementation on the Plasma Metabolome in ADHD Youth
95	Casey Chamberlain	A multi-modal metabolomics platform in zebrafish reveals organ-specific drug effects
Biomedical 2 (Tuesday October 19th)		
100	Alexandra Cheney	Fecal microbial transplantation as a potential therapeutic strategy to mitigate the debilitating effects of the neurodegenerative disease, Familial dysautonomia
104	Stephanann Costello	Investigating the gut microbiome-brain-metabolism axis in Familial dysautonomia; Implications of choline and taurine metabolism in disease phenotype and progression
107	Charles Evans	Inter-Laboratory Comparison of Known and Unknown Features in Untargeted Lipidomics Data
131	Rupasri Mandal	Targeted Metabolomics Identifies High Performing Diagnostic and Prognostic Biomarkers for COVID-19

Biomedical 3 (Tuesday October 19th)		
133	Susan McRitchie	How exposome research is informing precision medicine and precision nutrition
134	Kevin Milton Mendez	Metabolomics analysis of asthma phenotypes across multiple cohorts
164	Insha Zahoor	Profiling blood-based markers using combination of highly sensitive single molecule array technology (SIMOA) and untargeted metabolomics in multiple sclerosis
165	Lun Zhang	A comprehensive targeted metabolomic sssay for uremic toxin quantification
Cancer 1 (Tuesday October 19th)		
24	Raichel Cohen-Harazi	Hierarchical clustering of CRC metabolic profiling data reveals putative pathways involved in metastatic progression
87	Catherine Yu	An evaluation of the National Institutes of Health Grants Portfolio: identifying opportunities and challenges for multi-omics research that leverage metabolomics data
96	Ya-Chun Chan	Metabolomics on a Chip: Development of an Impedance-Based Metabolite Biosensor for Early Diagnosis of Colon Cancer
99	Ciao-Sin Chen	Metabolomics and nutritional biomarkers of motor chemotherapy-induced peripheral neuropathy
101	Leo Cheng	Screening Human Lung Cancer with Predictive Models of Serum Magnetic Resonance Spectroscopy Metabolomics
Cancer 2 (Tuesday October 19th)		
105	Ronald Eldridge	Hierarchical clustering analysis of plasma metabolomics identifies biologically distinct subtypes of head and neck cancer associated with overall survival
109	Brian David Fries	Lipidomics of generation 1 and 2 FASN inhibitors in p53 and KRAS mutant colorectal cancer tumor spheroids
152	Pratik Shriwas	A novel pan-class I Glucose transporter inhibitor targets metabolism of A549 cancer cells in vitro and A549 xenograft tumors in vivo
155	Harini Sridharan	Analyzing the Crabtree effect through metabolomics
158	Dorna Varshavi	Metabolic Reprogramming in Mutant KRAS Colorectal Cancer Cells
Computational Approaches 1 (Tuesday October 19th)		
2	Amity Andersen	Predicting ion fragmentation pathways for ìsoftî ionization mass spectrometry using hyperdynamics methods
10	Richa Batra	The metabolic landscape of brain alterations in Alzheimer's Disease
46	Samantha Johnson	Using Computation to Probe Microsolvated Phases during Soft Ionization Processes in Mass Spectrometry
56	Yuanyue Li	Spectral entropy outperforms MS/MS dot product similarity for small molecule compound identification
93	Christopher Brydges	Metabolomic Dysregulation in Myalgic Encephalomyelitis/Chronic Fatigue Syndrome: A Multi-study Bayesian Analysis
114	Yue Han	Towards inferring absolute concentrations from relative abundances in metabolomics data

Computational Approaches 2 (Tuesday October 19th)		
128	Yunfei Liao	A Prescreening Algorithm to Speed Up Spectra Search in the ADAP-KDB Spectral Knowledgebase
140	Andrew Christopher Patt	MetaboSPAN: an R package for network-based pathway analysis of metabolite data
160	Shunyang Wang	Predicting electron ionization mass spectra by excited state molecular dynamics
162	Janis Wigginton	A simple, iterative algorithm for RT alignment and integration of multi-batch untargeted LCMS data.
163	Rui Xu	Molecular network-oriented parameter optimizations enhanced the annotation of small molecules in metabolomics
Lipidomics 1 (Tuesday October 19th)		
13	Kamil Borkowski	Soluble epoxide hydrolase metabolites and ethanolamides associations with cognition an Alzheimerís phenotype.
15	Tomas Cajka	MetaboAtlas21: A Comprehensive Metabolome Atlas of Mouse Tissues and Biofluids
20	Li Chen	Lipidomics Analysis Elucidates Postprandial Metabolic Excursions Following Milk Protein Ingestion in Individuals with Prediabetes
41	Kelly Hines	Assessing membrane fluidity of antibiotic-resistant Staphylococcus aureus using an RPLC-IM-MS method for isomeric phospholipid separations
89	Xueyun Zheng	Lipid isomer separation and C=C double bond localization using ozone-induced dissociation and ion mobility spectrometry
Lipidomics 2 (Tuesday October 19th)		
97	Li Chen	Lipidomics Analysis Elucidates Postprandial Metabolic Excursions Following Milk Protein Ingestion in Individuals with Prediabetes
117	Giorgis Isaac	Application of hybrid surface technology for improving sensitivity and peak shape of phosphorylated and carboxylate lipids
126	Dmitry Leontyev	Ultrahigh Resolution Imaging Mass Spectrometry Unveils Lipidome Alterations in Mild Traumatic Brain Injury
130	.isaura Maldonado-Pereir	Effects of Food Processing on the Fatty Acids Composition of Ultra-Processed Foods
143	Lisa Reid	A complete solution for targeted lipid analysis using a smart compact LC-ToF
Lipidomics 3 (Tuesday October 19th)		
150	Tong Shen	Oxidized complex lipids in bio-specimen by LC-DDA-MS/MS and iterative MS/MS
151	Bindesh Shrestha	Enhanced Metabolite and Lipid Imaging by Desorption Electrospray Ionization (DESI) MS using Novel High-Performance Sprayer and Heated Transfer Line
156	Nathanial Chase Stevens	Untargeted identification and analysis of oxidized complex lipids in mouse organs
161	Jeffrey Waters	Altered omega fatty acid derived lipid mediators in relapsing-remitting multiple sclerosis patients
170	Emmanuelle Claude	Advantages of High Resolution Ion Mobility on the Cyclic IMS for DESI Mass Spectrometry Imaging

Microbial Metabolomics 1 (Tuesday October 19th)		
16	Kieran Tarazona Carrillo	Evaluating stool sample preparation for analysis of short-chain fatty acids by comprehensive two-dimensional gas chromatography ñ time-of-flight mass spectrometry
62	Seo Lin Nam	Evaluation of fresh, frozen, and lyophilized fecal samples by SPME and derivatization using GC◊GC-TOFMS
72	Sarangapani Sreelatha	Metabolomics unveils the complexity of lipid signatures in bacteria exposed to functional chitosan nanoparticles and its membrane active mechanism
108	Russell Fling	2,3,7,8-Tetrachlorodibenzo-p-dioxin Elicits Changes in Gut Microbiome Consistent with Progression of Non-alcoholic Fatty Liver Disease
113	Douglas Guzior	Analyzing the Diversity of in vitro Microbially Conjugated Bile Acid Products
Microbial Metabolomics 2 (Tuesday October 19th)		
135	Chathuri Mohottige	Solvent extraction method optimization for mycorrhizal fungal metabolome analysis
139	Wimal Pathmasiri	Assessment of metabolism and microbiome of infants at 6 weeks of age and the relationship to delivery mode and feeding type
145	Blake Rushing	Using UHPLC High Resolution Mass Spectrometry to Analyze Stool and Seminal Plasma
154	Kyle Spencer	The effects of a prebiotic-supplemented diet on the bacteriome and metabolome
166	Shiqi Zhang	Untargeted Metabolomics Sensitively Differentiates Gut Bacterial Species in Single Culture and Co-culture Systems
Ecology and Environment (Wednesday October 20th)		
85	Mengxue Xia	Global Effects of Arbuscular Mycorrhizal and Ectomycorrhizal Associations on Root Metabolomes Feature Alterations on Carbon Partitioning and Flavonoid Profiles
118	Trevor Johnson	Analysis of Geosmin Consumed by Bacterial Cultures by HSPME-GC-MS/MS
124	Jisun Lee	Exploring Dose-Response in Glyphosate-Resistant and -Susceptible Palmeri Amaranth by UHPLC-HRMS based Untargeted metabolomics
125	Elena Legrand	Metabolomics analysis of American lobster (Homarus americanus) larvae after exposure to conventional heavy crude oil
137	Galen O'Shea-Stone	NMR Based Metabolic Profiling Distinguishes the Differential Impact of Capture Techniques on Wild Bighorn Sheep
Food and Nutrition 1 (Wednesday October 20th)		
30	Ryan Dias	Aroma and Flavour Profiling of Blueberries using Comprehensive Two-Dimensional Gas Chromatography
45	Bhupinder Singh Jatana	Global Metabolomics reveal†Nitrogen Fertilization†as a Robust tool to Enhance Phytonutrient Content in Strawberry Fruits
53	Matthias Klein	Metabolite profiles distinguish common food microbes in a rapid NMR metabolomics experiment
75	Fenfen Tang	NMR-based metabolomics for olive oil cultivar classification: a comparison with standard targeted analysis of fatty acids and triglycerides.
79	Dajana Vuckovic	Effect of diet and probiotics on plasma oxylipin concentrations during the development of atherosclerosis in ApoE-knockout mice
102	Rachel Coble	Why Measuring Choline and Related Metabolites Matters in Nutrition Research

Food and Nutrition 2 (Wednesday October 20th)		
119	Hansani Karunarathne	Tocotrienols improve the metabolomic profile of maintenance hemodialysis (MHD) patients
120	Colin Kay	MetaboFood-KDB: A cloud knowledgebase for searching metabolomics data for nutritionally relevant compounds
127	Yuan Li	The Dietary Exposome Matters for Developing Nutritional Interventions
132	Shyamchand Mayengbam	Investigating the mechanistic roles of oligofructose-enriched inulin in reducing body fat of overweight and obese children
142	Mary Playdon	Blood metabolite profile and risk of estrogen receptor negative breast cancer: A meta-analysis
147	Isin T Sakallioglu	Glucuronidation of metabolites and triglyceride accumulation as specific biomarker for alcohol induced liver disease in rat hepatocytes
Metabolite ID 1 (Wednesday October 20th)		
5	Carter Asef	Annotation of Unknowns in Metabolomics: A Comprehensive Workflow Leveraging Machine Learning and In Silico Ion Mobility Collision Cross Sections Predictions
32	Susan Egbert	A New Perspective on Usnic Acid Biosynthesis in Cladonia rangiferina
42	Robert Hood	Characterizing the follicular fluid metabolome: quantifying the correlation across ovarian follicles and with the serum metabolome
69	Ayesha Seth	Development of signal amplification strategy for mass spectrometry using Gold nanoparticles (AuNPs) bioconjugates
70	Amanda Shaver	A meta-analysis model approach to identifying metabolites in untargeted metabolomics studies
Metabolite ID 2 (Wednesday October 20th)		
71	Zyad Shehadeh	MetaLINCSR: An intuitive R-Shiny application for metabolomics processing, biomarker discovery, and analysis
78	Arpana Vaniya	Extending the MassBank.us natural product reference mass spectral library with more than 4,400 new reference standards
94	Daniel Cardenas	Using KBase and PickAxe to build a metabolic model and predict novel metabolic pathways in Bacillus subtilis
111	Jasmine Gill	Digging deeper into the root metabolome of Sorghum accessions to evaluate the mechanisms of phosphorus acquisition
116	Jiri Hricko	Characterization of NIST Standard Reference Material 1950 — Metabolites in Frozen Human Plasma
Metabolite ID 3 (Wednesday October 20th)		
123	Sharie Kwok	Comparing MS-FINDER, NIST MS Interpreter and CFM-ID to evaluate substructure annotation capabilities
144	Nick Rigel	Elucidation of unknown metabolites using a new NMR/MS combinatorial approach
146	Madison Rzepka	Impact of Microbially Conjugated Bile Acids on the Cultured Murine Gut Microbiome
159	Dorsa Varshavi	Discovery of an anti-obesogenic agent from the microbiome of a genetically modified mouse
168	Lisa Zou	Lipid oxidation as critical contributor of the cholesterol dysregulation in and its Implications in Neurological Diseases

New/Experimental Methods and Technology (Wednesday October 20th)		
1	Enoch Amoah	Differentiation of Isomeric Monosaccharides through Plasma Reaction Studies by Ambient Mass Spectrometry.
48	Marissa Jones	Zooming into your favorite metabolic pathway: Combining broad and targeted metabolite profiles for deeper understanding of the metabolome
76	Michael James Taylor	Understanding the role of the substrate on laser desorption/ionization and primary ion beam analysis of lipids through molecular modeling
77	Jonathan Turner	Characterization of Zwitterionic HILIC Columns Based on Hybrid Particles
136	Samuel G Moore	Enzymatic synthesis assisted discovery of proline-rich macrocyclic peptides in marine sponges
Novel Mass Spectrometry Instrumentation and Approaches (Wednesday October 20th)		
14	Benjamin Burris	Orthogonal solid-phase extraction-contained-electrospray ionization mass spectrometry for complex lipid mixture analysis
18	Li Chen	Metabolite discovery through global annotation of untargeted metabolomics data
112	Raúl González-Domíguez	Comprehensive and quantitative metabolomics platform for characterizing the human exposome
121	Adam King	Combining a suite of rapid profiling LC-MS/MS methods and ion mobility workflows to investigate the metabolome of prostate cancer patients.
141	Xuejun Peng	Lipidomic Profiling of COVID-19 Patients' plasma by timsTOF Pro PASEF
Plants 1 (Wednesday October 20th)		
36	Ryland Giebelhaus	A Metabolomics Snapshot in Time: Metabolomic comparison of the living fossil Wollemia nobilis and Araucaria heterophylla
55	Xingxing Li	Switchgrass Metabolomics Reveals Striking Genotypic and Tissue/Developmental Differences in the Specialized Metabolites
73	Robert Stanley	Untargeted metabolomics and controlled progeny tests reveal that green ash (Fraxinus pennsylvanica) presents a multigenic metabolite-based resistance phenotype against the invasive emerald ash borer (Agrilus planipennis)
86	Honglian Ye	Response of metabolic and lipid synthesis gene expression changes in Camellia oleifera to mulched ecological mat under drought conditions
110	Xinyu Fu	Integrated metabolic flux and pool size analysis of plant central metabolism under varying photorespiratory conditions
115	Mickel Hiebert	The Cannabis Compound Database and Novel Cannabis Metabolite Assays
Plants 2 (Wednesday October 20th)		
122	Clayton Kranawetter	Metabolomics of Secreted Specialized Metabolites from Isolated Medicago truncatula Border Cells
129	Armando Alcazar Magana	Characterization of Withania somnifera extracts using LC-HRMS/MS in combination with GNPS networking analysis
138	Hannah Parks	Expanding the tropane alkaloid metabolic network: Metabolite discovery in Atropa belladonna
157	Alanna Sun	Evaluation of natural products in Mamaki Tea using untargeted metabolomics
167	Jiamin Zheng	A Comprehensive Targeted Metabolomics Assay for Crop Plant Sample Analysis

Standards and Data Quality (Wednesday October 20th)		
7	Jessica Bade	Understanding and predicting†drift time shifts of†fragment†ions†in ion mobility†spectrometry†measurements
28	Clay Davis	Evaluation of a NIST Human Prenatal Serum Suite as a Metabolomics Quality Control Material
80	Jacob Walsh	Synthesis of deoxynivalenol metabolites for use as analytical standards
148	Wasim Sandhu	Accurate Mass Spectral and Retention Index Library for Metabolomics Based on Quadrupole Time-of-flight Gas Chromatography/Mass Spectrometry
149	Tracey Schock	Qualitative characterization of NIST RM 8231- Frozen Human Plasma Suite for Metabolomics
169	Sandra Da Silva	NIST Interlaboratory study of human stool reference materials: towards metabolomics analysis harmonization

Differentiation of isomeric monosaccharides through plasma reaction studies by ambient mass spectrometry.

Enoch Amoah[1], Abraham Badu-Tawiah[2]

[1]Department of Chemistry and Biochemistry, The Ohio State University, USA
[2] Department of Chemistry and Biochemistry, The Ohio State University, USA
Amoah.23@osu.edu

Abstract

This study takes advantage of accelerated reaction rates, typical under solvent-limited environments (e.g., charged microdroplets and gas-phase ion reactions) to examine that reactivity of isomeric species of monosaccharides toward halide adduction and phenylboronic acids. Currently, the difficulties of monosaccharide analysis mainly lie in the fact that (i) monosaccharides have similar structures, and many of them are epimers of each other, differing from each other in the configuration of C-1 (for aldoses) or at C-2 (for ketoses) and (ii) monosaccharides are highly hydrophilic, lacking ionizable groups. These properties make saccharide analysis by mass spectrometry very challenging. This presentation will discuss a fundamentally new MS approach to saccharides analysis by combining ion generation and reaction into a single step. The selected reactions not only offer sensitive detection of monosaccharides, but they can distinguish isomers, especially when the reaction product is subjected to tandem MS via collision-induced dissociation (CID). For example, while Cl^- adducts of both reduction and non-reducing sugars can be sensitively detected in the negative-ion mode, their CID patterns are markedly different. Similarly, since phenylboronic acids prefer to react with *cis*-diol moieties, we expect reaction rates for *trans*-diol and 1,3-diol isomeric species to differ. Results will be presented that discuss the analysis of Fructose, Ribose, Mannose, Arabinose, Galactose, Xylose, and Rhamnose based on their reaction with halides and phenylboronic acids. We will also showcase the applicability of the method to analyze a variety of saccharides, including disaccharides (e.g., sucrose) and polysaccharides such as maltoheptaose (linear chain), and β-cyclodextrin (cyclic heptasaccharide).

Funding Sources (if applicable):

2

Predicting ion fragmentation pathways for "soft" ionization mass spectrometry using hyperdynamics methods

Amity Andersen[1], Edoardo Apra[1]

[1]Environmental Molecular Sciences Laboratory and Biological Sciences Division, Pacific Northwest National Laboratory, USA
amity.andersen@pnnl.gov

Abstract

Molecular dynamics techniques have been shown to be an invaluable tool in predicting fragmentation in electron impact ionization mass spectrometry. Conventional molecular dynamics, however, cannot predict mass spectrometry fragmentation patterns in "soft" ionization setups (e.g., electrospray ionization) where time to fragmentation can extend beyond picoseconds. For "soft" ionization, we propose applying recent collective variable-driven hyperdynamic (CVHD) techniques (Bal and Neyts 2015) to accelerate the time evolution of ionic species to possible fragmentation events. In this presentation, we will show our recent application of CVHD to amino acid ions in the gas phase.

References:

Bal, Kristof M., and Erik C. Neyts. "Merging metadynamics into hyperdynamics: accelerated molecular simulations reaching time scales from microseconds to seconds." *Journal of chemical theory and computation* 11.10 (2015): 4545-4554.

Funding Sources (if applicable):

5

Annotation of unknowns in metabolomics: a comprehensive workflow leveraging machine learning and in silico ion mobility collision cross sections predictions.

Carter K. Asef[1], Brianna M. Garcia[3,4], Goncalo J. Gouviea[2,3], Susanta Das[5], Kiyoto A. Tanemura[5], Markace Rainey[1], Amando O. Shaver[2,3], Franklin E. Leach III[4], Kenneth M. Merz Jr.[5], Alison M. Morse[6], Arthur S. Edison[2,3], Lauren M. McIntyre[6]

[1]School of Chemistry and Biochemistry, Georgia Institute of Technology, USA
[2]Departments of Genetics and Biochemistry and Molecular Biology, University of Georgia, USA
[3]Complex Carbohydrate Research Center, University of Georgia, USA
[4]Department of Chemistry, University of Georgia, USA
[5]Depeartment of Chemistry, Michigan State University, USA
[6]Department of Molecular Genetics and Microbiology, University of Florida, USA
Casef3@gatech.edu

Abstract

To relate MS detected ion features to biological processes, annotation of unknowns is necessary to assign structures. This is typically performed after gathering high resolution LC-MS and MS/MS data to yield elemental formulas and compare against fragmentation databases of known metabolites. This approach often falls short as databases are still incomplete or fragmentation is uninformative. Our study leverages the advantages brought by multiple MS platforms to provide robust metabolomics data supplemented by experimental and predicted ion mobility collision cross sections (CCS) for the purpose of filtering potential candidate structures of unknown features.

Six *Caenorhabditis elegans* tricarboxylic acid cycle mutant strains were grown in tandem with growth matched PD1074 control strains and extracted to generate both polar and nonpolar fractions. UPLC-MS data sets were collected using a Thermo ID-X Orbitrap mass spectrometer using both HILIC and RP chromatography. Ranked-ANOVA was performed to determine differentially expressed features which were used to create oPLS-DA models and ordered by their VIP scores with the top features being selected for identification efforts. Pooled samples were analyzed on a Waters Synapt G2-S using matched chromatography to obtain CCS values for features of interest. Machine learning and in silico approaches were employed to predict CCS values and filter structures of candidate compounds.

These selected features were searched against in-house and public MS2 databases using Thermo Compound Discoverer 3.1 yielding structural identification for only 3 out of 40 top compounds. For those compounds without robust MS2 annotations, elemental formulas were used to propose candidate structures. Candidate structures were then eliminated by comparing experimentally measured CCS values against in silico predicted values. If multiple candidate structures remained, trap CID mobility-resolved experiments were performed to measure CCS values of fragment ions which were again compared against predicted values of theoretical fragment structures.

Funding Sources (if applicable):

We acknowledge support by NIH 1U2CES030167-01

7

Understanding and predicting drift time shifts of fragment ions in ion mobility spectrometry measurements

Jessica Bade[1], Sean Colby[2], Aivett Bilbao[3], Yehia M. Ibrahim[4], Richard D. Smith[5], Athena Schempoes[6], Kent Bloodsworth[7], Ryan Renslow[8], Thomas Metz[9]

[1, 2, 9] Earth and Biological Sciences, Pacific Northwest National Laboratory, USA,
[3-7, 9]Environmental and Molecular Sciences Laboratory, Pacific Northwest National Laboratory, USA,
jessica.bade@pnnl.gov

Abstract

In ion mobility spectrometry (IMS), the drift time (DT) is a measurement of the mobility of a molecule in the gas phase while collisions with a neutral buffer gas separates it from other analytes. The DT reflects the ion's collision cross sections (CCS), a property that represents the three-dimensional structure of the corresponding ion. In drift tube-IMS, collision-induced dissociation can be activated by applying high collision energy (CE) voltages after the mobility separation. Low and High CEs can be continuously alternated to collect precursor and fragment ion spectra within a single run. Fragment ions are then assigned to their corresponding precursor based on their DT. A DT "shift" is a delay relative to the precursor ion that has been observed in fragments due to passing the collision cell. Currently available software assumes a windowed approach to assigning fragment ions within a tolerance of the precursor m/z drift time. Real fragments of the precursor can be found within this window, but it is unknown if this approach is exhaustive enough to effectively separate enough true positive assignments from false positive ones. An expanded version of this approach has been observed, which combines applying an artificial shift (e.g. 12 ms) to fragment DTs and searching within a second window tolerance. Although it could be more effective, it still does not address the issue that fragmentation shift phenomenon is suspected to be a function of the ratio between precursor m/z and fragment m/z, with even more diversity across voltages and modes. Here we explore and discuss the complex relationship between fragment and precursor ions in data sets comprised of known, low complexity (non-overlapping DT distributions) samples run on IMS-MS/MS, positive and negative modes, at 5 CE voltages with 3 replicates. We have modeled the relationship between precursor and fragment ions across modes, voltages, and m/z. The results show that a more robust approach needs to be applied to properly capture real fragments and simultaneously filter false fragments. A future implementation of this work is incorporation of a dynamic drift time shift prediction applied to real-time, high-throughput IMS-MS/MS data processing for higher confidence in fragment assignment with data independent acquisition experiments.

Funding Sources (if applicable):

Common Fund Metabolomics Program Phase II, PNACIC Grant: NIH CIDC U2CES030170

10

The metabolic landscape of brain alterations in Alzheimer's Disease

Richa Batra[1], Matthias Arnold[2,3], Gabi Kastenmüller[3], Mariet Allen[4], Xue Wang[4], David A. Bennett[5], Nilüfer Ertekin-Taner[4], Rima F. Kaddurah-Daouk[2] and Jan Krumsiek[1]

[1]Weill Cornell Medicine, USA
[2]Department of Psychiatry and Behavioral Sciences, Duke University, USA
[3]Helmholtz Center Munich, Neuherberg, DE
[4]Mayo Clinic, Jacksonville, Florida, USA
[5]Rush Alzheimer's Disease Center, Rush University, USA
rib4003@med.cornell.edu

Abstract

Impairment of brain glucose metabolism has been frequently described in Alzheimer's disease (AD). Moreover, the strongest predictor of the lifetime incidence of AD is the ε4 allele of *APOE,* a protein involved in lipid metabolism. These connections between AD and metabolism provide motivation to perform in-depth metabolic profiling of human brain tissue for different stages of AD pathophysiology. 514 Brain tissue samples were obtained from the Religious Orders Study and Memory and Aging Project (ROS/MAP) at the Rush Alzheimer's Disease Center. ROS/MAP collects extensive phenotyping of the participants' cognitive trajectories as well as postmortem pathology. Metabolic profiling was performed on Metabolon's untargeted platform, yielding 667 metabolites after preprocessing. Generalized linear models with appropriate linkage functions for 8 continuous or categorical AD-related phenotypes were used to discover the association of metabolic profiles with AD-related phenotypes, such as amount of amyloid and tangles in brain, global burden of pathology, NIA-Reagan score, diagnosis (derived from Braak and CERAD scores), clinical diagnosis at the time of death, global cognition assessed during the visit before death, estimated decline of global cognition over lifetime. The models included confounder correction for age, gender, body mass index, years of education, postmortem interval, number of APOEε4 alleles, and medications. We found 321 metabolites significantly associated (adjusted p-value <0.05) with one of the AD phenotypes. The associated metabolites are involved in various metabolic processes known to be involved in AD pathogenesis, such as bioenergetics, cholesterol metabolism, neuroinflammation. In addition, we showcased detailed biochemical alterations in a pathway cascade downstream of neurotransmitters glutamate and GABA, which includes urea cycle, polyamine metabolism, and methionine metabolism. We have generated a comprehensive landscape of AD-associated metabolites and associated processes. These will be instrumental to fill the gap in our understanding of the metabolic components of AD pathophysiology.

Funding Sources (if applicable):

U19AG063744, U01AG061359

12

Spatial SILAC, establishing selective pulse chase-SILAC labeling of three-dimensional multicellular spheroids

Nicole C. Beller[1], Jessica K. Lukowski[2], Amanda B. Hummon[1]

[1]Department of Chemistry and Biochemistry, The Ohio State University, USA
[2]Department of Chemistry and Biochemistry, University of Notre Dame, USA
beller.25@osu.edu

Abstract

Accurate model systems are crucial in cancer research for effectively testing potential chemotherapeutics. Three-dimensional multicellular spheroid model systems are quickly gaining popularity for their closer resemblance to the in vivo tumor. Spheroids exhibit pathophysiological and chemical gradients, forming three distinct cellular populations: a necrotic core, quiescent middle, and proliferating outer region of cells. These layers form during the spheroids' radially symmetric growth and can be discretely labeled using stable isotope labeling of amino acids in cell culture (SILAC). To establish the spatial SILAC platform, initial testing was performed to determine the labeling efficiencies and robustness of heavy labeling in each layer of the spheroids. Utilizing duplex SILAC labels, the heavy label was pulsed discretely into a single layer of the spheroids, with light labels pulsed into the remaining layers. These duplex spheroids, in addition to a fully heavy and light control, were serial trypsinized, lysed, digested, and analyzed by mass spectrometry. The resulting peptide identifications were used to determine the heavy labeling efficiency within each layer of the spheroids. Next, the proteins were analyzed to determine whether the labeling could be correlated to a specific cellular subpopulation. The results showed significant correlations confirming that duplex spatial SILAC can be used to trace proteomic changes to their origin in the spheroids. To further this platform, triplex labeling permutations were generated and tested in a similar manner. These very promising results suggest a future of combination pharmacodynamic and pharmacokinetic studies. Moving forward, as we begin testing chemotherapeutics, the spatial SILAC spheroid model will allow for more accurate proteome depictions with greater insight into spatial distributions, ultimately improving the accuracy of testing.

Funding Sources (if applicable):

Nicole Beller and Amanda Hummon are supported by R01GM110406 from the National Institutes of General Medical Sciences. Jessica Lukowski was supported by Career award 1351595 from the National Science Foundation.

13

Soluble epoxide hydrolase metabolites and ethanolamides associations with cognition an Alzheimer's phenotype.

Kamil Borkowski[1], Theresa L. Pedersen[2], Nicholas T. Seyfried[3], James J. Lah[4], Allan I. Levey[4], Chadwick M. Hales[4], Eric B. Dammer[3], Colette Blach[5], Gregory Louie[6] Rima Kaddurah-Daouk[7], John W. Newman[1,8,9] and Alzheimer's Disease Metabolomics Consortium

[1]West Coast Metabolomics Center, Genome Center, University of California - Davis, USA
[2]Dept Food Science and Technology, University of California - Davis, USA
[3]Department of Biochemistry, Emory University School of Medicine, USA
[4]Department of Neurology, Emory University, USA
[5]Duke Molecular Physiology Institute, Duke University, USA
[6]Department of Psychiatry and Behavioral Sciences, Duke University, USA
[7]Department of Psychiatry and Behavioral Sciences, Duke Institute for Brain Sciences and Department of Medicine, Duke University, USA
[8]Western Human Nutrition Research Center, United States Department of Agriculture - Agriculture Research Service, USA
[9]Department of Nutrition, University of California - Davis, USA

Alzheimer's disease and dementia together with cardiovascular disease and other cardiometabolic disorders may share inflammatory origins. Lipid mediators, including oxylipins, endocannabinoids, bile acids and steroids regulate inflammation, vascular system, energy metabolism and cell proliferation with well-established involvement in cardiometabolic diseases. However, their roles in Alzheimer's disease and cognition and their potential as biomarkers are poorly understood. Here we describe the analysis of two cohorts: A) cross-sectional analysis of serum from elderly subjects (n=210) with or without mild cognitive impairment (MCI) to probe associations of lipid mediators with cognition; B) case control comparison of plasma and matched cerebrospinal fluid from Alzheimer's disease (AD) patients (n=150) and healthy controls (n=135) for new AD lipid mediator biomarker discovery. **Methods**: Lipid mediators were measured using state of the art targeted quantitative mass spectrometry. Data were analyzed using analysis of covariates, adjusting for sex, age, and ethnicity. Additionally, we used machine learning as a variable reduction technique, followed by stepwise regression (linear for cognition measures and logistic for AD phenotype) to identify independent lipid mediator predictors of cognition and AD phenotype. **Results**: We found associations with both cognition and AD phenotype with members of two metabolic pathways: soluble epoxide hydrolase (sEH) being negatively associated with cognitive measurement of perceptual speed and higher in AD patients; fatty acids ethanolamides, a class of endocannabinoids, manifesting positive associations with perceptual speed and lower levels in AD patients. Both pathways regulate inflammation with sEH additionally regulating vascular tone, a potential implication for blood brain barrier. **Conclusions**: Current work shows association of cognition and AD phenotype with both central and peripheral markers of inflammation and vascular regulators. Previous studies report AD-associated sEH upregulation in the brain and that endocannabinoid metabolism provides an adaptive response to neuroinflammation. This study supports the involvement of P450-dependent and endocannabinoid metabolism in Alzheimer's disease, yielding potential biomarkers of the disorder. The results further suggest that combined pharmacological intervention targeting both metabolic pathways may have therapeutic benefits for Alzheimer's disease.

Funding Sources (if applicable):

Metabolomics data is provided by the Alzheimer's Disease Metabolomics Consortium (ADMC) and funded wholly or in part by the following National Institute on Aging grants and supplements, components of the Accelerating Medicines Partnership for AD (AMP-AD) and/or Molecular Mechanisms of the Vascular Etiology of AD (M2OVE-AD): NIA R01AG046171, RF1AG051550, RF1AG057452, R01AG059093, RF1AG058942, U01AG061359, U19AG063744 and FNIH: #DAOU16AMPA awarded to Dr. Kaddurah-Daouk at Duke University in partnership with a large number of academic institutions. As such, the investigators within the ADMC, not listed specifically in this publication's author's list, provided data but did not participate in analysis or writing of this manuscript. A complete listing of ADMC investigators can be found at: https://sites.duke.edu/adnimetab/team/. Metabolomics data and pre-processed data are accessible through the AMP-AD Knowledge Portal (https://ampadportal.org). The AMP-AD Knowledge Portal is the distribution site for data, analysis results, analytical methodology and research tools generated by the AMP-AD Target Discovery and Preclinical Validation Consortium and multiple Consortia and research programs supported by the National Institute on Aging. Additional support was provided by Emory ADRC P30 AG066511 awarded to Allan I. Levey, Emory EHBS R01 AG070937 awarded to James J. Lah and USDA Intramural Projects 2032-51530-022-00D and 2032-51530-025-00D awarded to John W. Newman. The USDA is an equal opportunity employer and provider

14

Orthogonal solid-phase extraction-contained-electrospray ionization mass spectrometry for complex lipid mixture analysis

Benjamin J. Burris[1], Nava Ramazanali[1], Abraham K. Badu-Tawiah[1]

[1]Department of Chemistry and Biochemistry, The Ohio State University, United States
Burris.186@osu.edu

Abstract

While it is known that reactions within microreactors, such as charged microdroplets, occur at accelerated rates, the application of such techniques in clinical settings have not manifested. We present a modification of contained-electrospray ionization (contained-ESI) mass spectrometry (MS) platform is proposed to perform rapid analysis of complex lipid solutions. Previously, contained-ESI platform has been shown to be capable of accelerating the rates of lipid hydrolysis by lipase by up to three orders of magnitude. Beyond this, solid-phase extraction (SPE) and contained-ESI MS could be coupled, to enable lipids analysis via on-line analysis. The contained-ESI platform allows for the introduction of two separate reagents (lipase and lipids) into a reaction cavity through separate deactivated fused silica capillaries whereupon these reagents mix as charged microdroplets and as discontinuous thin-films. Contained-ESI has other adjustable parameters that allow for modulating reaction conditions: 1) nebulizer gas helps to transport droplets to the mass spectrometer and creates turbulent mixing conditions within the cavity to induce reaction, and 2) a volatile reagent can introduce a headspace vapor which can change the reaction environment within cavity. After the spray of the coalesced lipid and lipase solutions, free fatty-acid products from the hydrolysis of lipids catalyzed by lipase are readily detected in negative-ion mode mass spectrometry. To enable online lipid mixture analysis, SPE cartridges fitted with different sorbents are used to fractionate the sample into individual lipid components. The lipid species are eluted and transferred to the contained-ESI platform where particular classes of lipids are analyzed at any given time. Both an on-line separation of dissimilar lipids and the subsequent hydrolysis of the separated lipids using the introduction of lipase allows for a complete characterization of the lipid profile from a complex mixture.

Funding Sources (if applicable):

National Science Foundation (NSF)

15

MetaboAtlas21: a comprehensive metabolome atlas of mouse tissues and biofluids

Tomas Cajka[1], Michaela Novakova[1], Lucie Rudl Kulhava[1], Jiri Hricko[1], Ondrej Kuda[1], Michaela Paucova[1]

[1]Institute of Physiology of the Czech Academy of Sciences, Czech Republic
tomas.cajka@fgu.cas.cz

Abstract

Genome, transcriptome, and proteome atlases that comprehensively characterize various tissues, biofluids, or cells have become available over the last decade. However, similar resources mapping metabolites are very rare and only a fraction of the metabolome is captured by analytical platforms used. This shows a lack of sufficient data on the metabolome characterizing different tissues and biofluids.
Here, we present a specific atlas of mouse metabolome and lipidome (MetaboAtlas21) in the context of systemic energy balance (chow diet) and under chronic nutrient stress (high-fat diet). Male mice were fed a control (chow) diet for 2 months or a high-fat diet for 2 months and 10 months. Urine, plasma, feces, and 18 different tissues were collected from each animal for metabolomics and lipidomics analysis. These matrices cover digestive, excretory, respiratory, reproductive, endocrine, muscular, cardiovascular, and nervous systems. Also, chow and high-fat diet feeds were analyzed along with quality control human plasma/serum materials (NIST SRM 1950 plasma, Merck S1-100ML serum, Sigma–Aldrich S7023 serum). In total, 408 samples were included in this study.
An 'all-in-one' extraction protocol LIMeX using methyl *tert*-butyl ether, methanol, and water was used to isolate metabolite fractions and analyzed using a multiplatform LC-MS-based approach (7 platforms for non-fat tissues and biofluids; 8 platforms for adipose tissues). Raw data files were processed using MS-DIAL 4. Metabolites were annotated using in-house retention time–*m/z* library and using MS/MS libraries available from commercial and open sources (NIST20, MassBank, MoNA). Lipids were annotated using LipidBlast in MS-DIAL.
Ultimately, we annotated over 3,000 unique polar metabolites and complex lipids. To better understand the structure of generated data, we provide a user-friendly data visualization tool (metaboatlas21.metabolomics.fgu.cas.cz) to easily access and analyze the different combinations of tissues and biofluids in response to the metabolic challenge.

Funding Sources (if applicable):

Czech Science Foundation (20-21114S), Ministry of Education, Youth and Sports (LTAUSA19124), Czech Health Research Council (NU20-01-00186)

16

Evaluating stool sample preparation for analysis of short-chain fatty acids by comprehensive two-dimensional gas chromatography – time-of-flight mass spectrometry

Kieran Tarazona Carrillo[1], Seo Lin Nam[1], A. Paulina de la Mata[1], Olle de Bruin[2], Evgueni Doukhanine[2], James Harynuk[1]

[1]Department of Chemistry, University of Alberta, Canada
[2]DNA Genotek Inc., Canada
ktarazon@ualberta.ca

Abstract

Short chain fatty acids (SCFAs), produced by the bacterial fermentation of non-digestible fibre in the gut, are volatile organic acids with a chain length of up to 6 carbon atoms. These metabolites provide insight into the interactions between the gut microbiome and host health. Their presence and levels have been studied extensively to identify links to various health conditions, including inflammatory bowel disease. As such, reliable and sensitive methods for accurate quantification of SCFAs are required. However, the analysis of SCFAs in stool is challenging given the complexity of the matrix and the high volatility of the compounds.

Due to the volatile nature of SCFAs, care must be taken to ensure they are not lost through evaporation during sample preparation, and an appropriate analytical technique is required. Gas chromatography (GC) is the most suitable instrumentation for the analysis of volatile compounds. Headspace extraction techniques, such as solid-phase microextraction, are applicable to the analysis of volatiles and are easily coupled to GC; however, quantification is difficult due to the principles of partitioning equilibria involved in the method. Techniques based on liquid extraction followed by chemical derivatization can overcome these difficulties and previously developed methods have been shown to have a high level of sensitivity, reproducibility, and recovery of SCFAs. Comprehensive two-dimensional gas chromatography coupled to time-of-flight mass spectrometry (GC×GC-TOFMS) provides enhanced separation of complex mixtures and superior sensitivity compared to one-dimensional GC techniques.

This work presents an evaluation of SCFA extractions from stool as well as derivatization and derivatization-free methods for their analysis by GC×GC-TOFMS.

Funding Sources (if applicable):

Canada Foundation for Innovation, Genome Canada, Genome Alberta, MITACS.

18

Metabolite discovery through global annotation of untargeted metabolomics data

Li Chen[1,2,*], Wenyun Lu[2,3], Lin Wang[2,3], Xi Xing[2,3], Ziyang Chen[1,4], Xin Teng[2], Xianfeng Zeng[2,3], Antonio D.Muscarella[2], Yihui Shen[2], Alexis Cowan[2,4], Melanie R. McReynolds[2,3], Brandon Kennedy[5], Ashley M. Lato[6], Shawn R. Campagna[6], Mona Singh[2,7], Joshua Rabinowitz[2,3,4,#]

[1]Shanghai Key Laboratory of Metabolic Remodeling and Health, Institute of Metabolism & Integrative Biology, Fudan University, CN

[2]Lewis-Sigler Institute for Integrative Genomics,

[3]Department of Chemistry,

[4]Department of Molecular Biology,

[5]Lotus Separation LLC, Department of Chemistry,

[7]Department of Computer Science, Princeton University, Princeton, USA

[6]Department of Chemistry, The University of Tennessee at Knoxville, USA

Abstract

Liquid chromatography-high resolution mass spectrometry (LC-MS)-based metabolomics aims to identify and quantitate all metabolites, but most LC-MS peaks remain unidentified. Here, we present a global network optimization approach, NetID, to annotate untargeted LC-MS metabolomics data. The approach aims to generate, for all experimentally observed ion peaks, annotations that match the measured masses, retention times, and (when available) MS/MS fragmentation patterns. Peaks are connected based on mass differences reflecting adducting, fragmentation, isotopes, or feasible biochemical transformations. Global optimization generates a single network linking most observed ion peaks, enhances peak assignment accuracy, and produces chemically-informative peak-peak relationships, including for peaks lacking MS/MS spectra. Applying this approach to yeast and mouse data, we identified five previously unrecognized metabolites (thiamine derivatives and N-glucosyl-taurine). Isotope tracer studies indicate active flux through these metabolites. Thus, NetID applies existing metabolomic knowledge and global optimization to substantially improve annotation coverage and accuracy in untargeted metabolomics datasets, facilitating metabolite discovery.

Funding Sources (if applicable):

This work was supported by a Department of Energy (DOE) grant (no. DE-SC0012461 to J.D.R.), the Center for Advanced Bioenergy and Bioproducts Innovation (grant no. DE-SC0018420, subcontract to J.D.R.), NIH grant R50CA211437 to W.L and the Howard Hughes Medical Institute and Burroughs Wellcome Fund via the PDEP and Hanna H. Gray Fellows Programs to M.R.M. The Center for Advanced Bioenergy and Bioproducts Innovation and the Center for Bioenergy Innovation are both U.S. Department of Energy Bioenergy Research Centers supported by the Office of Biological and Environmental Research in the DOE Office of Science. Any opinions, findings, conclusions or recommendations expressed in this publication are those of the authors and do not necessarily reflect the views of the U.S. Department of Energy.

19

Gut microbial metabolism of aromatic amino acids under chemical and microbiota interventions

Chi Chen[1], Yuyin Zhou[1]

[1] Department of Food Science and Nutrition, University of Minnesota, USA

chichen@umn.edu

Abstract

The acceptance of gut microbiota as an important metabolic and endocrine organ is mainly based on the energy supply and regulatory functions of their microbial metabolites. Unabsorbed aromatic amino acids (AAAs), undergoing oxidation and reduction as well as lyase-mediated elimination and decarboxylation in microbial metabolism, can form various aryl metabolites with diverse bioactivities, but the contribution of individual metabolic pathways to the formation and distribution of these metabolites as well as their responses to chemical and microbial interventions are not well examined. In this study, deuterated AAAs were used as the sole source of dietary AAAs to trace their metabolic fates in mouse. The results showed that besides dietary AAAs, endogenous AAAs are also the major contributor of microbial metabolites. Tyrosine and phenylalanine undergo both oxidative and elimination reactions, producing phenylacetate (PAA), 4-hydroxyphenylacetate (4HPAA), p-cresol, phenol, and their conjugates as their major microbial metabolites, while tryptophan is mainly degraded by elimination reaction, producing indoxyl sulfate as a major microbial metabolite. Modulation of microbial AAA metabolism by microbiota and chemical interventions were investigated by the treatments of antibiotics, fecal microbiota transplantation (FMT), and green tea polyphenols (GTP). In both humans and mice, the production of p-cresol is highly sensitive to antibiotics and FMT since urinary p-cresol sulfate was depleted by antibiotics and then quickly restored by FMT through drastic changes in the gut microbiota. In contrast, chronic GTP consumption in humans did not significantly alter the microbial composition, but decreased urinary hippuric acid, indoxyl sulfate, and phenylacetylglutamine, potentially through competitive inhibition in the microbial metabolism of GTP and AAAs. Overall, microbial metabolism of AAAs is dominated by oxidative and elimination reactions. Because of its susceptibility to microbiota and chemical interventions, microbial metabolism of AAAs could be a target for dietary and therapeutic modulations of gut microbiota in humans and animals.

Funding Sources (if applicable):

This research was supported, in part, by NIH Grants R21AI091907 and R01DA043252, and NIFA project MIN-18-125.

20

Lipidomics Analysis Elucidates Postprandial Metabolic Excursions Following Milk Protein Ingestion in Individuals with Prediabetes

Li Chen[1,2], Rui Xu[1], Joshua D. McDonald[1], Richard S. Bruno[1], Shiqi Zhang[1], Fouad Choueiry[1], Jiangjiang Zhu[1,2]

[1] Human Nutrition Program, Department of Human Sciences, The Ohio State University, USA
[2] James Comprehensive Cancer Center, The Ohio State University, USA
Chen.10289@osu.edu

* presenting author: chen.10289@osu.edu

Dietary intervention is an effective approach to attenuate postprandial hyperglycemia (PPH) and lower cardiovascular disease (CVD) risk. Among many healthy choices, diary milk and its protein contents have been suggested to attenuate PPH-induced oxidative stress, however, how these protein contents alter the metabolic excursions in at risk population is unclear. Therefore, in this study, our objective is to examine the differential plasma lipidomic responses to whey or casein proteins in a group of twenty-three adults (17 males and 6 females) with prediabetes. In this randomized cross-over study, the participants consumed glucose alone or with dairy proteins (whey or casein proteins), and 207 plasma samples were collected at multiple time points post-consumption (0, 90 and 180 mins) for an untargeted lipidomics analysis. The UPLC-HRMS analysis was performed to identify a total of 764 lipids from 7 lipid categories in all samples. Linear and logistic regression models were used to assess the lipidomes and their relationships to other health outcomes after adjusted for BMI, age and gender. Our analyses revealed that milk proteins could significantly modulate the lipid profiles to potentially reduce the CVD risks of the participants with prediabetes, and casein protein have a quicker and different impact to the lipidome after ingestion than whey protein. Additionally, multiple significant correlations between lipidomics-based biomarkers and participant characteristics were identified. For instance, PE (36:1), a lipid that was associated with increased risk of diabetes, was significant decrease in our study and positively correlated with both the fasting plasma insulin and HOMA-IR in the casein group at 180 min. Collectively, our study suggested that milk proteins could protect against lipid changes induced by PPH and mediate CVD risks, and different milk proteins (whey vs. casein) could impact the lipid metabolism from different pathways in adults with prediabetes.

Funding Sources (if applicable):

The study was partially supported by the National Institute of General Medical Sciences of the National Institutes of Health under Award Number R35GM133510 (to JZ), and by the National Dairy Council (to RSB).

22

A longitudinal investigation of the metabolomic architecture of ADHD: developmental metabolomics in *utero* through early childhood

Su H. Chu[1,2], Rachel S. Kelly[1,2], Mengna Huang[1,2], Augusto Litonjua[3], Scott T. Weiss[1,2], Jessica Lasky-Su[1,2]

[1]Channing Division of Network Medicine, Brigham and Women's Hospital, USA
[2]Department of Medicine, Harvard Medical School, USA
[3]Division of Pediatric Pulmonary Medicine, Department of Pediatrics, University of Rochester Medical Center, USA
su.chu@channing.harvard.edu

Abstract

ADHD is a highly prevalent neuropsychiatric disorder with a complex, multifactorial etiology. However, its molecular underpinnings remain poorly understood. Longitudinal metabolomic profiling spanning fetal development and early life has the potential to deepen mechanistic understanding of ADHD pathophysiology.

We performed longitudinal plasma metabolomic profiling via UPLC-MS/MS among mother-child pairs in the Vitamin D Antenatal Asthma Reduction Trial (VDAART). To identify sensitive developmental periods for metabolomic ADHD risk, we conducted metabolome-wide association scans (metaboWAS) via logistic regression at each time point among: 1) 652 mothers with plasma at 10-18 and/or 32-38 weeks gestation, adjusting for baseline age, race, smoking, and study site; 2) 687 children with plasma at ages 1, 3, and/or 6 years, adjusting for the above maternal variables as well as child sex and race. Finally, mixed effects models were used to identify longitudinal trends in offspring metabolomic profiles associated with ADHD case status.

In the maternal metabolome, we identified protective associations between 1-methylnicotinamide ($p_{10\text{-}18wks}$=0.029; $p_{32\text{-}38wks}$=0.004) and ADHD risk, and generalized long-chain fatty acid dysregulation between 10-18 weeks. In the child metabolome, we observed global patterns of carnitine dysregulation at ages 1 and 6. Metabolites of tryptophan and glutathione metabolism were observed at age 3 (5-hydroxyindoleacetate, p=0.005; 2-aminobutyrate, p=0.005), along with several dietary metabolites associated with gut microbiota, possibly reflecting the transition from liquid to solid foods in early life. Corroborating the cross-sectional analyses, longitudinal ADHD-associated differential shifts in relative metabolite levels were identified for 1-methylhistidine (p=0.003), 1-methylnicotinamide (p=6.5e-04), and cysteine-glutathione disulfide (p=0.002).

As the first study to conduct a metabolomic interrogation of ADHD across multiple stages of development, we provide preliminary evidence of both distinct and common metabolic activity across multiple phases of child development, such as metabolites of carnitine, tryptophan, and glutathione metabolism, in the molecular etiology of ADHD. Further investigation of these mechanisms is warranted.

Funding Sources (if applicable):
K01HL153941
UG3 OD023268

24

Hierarchical clustering of CRC metabolic profiling data reveals putative pathways involved in metastatic progression

Brian Kirk[1], Igor Koman[2], Raichel Cohen-Harazi[2]

[1]Independant researcher
[2]Insititute for Personalized and Translational Medicine, Ariel University, Israel
raichelha@ariel.ac.il

Abstract

Metastatic progression is commonly modeled as a linear process from primary Stage I to Stage IV metastasis. Classic study design compares metastatic to primary samples, but this setup cannot resolve initiating metastatic events at the primary level. This leaves unanswered the influence of such events on the development of later stage metastasis. To address this issue we initially performed p-value based hierarchical clustering (pvclust) on the colorectal cancer subset of cell line metabolic profiling data published by Li, et al. and resolved three statistically significant clusters, characterized by varying proportion of cell lines with metastatic versus primary origin (High Primary (HPrim); High Metastasis (HMet); Mixed (Mxd)). Our methodology ensured that although the set is composed of cell lines grown in diverse media, these clusters exhibit no dependence on culture media (p=.83). We do observe, however, statistically significant dependence between our clusters and metastatic composition, pathological cancer staging, proliferation rates, and cell adhesion properties, indicating that the clustering is relevant to both metastatic and physiological properties. Univariate analysis with SAM identified 101 statistically significant metabolites, which served as the basis for MSEA in which 7 statistically significant pathways were identified. Multivariate analysis with an ensemble of 100 elastic nets recapitulated much of the univariate analysis but prioritized pathways and key metabolites. Mapping our results to metabolic pathways revealed putative 2-step mechanisms in a progression from HPrim → Mxd → HMet, including a network of linking pathways extending from glycolysis/TCA, and implicated a small number of putative signaling molecules. These results indicate that metabolic pathways and mechanisms relevant to and distinguishing later stage metastasis can be observed in primary derived samples. This points to the role of primary alterations in late-stage metastasis and opens the possibility of clinical characterization of the potential degree of metastasis from a primary sample alone.

Funding Sources (if applicable):

This work was funded by private donations to The Institute for Personalized and Translational Medicine.

25

DEIMoS: an open-source tool for processing high-dimensional mass spectrometry data – updates and improvements

Sean Colby[1], Jamie Nunez[1], Madison Blumer[1], Christine Chang[1], Marjolein Oostrom[1], Daniel Orton[1], Kent Bloodsworth[1], Ernesto Nakayasu[1], Ryan Renslow[1], Thomas Metz[1]

[1]Earth and Biological Sciences, Pacific Northwest National Laboratory, USA
sean.colby@pnnl.gov

Abstract

Here we present DEIMoS: Data Extraction for Integrated Multidimensional Spectrometry, a Python application programming interface and command-line tool for mass spectrometry data analysis workflows, offering ease of development and access to efficient algorithmic implementations. Functionality includes peak detection, alignment, isotope detection, MS/MS spectral deconvolution, and data slicing, utilized in concert to return detected features aligned across study samples, as well as associated mass fragmentation patterns and/or isotopic signatures, if available. Notably, DEIMoS operates on data of arbitrary dimension, regardless of acquisition instrumentation: algorithm implementations utilize all dimensions simultaneously to (i) offer greater separation between features, improving detection sensitivity, (ii) increase alignment/feature matching confidence among datasets, and (iii) mitigate convolution artifacts in mass fragmentation patterns. We evaluate DEIMoS with LC-IMS-MS/MS data, highlighting peak detection, alignment, isotope detection, and MS/MS spectral deconvolution, demonstrating the advantages of our multidimensional approach in each data processing step, noting important changes and improvements resulting from the last year of development activity.

Funding Sources (if applicable):

Common Fund Metabolomics Program Phase II, PNACIC Grant: NIH CIDC U2CES030170

28

Evaluation of a NIST human prenatal serum suite as a metabolomics quality control material

W. Clay Davis[1], Ashley S.P. Boggs[1], Tracey B. Schock[1]

[1]Chemical Sciences Division/ National Institute of Standards and Technology, USA
clay.davis@nist.gov

Abstract

Biomarker discovery hinges on the development and use of robust analytical measurements and workflows. Sample preparation, data acquisition, data processing, identification, and statistical analysis of analytical data from omics platforms often produces more variability than actual biological change, hindering biomarker identification and validation. The lack of quality control (QC) materials for determining analytical accuracy and precision in the non-targeted discovery phase of differential studies limits the true assessment of potential health and disease status markers. QA/QC materials aid in controlling systematic variance due to analyst, batch, extended analysis time, and multi-laboratory studies. The limited availability of appropriate QA/QC control materials in metabolomics applications poses numerous challenges for the advancement of both metabolomic and lipidomic research.

While it is important to incorporate QC materials that are specific to an experimental study, such as a matrix-matched experimental sample pool, it is also imperative to include a well-defined, stable, homogeneous sample that enables measurement harmonization, comparability, and commutability across instrumentation, software, and laboratories. NIST is currently developing both biofluid and tissue-based quality control materials that suit multiple omics disciplines, NMR- and MS-based metabolomic, lipidomic and proteomic measurements. In addition to the development of new materials, NIST is currently evaluating several existing SRM and RMs as useful materials in non-targeted and semi-targeted metabolomic, lipidomic, and proteomic applications.

One of these materials, SRM 1949 Frozen Human Prenatal Serum, is a 4-cohort suite consisting pools of serum from reproductive-age women for the base level (non-pregnant), and pools of maternal serum for the three pregnancy trimester levels (trimester 1, trimester 2 and trimester 3). SRM 1949 has the potential to serve the metabolomics community as an additional QC material to aid in the validation of deep biomolecular profiling, differential analysis, and data analytics for untargeted clinical assessments.

Funding Sources (if applicable):

30

Aroma and flavour profiling of blueberries using comprehensive two-dimensional gas chromatography

Ryan P. Dias[1], Trevor A. Johnson[1], L. Felipe V. Ferrão[2], Patricio R. Munoz[2], A. Paulina de la Mata[1], First Name Last Name[1]

[1]Department of Chemistry, University of Alberta, CA
[2]Blueberry Breeding and Genomics Lab, University of Florida, USA
dias1@ualberta.ca

Abstract

Aroma and flavour profiles of consumer products are comprised of diverse mixtures of volatile organic compounds (VOCs). Most compounds are endogenous to the consumable, while others are added intentionally (e.g., flavour or fragrance augmentation); and some other compounds are responsible for "off" scents or flavours. Headspace sampling techniques have been shown to be the most effective approaches to extraction of VOCs from a variety of matrices (wine, bread, milk, etc). The most commonly employed technique for food applications is headspace solid-phase microextraction (HS-SPME), though it has not been applied to blueberry VOCs. Chemical profiles can be obtained with little disturbance to the bulk sample during headspace extractions. Similarly, only a small amount (mass) of representative sample is required for a comprehensive profile of fruit volatiles. Though HS-SPME has been extensively applied due to simplicity/relatively low cost, the fibre coating introduces selectivity into the extraction process, preferentially sorbing certain compounds over others. Additionally, the extraction equilibria can be highly susceptible to matrix effects. Dynamic headspace (DHS) has been proposed as a more powerful substitute to HS-SPME, citing improved sensitivity and fewer concerns related to extraction selectivity and matrix effects. DHS extractions yield a larger number of compounds and require even less mass of sample than HS-SPME. These two extraction techniques were applied to homogenized blueberries; major chemical classes were identified with automatic filtering scripts for GC×GC-TOFMS data. Optimized HS-SPME and DHS methods were assessed for extraction reproducibility and chemical diversity (i.e., profiled chemical classes).

Funding Sources (if applicable):

TMIC (The Metabolomics Innovation Centre)
Genome Canada
Genome Alberta

32

A new perspective on Usnic acid biosynthesis in *Cladonia rangiferina*

Susan Egbert[1], Jordan Hoffman[2], James Lendemer[2], John L. Sorensen[1]

[1]Department of Chemistry, University of Manitoba, CA
[2]Institute of Systematic Botany, New York Botanical Garden, USA
Sbegbert80@gmail.com

Abstract

One of the most prevalent natural products in lichen fungi is usnic acid, which has been shown to possess a broad array of bioactivity. Although usnic acid was originally isolated from the lichens *Ramalina fraxinea* and *Usnea barbata*, the only putative biosynthetic gene cluster (BGC) was reported from the known producer *Cladonia uncialis*. This presentation will describe how we have used the gene cluster from *C. uncialis* as a guide to explore usnic acid biosynthesis in other *Cladonia spp.*

We investigated strains of *C. rangiferina* and *C. stygia*, reported in the literature as lacking an ability to produce usnic acid. We first examined genomic data for several *C. rangiferina* strains and used genome annotation tools to identify the usnic acid BGC in these samples. A comparison of these BGC's to the usnic acid BGC's from a number of *C. unicalis* strains displayed high homology, suggesting that these genes should be functional. *Cladonia stygia* on the other hand lacked the usnic acid BGC entirely. Subsequent LC-MS analysis revealed readily detectable amounts of usnic acid in the *C. rangiferina* extracts.. The conclusions of this study demonstrate that the fundamental lack of knowledge on lichen secondary metabolism and biosynthesis represent a unique opportunity to discover new chemistry, evaluate phylogenetic relationships, and potential ecological functions of lichen chemistry

Funding Sources (if applicable):

36

A metabolomics snapshot in time: metabolomic comparison of the living fossil *Wollemia nobilis* and *Araucaria heterophylla*

Ryland T. Giebelhaus[1], Lauren A.E. Erland[1], Susan J. Murch[1]

[1]Department of Chemistry, University of British Columbia, Kelowna, Canada
giebelhausryland@gmail.com

Abstract

Wollemi pine (*Wollemia nobilis*) is a living fossil, known only through fossil records until its 1994 discovery in Australia. Phylogenetic studies have found that Wollemi is closely related to Norfolk pine (*Araucaria heterophylla*), making comparisons between Wollemi and Norfolk pine an interesting system for exploring metabolic evolution in plants. Wollemi has distinct botanical characteristics including unique changes in leave physiology with age. Our objectives were to (a) compare the metabolomes of the two related species (b) to identify age-related patterns of metabolites which may be associated with the unique morphological characteristics of Wollemi and (c) test these hypotheses using targeted assays. We employed a liquid chromatography time of flight mass spectrometry-based (LC-TOF) untargeted metabolomics approach. Multivariate and univariate statistical tools, pathway analysis, discovery, and putative identification tools were used. A total of 9,213 unique features were identified by ion masses and retention times, with 459 features that were unique to Wollemi, and 332 features found exclusively in Norfolk pine. ~1000 features were determined to show a linear increase or decrease with age in Wollemi. Based on hormonomics analysis we generated two hypotheses which were investigated through targeted LC-MS analysis (i) Wollemi pine is more reliant on and will have higher concentrations of brassinosteroids and (ii) indolamine metabolism is altered between the two species. Contrary to our hypothesis Wollemi pine and Norfolk pine tissues were found to have similar concentrations of brassinolide. Wollemi pine was, however, found to have significantly higher melatonin levels as compared to Norfolk pine. Melatonin has previously been hypothesized to be an ancient signaling molecule, and presence of high levels of this compound in the Wollemi pine samples supports this hypothesis. We hypothesize that (1) brassinolides are an early class of phytohormone and (2) that melatonin may have conferred an evolutionary advantage in this species which has allowed it to persist.

Funding Sources (if applicable):

National Science and Engineering Research Council (NSERC)

38

Direct mass spectrometry analysis of isomeric lipids and free fatty acids via online plasma-droplet derivatization

Alexander J. Grooms[1], Abraham K. Badu-Tawiah[1]

[1]Department of Chemistry and Biochemistry, The Ohio State University, USA
grooms.92@osu.edu

Abstract

The presence of isomeric species is prevalent in lipidomics and presents an analytical challenge that standalone mass spectrometers cannot address. Carbon-carbon double bond configurations within the acyl chains of lipids contributes to a wide array of structural diversity within the lipidome that alters biological function including membrane permeability, thickness, and plasticity. The number and position of double bonds is difficult to determine with mass spectrometry (MS), even when utilizing collision-induced dissociation (CID) in tandem MS. The process often requires additional chemical derivatization or alternative analytical techniques.

Herein, we employed the use of non-thermal plasma generated in-situ via chemically etched silica capillaries to produce reactive oxygen species for lipid derivatization, which was followed by CID tandem MS for double bond localization. Epoxidation across the C=C bond is a common occurrence in the presence of the reactive oxygen species produced in corona discharge plasma. Etched capillaries for reactive species generation were integrated into a contained electrospray emitter with four inputs and one configurable output consisting of multiple operational modes.

Initially, the epoxidation of the C=C bond in oleic acid was chosen to evaluate reactive oxygen species generation. Preliminary results show the epoxidation product $[M+O-H]^-$ at *m/z* 297 alongside the pseudomolecular ion $[M-H]^-$ at *m/z* 281. This result was not observed using conventional electrospray ionization suggesting the importance of the etched silica emitters for reactive species production. The epoxidation product was supported by CID tandem MS, which produced characteristic fragment ions. Breakage of the epoxide peak at *m/z* 297 gave fragments *m/z* 155 and 171, indicating a double bond at the 9Z position. Analogous double bond configurations were evaluated for mixtures of relevant isomeric fatty acids. Detection of lipids, including phosphatidylethanolamine containing more than one acyl chain using the plasma-droplet emitter will be discussed.

Funding Sources (if applicable):

This research was supported by the U.S. Department of Energy, Office of Science, Office of Basic Energy Sciences, Condensed Phase, and Interfacial Molecular Science, under award number DE-SC0016044.

39

Development of a computational software for ultra-high precision collisional cross section measurements

Christopher Harrilal[1], Jaehun Chun[1], Nikhil Devanathan[1], Sandilya Garimella[1], Gregory Schenter[1]

[1]Biological Science Division/Pacific Northwest National Laboratory, USA
Christopher.Harrilal@pnnl.gov

Abstract

Ion mobility is a well-established analytical technique that separates gas phase ion based on their size to charge ratio. Recent advancements in technology, mainly pioneered by the development of Structures for Lossless Ion Manipulations (SLIM), has allowed for ultra-high-resolution separations where resolving powers approach 700-1200. Under these conditions, ions with mobility differences of a few 100 parts-per-million can be resolved.

Of interest are the mobility differences observed between isotopomers under ultra-high-resolution conditions. Isotopomers are isotopic isomers that have identical mass/structure but differ in the position of their nuclides. As such, they are not expected to have mobility differences. Experimentally, however, mobility differences are observed and correlate well to changes in the ions center-of-mass (COM) and moments-of-inertia (MoI). This indicates changes to rotational properties, arising from differences in the distribution of mass, may result in subtle mobility differences. To explain the origin of these mobility differences we have developed a collisional cross section (CCS) calculator that accounts for the CoM and MoI of each isotopomer by modelling collisions between a rotating ion and neutral buffer gas. In most traditional codes rotational motion is largely ignored. Our initial results successfully reproduce the experimental mobility shifts. Here we describe the framework of the code and provide an analysis of the scattering differences that lead to the mobility shifts. We ultimately aim to provide a tool that predicts mobility differences quickly and precisely between ions of similar mobility for future ultra-high-resolution mobility separations.

Funding Sources (if applicable):

41

Assessing membrane fluidity of antibiotic-resistant *Staphylococcus aureus* using an RPLC-IM-MS method for isomeric phospholipid separations

Christian Freeman[1], Jana Carpenter[1], Keerthi Appala[1], Kelly M. Hines[1]

[1]Department of Chemistry, University of Georgia, USA
Kelly.Hines@uga.edu

Abstract

Staphylococcus aureus varies its membrane fluidity in response to environmental stresses by changing the ratio of branched-chain fatty acids (BCFAs) to straight-chain fatty acids (SCFAs) in its membrane lipids. Altered membrane fluidity has been associated with an increased tolerance of membrane-targeting antibiotics, including daptomycin. We recently demonstrated a reversed-phase liquid chromatography method that can separate lipid isomers having branched-branched, branched-straight, or straight-straight fatty acyl tail combinations. When coupled with ion mobility-mass spectrometry, this approach can resolve the FA compositions of co-eluting lipid classes for high-confidence identifications. We used this method to examine the distribution of FA isomers in the lipids of a *S. aureus* strain with daptomycin resistance. As previously reported, N315-Dap8 had decreased levels of PGs and increased lysylPGs compared to N315 as a result of the *pgsA* and *mrpF* mutations. However, the RPLC method revealed that the PGs present in N315-Dap8 contained only branched-branched and branched-straight FA combinations, whereas N315 contained PGs with all three combinations of FAs. PGs with two straight-chain FAs appeared in N315-Dap8 when bacteria were cultured in broth containing SCFAs, but the extent of incorporation was substantially lower than observed for N315. However, LysylPGs lacked straight-straight isomers in both N315 and N315-Dap8 even when straight FAs were provided in the culture media. These results indicate that daptomycin resistance is facilitated in-part by increased membrane fluidity. Future experiments will include metabolomics analyses and measurements of membrane fluidity in order to correlate the lipidomic measurements with levels of BCFA precursors and membrane physiology, respectively.

Funding Sources (if applicable):

NIAID K22AI143919

42

Characterizing the follicular fluid metabolome: quantifying the correlation across ovarian follicles and with the serum metabolome

Robert B Hood[1], Donghai Liang[2], Jennifer Ford[3], Irene Souter[4], Dean Jones[5], Russ Hauser[3,6], Audrey J Gaskins[1]

[1]Department of Epidemiology, Emory University, USA
[2]Gangarosa Department of Environmental Health, Emory University, USA
[3]Department of Environmental Health, Harvard University, USA
[4]Vincent Obstetrics and Gynecology, Massachusetts General Hospital and Harvard University, USA
[5]Division of Pulmonary, Allergy, & Critical Care Medicine, Emory University, USA
[6]Department of Epidemiology, Harvard University, USA
rhood2@emory.edu

Abstract

Background. Metabolomics has been used to characterize the small molecules present in many biological fluids and tissues; however, one matrix that has been understudied is the ovarian follicular fluid. Follicular fluid is the immediate microenvironment for oocytes and plays a critical role in ovarian steroidogenesis and gametogenesis. We sought to characterize the overlap in metabolomics features between serum and follicular fluid as well as the overlap in features between different follicles collected from the same woman. **Methods.** 135 women undergoing in vitro fertilization provided a serum sample during controlled ovarian stimulation and up to three follicular fluid samples during oocyte retrieval. Serum and follicular fluid samples were analyzed separately using liquid chromatography and high-resolution mass spectrometry with two technical columns. Features appearing in at least 20% of samples were matched between matrices based on mass-to-charge ratio within 10 ppm. Three correlation coefficients (overall, feature-specific, and subject-specific) were then used to describe how well the concentration of the overlapping features were associated between the matrices. **Results.** In the C18 negative and HILIC positive columns, we identified 3970 and 2352 metabolic features that overlapped between the serum and follicular fluid; however, the overall (C18:0.258; HILIC:0.520), feature-specific (C18:0.348, HILIC:0.353), and subject-specific (C18:0.390, HILIC:0.500) correlations were low. In contrast, when comparing metabolite concentrations across follicles within a woman, the overall (C18:0.982-0.999; HILIC:0.984-0.999), feature-specific (C18:0.741-0.811, HILIC:0.781-0.847), and subject-specific (C18:0.877-0.891, HILIC:0.901-0.914) correlations were high. **Conclusions.** We observed low to moderate correlation between serum and follicular fluids metabolomics features but observed high correlation across ovarian follicles. Based on these results, the follicular fluid appears to represent a novel matrix, distinct from serum, that may be a more optimal source for biochemical predictors of female fertility. Given the high correlation in features across follicles, a single follicular fluid sample may be sufficient for estimating many subject-specific biomarker concentrations.

Funding Sources (if applicable):

This work was supported by the following grants from the NIEHS (P30-ES019776, R01-ES009718, R01-ES022955, P30-ES000002, and R00-ES026648) and NIDDK (P30DK046200). The funding sources had no involvement in the study design, collection, analysis, or interpretation of the data; in the writing of the report; and in the decision to submit the article for publication.

45

Global Metabolomics reveal Nitrogen Fertilization as a Robust tool to Enhance Phytonutrient Content in Strawberry Fruits.

Bhupinder Singh Jatana[1], Jisun J.H. Lee [1], Christopher Kitchens[2], Christopher L. Ray[3], Pattrick Gerard[4], Nishanth Tharayil[1]

[1]Department of Plant and Environmental Sciences, Clemson University, USA
[2]Department of Chemical Engineering, Clemson University, USA
[3]Edisto Research and Education Center, Clemson University, USA
[4]School of Mathematical and Statistical Sciences, Clemson University, USA
bjatana@g.clemson.edu

Abstract

In the present day, where human health drives the consumer's food choices, increased attention is given to the quality of fruits and vegetables due to their abundance of nutraceuticals. These bioactive compounds offer various antioxidant, anticancer, antimutagenic, antimicrobial, antiinflammatory, and antineurodegenerative properties. However, the global intake of phytonutrients per person is 50% lower than the WHO recommendation, primarily due to their lower content in the produce. This paradigm is now changing toward breeding for higher phytonutrient content. One of the potential ways to improve the phytonutrient content in the fruits is by fertilizing the crops with different mineral forms of nitrogen (N). However, the effect of different N minerals on the phytonutrient content of fruits is less known. Here we investigated the effect of different mineral forms of N (NH_4^+, NO_3^-, NH_4NO_3, and organic N) on the phytonutrient and aroma profile of fruits using the untargeted metabolomics approach.

We analyzed the ripe fruits from strawberries fertilized with different forms of N for volatile, primary, and secondary metabolites. Fertilization with organic N resulted in higher aroma-related volatile compounds in strawberry fruits. The plants fed with NH_4NO_3 and NO_3^- had higher sugars, sugar alcohols and amino acids accumulation in fruits. On the other hand, NH_4^+ and organic N fertilization resulted in higher TCA cycle organic acids, shikimic acid, aromatic amino acids, and volatile aroma compounds in fruits. The NH_4^+ and organic form of N significantly increased the cellular content of phenolic acids, flavonoids, and ellagic acid derivatives in the fruits, which have anti-carcinogenic and antineurodegenerative properties. The hydrolyzable tannins, which are branched from dehydroshikimic acid, were abundant in the fruits of plants fed with NH_4NO_3 and NO_3^-. Overall, our results reveal that different mineral forms of N differentially regulate the phytonutrient content in strawberry fruits, which can inform agronomic and breeding efforts.

Funding Sources (If applicable)**:**

This research project was funded by the United States Department of Agriculture (USDA NIFA award: 2016-67020-25264).

46

Using computation to probe microsolvated phases during soft ionization processes in mass spectrometry

Samantha I. Johnson[1], Wilma Rishko[2], Rahul Somni[1], Marcel Baer[1], Simone Raugei[1]

[1]Physical and Computational Sciences Directorate, Pacific Northwest National Laboratory, USA
[2]Department of Physics, University of Illinois, Urbana-Champaign, USA
samanthajo.johnson@pnnl.gov

Abstract

Identification of molecules and elucidation of their chemical structure are ubiquitous problems in chemistry. Mass spectrometry (MS) has frequently been applied for these purposes due to its sensitivity and versatility, as it can be applied to single compounds or complex mixtures. Ideally, MS patterns are chemical fingerprints that can be used to detect known compounds or characterize new molecules. However, the process is sensitive to the nature of parent medium (matrix effects) and instrument conditions, making unambiguous identification often difficult. Toward improved MS detection and analysis, we are developing atomistic computational approaches to understand and predict how the original environment and MS conditions regulate the ionization fate of analytes.

In order for detection to occur, analytes must be charged and must transfer from the condensed phase to the gas phase, in a process called ionization. We focus on soft ionization processes, such as electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI), for detection of moderately polar and nonpolar analytes. Although these methods tend to retain the analyte's structure, rather than fragmenting it as in hard ionization approaches, chemical transformations can still occur in the microsolvated phase between condensed and gas phases, and may even be accelerated relative to the condensed phase. To understand these processes, we use computational methods (density functional theory, molecular dynamics and enhanced sampling approaches) to probe ionization propensity of analytes, as well as microsolvated cluster energetics and structures. We will discuss how computational methods provide precious insights on how the nature of parent condensed phase influences the fate of an analyte in the gas phase (the so-called matrix effects). Using amino acids in water clusters as a testbed, we show how microsolvated environments can change the ionization propensity as well as the tautomeric form of the analyte, which dictates which species enter the mass spectrometer.

Funding Sources (if applicable):

Laboratory Directed Research and Development as provided by the m/q Initiative at Pacific Northwest National Lab

48

Zooming into your favorite metabolic pathway: Combining broad and targeted metabolite profiles for deeper understanding of the metabolome

Marissa A. Jones[1], Tuan Hai Pham[1], Barbara Ustaszewski[1], Katherine Black[1], Therese Koal[1]

[1]Biocrates Life Sciences AG, USA and AT
Marissa.jones@biocrates.com

Abstract

Metabolomics and lipidomics face two main challenges: comprehensive coverage and interpretation of the results in a broader context. Comprehensive targeted metabolomics solutions go beyond broad coverage and provide reliable, reproducible, and quantitative analysis. However, even when these requirements are met, interpretation and biological relevance can remain a challenge. For an experiment to move beyond analytical measurement, metabolites must be placed in context through pathway analysis. Through incorporating multiple metabolites across various pathways, results are not limited to the analysis of individual metabolite concentrations. Rather, pathway integration enables results to be interpreted in the broader context of diverse, and often interconnected, pathophysiological processes. Here, we suggest a workflow maximizing the output of metabolomics experiments by using broad and targeted profiling approaches synergistically with innovative approaches for functional data interpretation.

As no single method can quantify the complete metabolome, a multiplexed method covering up to 630 metabolites and lipids from 26 compound classes (MxP® Quant 500 kit) offers an attractive solution for high-throughput hypothesis generation. Functional interpretation of results using the MetaboINDICATOR™ software tool allows for the inclusion of 234 pre-defined sums and ratios, as well as user-defined sums and ratios, into the statistical analysis, providing immediate links to alterations in pathophysiological processes (e.g. inflammation, gut microbial dysbiosis, etc.). This initial broad, hypothesis-generating analysis covers key metabolites and can reveal elevated and decreased levels of various metabolites, allowing for further "zooming" into individual pathways. These individual pathways can be subsequently analyzed with specific assays (e.g. for bile acids, tryptophan metabolites, acylcarnitines) to verify and strengthen the generated hypotheses. Through the combination of broad and pathway-specific targeted assays, biocrates addresses the two main challenges of metabolomics. Specifically, biocrates' kit technology offers improved analytical sensitivity and biocrates' MetaboINDICATOR™ software aids in the interpretation of results within a broader context.

Funding Sources (if applicable):

51

Combining electron activation and collision-induced dissociation yields massively enriched mass spectra for >3,000 metabolites

Uri Keshet[1], JongCheol Lee[1], Tong Shen[1], Oliver Fiehn[1]

[1]West Coast Metabolomics Center, University of California Davis, USA
ukeshet@ucdavis.edu

Abstract

Around 100,000 small molecule compounds have available CID mass spectra in libraries. However, CID spectra often lead to only 2-6 fragment ions per compound. This sparse fragmentation easily results in erroneous compound identifications. In addition, metabolomics and lipidomics span a very wide range of compound classes. It is challenging to find CID collision settings that will provide optimal fragmentations for these structures. Recently, a new commercial instrument has been introduced to enrich MS/MS spectra of small molecules, using electron activated dissociation (EAD) by the impact of energetic (12eV) electrons as an orthogonal fragmentation technique to CID. The new Sciex ZenoTOF 7600 combines EAD and CID in series, to provide fragments-rich MS/MS spectra for small molecules.

We analyzed more than 4,000 chemical standards of polar metabolites and natural products for the library by LC-MS with EAD+CID in positive ESI mode. $[M+H]^+$, $[M+Na]^+$, $[M+NH4]^+$ adduct ions were included in information-dependent analysis mode, resulting in a total of 5,000 MS/MS spectra in the library, publicly available on MassBank.us. We validated the use of this instrument and the new library by analyzing eight biological samples from human gut intestinal tract samples, a pooled plasma sample, and vegan and omnivore human fecal matter samples. Additionally, these samples were analyzed in CID-only mode and were similarly annotated in the same process. The new EAD+CID library provided five times more annotated compounds compared to using a library of CID-only mass spectra of the same compounds. The ion-rich EAD+CID MS2 spectra also provided higher confidence in annotations, and a reduced number of false positive annotations compared to their CID-only counterparts. Additionally, Zeno pulsing benefits were recognized in the new Sciex ZenoTOF instrument over the classic Sciex TTOF 6600, providing increased intensity in MS2 despite the higher degree of fragmentation, and thus better ion statistics.

Funding Sources (if applicable):

NIH U2C ES030258

52

Combinational open-source software application for unknown feature analysis for LC-MS based untargeted metabolomics

Yongseok Kim[1], Vicki H. Wysocki[1]

[1]Department of Chemistry and Biochemistry, The Ohio State University, USA
kim.7263@buckeyemail.osu.edu

Abstract

Liquid chromatography coupled to mass spectrometry (LC-MS) is one of the most widely used methods for untargeted metabolomics. While LC-MS-based metabolomics results in an extensive list of the features referring to pairs of mass-to-charge ratio and retention time, one main limitation is the lack of annotation information for a large fraction of these features in available databases. As an alternative approach, *in silico* fragmentation has been suggested, facilitating the annotation of unknown features without any reference mass spectra. Various software such as MS-Finder, Sirius+CSI:Finger ID, and CFM-ID 3.0 have been developed by building on diverse rules such as hydrogen rearrangement rules or competitive fragmentation models. However, solving this limitation is still a challenging task due to ambiguities of the scoring system or the lack of validation tools for candidate compounds.

Here, we suggest a workflow for unknown feature annotation by using multiple open-source software packages. Level 2 annotation is performed with MS-Dial software before unknown feature analysis. The retention time (RT) of level 2 annotated features is input into QSRR Automator, to build RT prediction models. The features not matching to mass spectra library are analyzed with MS-Finder and Sirius + CSI:Finger ID for *in silico* fragmentation analysis. The retention time of suggested candidates from *in silico* fragmentation software packages was calculated with the model developed with QSRR automator based on Level 2 annotation. The calculated RT was compared to the actual RT. Potential candidates showing lower RT differences between predicted and observed values than the mean error of the predicted model are considered “validated” compounds. The retention time model lowered the false-positive feature annotation, especially when more than one compound was suggested from *in silico* fragmentation software. Also, RT model can suggest the score criteria of each software.

Funding Sources (if applicable):

NIH NIAID (RO1Al116119)

53

Metabolite profiles distinguish common food microbes in a rapid NMR metabolomics experiment

Danhui Wang[1], Peyton Greenwood[1], Matthias S. Klein[1]

[1]Department of Food Science and Technology, The Ohio State University, USA
klein.663@osu.edu

Abstract

Food microbes cause hundreds of millions of cases of foodborne disease and are responsible for hundreds of thousands of deaths each year worldwide. In addition, microbial food spoilage causes food waste and economic loss, worsening food insecurity and threatening the sustainability of the food system. It is evident that there is an urgent need to improve microbial food safety methods.
We suggest a new rapid detection method for food microbes based on untargeted metabolomics.
Common pathogenic and non-pathogenic food microbes such as E. coli and Salmonella were grown for four hours under identical conditions in a new, specifically developed, protein-free defined medium. ^{1}H 1D NOESY spectra of medium were collected on an 850MHz Bruker Avance III HD Ascend spectrometer and binned using the R package mrbin. After an optimized feature selection step, machine learning algorithms were trained to identify microbial strains. Outer cross-validation was used to estimate the true classification error rate. Metabolites were identified by database queries and measurements of pure compounds.
Results show that unknown samples were predicted with exceptionally high accuracy, with highest accuracies for *Salmonella enterica* serovar *Typhimurium*, a food pathogen of high concern. Predictions were mostly based on small organic acids and alcohols.
This new method is not only rapid, but also able to distinguish between viable and inactivated microbes, in contrast to currently used methods. This is especially important in food processing settings to avoid false positive results. The identified metabolites may be measured in a targeted way using other analytical platforms that are more readily available to allow broad application of the method. This research has the potential to become a game changer in the food sector, helping to rapidly identify and stop bacterial contaminations, reducing food recalls, foodborne disease, and food waste for a safer and more sustainable food system.

Funding Sources (If applicable):

Foods for Health Discovery Theme
USDA National Institute of Food and Agriculture, Hatch project 1018603

55

Switchgrass metabolomics reveals striking genotypic and tissue/developmental differences in the specialized metabolites

Xingxing Li[1, 2], A. Daniel Jones[1, 2], Robert L. Last[1, 2]

[1] DOE Great Lakes Bioenergy Research Center, Michigan State University, USA
[2] Department of Biochemistry and Molecular Biology, Michigan State University, USA
lixingxi@msu.edu

Abstract

Plant specialized metabolites are taxonomically restricted in plants and, while these molecules are not absolutely required for survival, they are important for host interactions within specific environmental niches. Hundreds of thousands of structurally diverse plant specialized metabolites might exist in the plant kingdom. We leveraged liquid chromatography (LC) mass spectrometry (MS) based untargeted metabolomics to investigate the metabolome of the North American bioenergy crop, switchgrass (*Panicum virgatum* L.). We documented both polyphenols and especially abundant terpenoid glycosides in switchgrass. These include 6 flavonoid glycosides, 73 steroidal/triterpenoid saponins and 17 di-/sesqui-terpenoids annotated based on their MS spectra. Seven previously unreported structurally distinct saponins were purified for NMR analysis. Further analysis revealed the total saponins varying within the switchgrass genetic diversity. In general, apparent accumulation differences for most metabolite features were found between cultivars from the northern upland and southern lowland ecotypes when individual tissue types were focused. This work sets the stage for us to understand the roles of these differentially accumulated metabolites in biotic and abiotic stress tolerance and shaping potentially beneficial plant-microbe interactions.

Funding Sources (if applicable):

This work was supported by the Great Lakes Bioenergy Research Center, U.S. Department of Energy, Office of Science, Office of Biological and Environmental Research under Award Number DE-SC0018409.

56

Spectral entropy outperforms MS/MS dot product similarity for small molecule compound identification

Yuanyue Li[1], Tobias Kind[1], Jacob Folz[1], Arpana Vaniya[1], Sajjan Singh Mehta[1,2], Oliver Fiehn[1]

[1]West Coast Metabolomics Center, UC Davis Genome Center, University of California-Davis, USA
[2]oloBion, Parc Científic de Barcelona, Barcelona, ES
gccli@ucdavis.edu

Abstract

Compound identification in small molecule research such as untargeted metabolomics or exposome research relies on matching MS/MS spectra against experimental or in-silico mass spectral libraries. Most software programs rely on dot product similarity scores. We introduce the novel concept of MS/MS spectral entropy to improve scoring results in MS/MS similarity searches via library matching. Entropy similarity outperformed 42 alternative similarity algorithms, including dot product similarity, when searching 434,287 spectra against the high quality NIST20 library. Entropy similarity scores proved to be highly robust even when adding different levels of noise ions. To find potential isomers with similar MS/MS spectra, we introduce “bond difference” to measure molecular similarity. Experimental spectra of natural products and human gut metabolome data were used to confirmed that entropy similarity largely improved the accuracy of mass spectrometry-based annotations in small molecule research to FDR <10%, annotated novel compounds and provided the basis to automatically flag poor quality, noisy spectra.

Funding Sources (if applicable):

Funding for the "West Coast Metabolomics Center for Compound Identification" was provided by the National Institutes of Health under award number NIH U2C ES030158 to OF.

61

Achieving comprehensive lipid profiling with a CCS, retention time and MS/MS library

Nyasha Munjoma[1], Sarah Lennon[1], Jeff Goshawk[1], Johannes PC Vissers [1], Giorgis Isaac[2], Steve Lai[2], Graham Mullard[1], Lee A. Gethings[1], and Robert S. Plumb[2]

[1] Waters Corporation, UK
[2] Waters Corporation, USA
Nyasha_munjoma@waters.com

Abstract

Collision cross section (CCS) values can be used in addition to other molecular identifiers, such as retention time (RT) and m/z as an orthogonal attribute to increase the specificity of metabolite and lipid identifications. Harmonisation of protocols and procedures to derive CCS values is of paramount importance for metabolomics and lipidomics, since reconciling CCS measurements from various ion mobility technologies such as DTIM-MS, TWIM-MS and TIM-MS remain an issue. Several in-silico and experimentally derived CCS databases have been published to date but suffer a high false-positive rate and often identifications lack biological relevance. Ideally, experimental measured CCS values should be used to populate CCS databases but due to a limited number of commercially available lipid standards, in-silico measurements are needed to enable adequate coverage.

Here we used a sample set consisting of 100 certified standards to validate a predictive $TWCCSN_2$ model by Broeckling et al. Data were processed against an initial library with threshold criteria of ≤5% error between measured and predicted CCS values and ≤0.1 min RT deviation. After correction for RT, to reduce false-positive identifications, 3.3% of positive ion signals fell outside the ±5% limit. This is consistent with previously reported data (96.8% within 5% error). Fewer ions were detected in negative mode with 4.5% being outside the 5% error limit. The validated prediction model and lipid class extracts (to increase biologically relevant hits) were used to generate a library containing >3200 lipids and screened against data resulting from heart, liver, and human plasma extracts.

Funding Sources (if applicable):

62

Evaluation of fresh, frozen, and lyophilized fecal samples by SPME and derivatization using GC×GC-TOFMS

Seo Lin Nam[1], Kieran Tarazona[1], A. Paulina de la Mata[1], Olle de Bruin[2], Evgueni Doukhanine[2], James Harynuk[1]

[1]Department of Chemistry, University of Alberta, CA
[2]DNA Genotek Inc., CA
seolin@ualberta.ca

Abstract

Feces is an immensely complex matrix containing thousands of metabolites from the host and the microbial community. It also contains live bacteria and enzymes, which makes the composition of feces vary greatly depending on factors such as diet, health, and metabolism by both the host and its intestinal microorganisms. In recent years, fecal metabolomics has received increasing attention for its potential in elucidating the convoluted interactions between health and gut microbiota. However, stool metabolomics is inherently challenging because fecal samples are alive and do not have a static chemistry. Consequently, proper control of pre-analytical parameters such as handling and storage is critical to ensure reliable and meaningful results for fecal studies by minimizing unwanted variations in the samples. However, no consensus currently exists on how fecal samples should be stored/processed prior to analysis.

In this work, the effects of sample handling conditions on fecal metabolite profiles and abundances were evaluated using a powerful separation technique, comprehensive two-dimensional gas chromatography coupled to time-of-flight mass spectrometry (GC×GC-TOFMS). Two different sample preparation methods, solid-phase microextraction (SPME) and derivatization via methoximation/trimethylsilylation, were employed as complementary techniques to evaluate fresh, frozen, and lyophilized fecal samples with expanded coverage of the fecal metabolome. Our work, a comprehensive comparison of the sample handling conditions, provides in-depth understanding of the physicochemical changes that occur within fecal samples during storage and handling. This work presents steps towards the standardization of sample handling protocols to stabilize and analyze fecal samples that are as representative as possible of their initial composition.

Funding Sources (if applicable):

63

Super-targeted mass spectrometry: Looking closely at methionine 100 years after its discovery

Cajetan Neubauer[1], Hannah Landecker[2]

[1]INSTAAR, University of Colorado Boulder, USA.

[2]Institute for Society and Genetics, University of California Los Angeles, USA

caj.neubauer@colorado.edu

Abstract

A wealth of metabolomics data highlights important roles of methionine metabolism in health and disease. Humans and many other organisms must derive methionine as an essential amino acid from food. Since its discovery in 1921, methionine – once a tightly regulated resource in ecosystems – has become a mass commodity in the global economy, with well over 1 million tons being produced annually from petroleum to fortify livestock feed. Methionine synthesis is an excellent exemplar of a planetary scale anthropogenic activity that manifests at the molecular scale of cellular metabolism, with potential systemic effects on human diets, health and environments. Taking a planetary health perspective, this talk will explore the scale and historical trajectory of the methionine industry and provide a preliminary model for tracing this amino acid through the food supply into the human body.[1] We now aim to test this model empirically using isotope analytics to quantify the synthetic proportion of methionine in foods and humans. The proposed approach is to use a highly targeted mass spectrometry developed in the last five years that is based on ESI-Orbitrap. The technique reveals naturally-occurring intramolecular stable isotope patterns in organic and inorganic metabolites.[2,3] We will also briefly illustrate topics where this kind of super-targeted mass spectrometry can complement metabolomics in the context of nutrition research and clinical diagnostics.

Related publications:

1. Neubauer C & Landecker H (2021): https://doi.org/10.1016/S2542-5196(21)00138-8
2. Neubauer C et al. (2018): https://doi.org/10.1016/j.ijms.2018.08.001
3. Hilkert A et al. (2021): https://doi.org/10.1021/acs.analchem.1c00944

Funding Sources (if applicable):

64

Game of Microbes: Natural Products as Weapons for Microbial Regulation in the Oral Cavity

Maribel Okiye[1,2,3], Michelle Velez[2], Ashotoosh Tripathi[3,5], David H. Sherman[3.5]

[1]Department of Chemistry, University of Michigan, USA
[2]Department of Computational Medicine and Bioinformatics, Life Sciences Institute, University of Michigan, USA
[3]Life Sciences Institute, University of Michigan, USA
[4]Department of Orthodontics, School of Dentistry, University of Michigan, USA
[5]Department of Medicinal Chemistry, College of Pharmacy, University of Michigan, USA
maribelo@umich.edu

Abstract

The human oral microbiome can contain over 700 different microbial species at a given time. How these microbial species interact can shape the microenvironment throughout the human body as these interactions are paramount to maintaining oral health and overall, systemic health. Several epidemiological studies have linked dysbiosis of oral microbes with cardiovascular disease, poor glycemic control in diabetics, low birth-weight preterm babies, rheumatoid arthritis, osteoporosis. Recent advances in technology, including next-generation sequencing (NGS) and bioinformatic tools, have been critical in unraveling the complexities of the oral microbiome. To understand how microbial interactions are being mediated within the oral cavity and how they affect oral and overall, systemic health we analyzed the untargeted metabolome of 2500 interval saliva samples from 20 healthy non-smoking individuals. Using a prospective, split mouth, experimental gingivitis model we were able to observe metabolite profile changes in the oral cavity during gingivitis progression and identify three molecules with regulatory properties in pathogenic oral microbial growth. Our study represents weekly characterization of the human oral metabolome, providing a high-resolution landscape for understanding oral health during various health implications.

Funding Sources (if applicable):

67

A distinctive metabolic pattern of metformin in a cohort of healthy subjects

Lina Dahabiyeh[1*], Muhammad Mujammami[2,3], Tawfiq Arafat[4], Hicham Benabdelkamel[5], Assim Alfadda[2,5], Anas Abdel Rahman[6-8*]

[1]Department of Pharmaceutical Sciences, School of Pharmacy, The University of Jordan, JO
[2]Endocrinology and Diabetes Unit, Department of Medicine, College of Medicine, King Saud University, SA
[3]University Diabetes Center, King Abdulaziz University Hospital, King Saud University, SA
[4]Jordan Center for Pharmaceutical Research, JO
[5]Proteomics Resource Unit, Obesity Research Center, College of Medicine, King Saud University, SA
[6]Metabolomics Section, Department of Clinical Genomics, Center for Genome Medicine, King Faisal Specialist Hospital and Research Centre (KFSHRC), SA
[7]Department of Biochemistry and Molecular Medicine, College of Medicine, Al Faisal University, SA
[8]Department of Chemistry, Memorial University of Newfoundland, CA
aabdelrahman46@kfshrc.edu.sa

Abstract

Abstract

Introduction: Metformin is a widely prescribed medication for the treatment of type 2 diabetes mellitus (T2DM). It possesses effective roles in various disorders, including cancer, dyslipidemia, and obesity. **Objectives:** The underlying mechanisms of metformin's multiple benefits are not fully understood, where we are investigating using the unique metabolic pattern in a controlled clinical study. **Methods:** A mass spectrometry-based untargeted metabolomics approach was used to investigate the metabolic changes associated with metformin administration in the plasma of 26 healthy subjects at five-time points; pre-dose, before the maximum concentration of metformin (C_{max}), C_{max}, after C_{max}, and 36 hours' post-dose. **Results:** A total of 111 metabolites involved in various biochemical processes were perturbed, with branched-chain amino acid (BCAA) being the most significantly altered pathway.

Additionally, the Pearson similarity test revealed that 63 metabolites showed a change in their levels dependent on metformin level. Out of these 63, the level of 36 metabolites was significantly altered by metformin. Significantly altered metformin-dependent metabolites, including hydroxymethyl uracil, propionic acid, glycerophospholipids, and eicosanoids pointed to fundamental biochemical processes such as lipid network signaling, energy homeostasis, DNA lesion repair mechanisms, and gut microbiota functions that could be linked to the multiple beneficial roles of metformin. **Conclusion:** The distinctive metabolic pattern linked to metformin administration can be used as a metabolic signature to aid in predicting and assessing the pharmacological effects of other drugs and chemical compounds. This study, is a unique model for generating a novel computational platform for drugs pharmacometabolomics and the associated side effects

Funding Sources (if applicable):

69

Development of signal amplification strategy for mass spectrometry using gold nanoparticles (AuNPs) bioconjugates

Ayesha Seth[1], C.K. Manju[1], Suji Lee[1], Dr. Abraham K. Badu-Tawiah[1]

[1]Department of Chemistry and Biochemistry, Ohio State University, USA
seth.64@buckeyemail.osu.edu

Abstract

Paper-based immunoassays are alternatives to traditional immunoassays like ELISA-based rapid diagnostic tests (RDTs) due to their affordability, portability, accessibility, and shorter assay times. We report the development of a highly selective lab-on-chip technology for the detection of malaria antigen: *plasmodium falciparum* histidine-rich protein-2 (PfHRP-2) in blood/serum using portable mass spectrometers. AuNPs were synthesized by utilizing sodium citrate as the reducing agent. Through a ligand exchange method, anti-malarial antibodies were immobilized on AuNPs (AuNP@Ab) and the thiol groups (−SH) of trimethylolpropane tris(3-mercaptopropionate) (TMTP) were simultaneously immobilized on the surface of the AuNPs through the formation of Au−S bonds. Due to high surface area of the AuNPs, numerous cleavable TMTP mass tags can be attached to AuNP@Ab. This results in the anticipated signal amplification by detection of these mass tags cleaved from the complex AuNP@Ab-TMTP using dithiothreitol (DTT). They were characterized using nano-electrospray and paper-spray ionization methods. Immunoassays with AuNP@Ab-TMTP will be performed on paper followed by on-chip detection by mass spectrometry (MS). Paper-based platform integrates the use of a sandwich immunoassay between the capture antibody, antigen, and detection antibody. Results discussed will showcase the characterization of the antibody-gold nanoparticle conjugates, AuNP@Ab and AuNP@Ab−TMTP, via ultraviolet−visible spectroscopy. We observed a shift in the absorption feature of citrate nanoparticle from 518 nm to 523 nm due to ligand exchange with antibody, and a further red shift of 3 nm after the attachment of TMTP. The main products of TMTP in MS were $[M+Na]^+$ at m/z 421 and $[M+NH_4]^+$ at m/z 416 following the addition of DTT/NH_4OH. Tandem MS was performed on each, and diagnostic peaks were m/z 315 and m/z 293, respectively. Unlike RDTs offering low performance at low-cost, the proposed signal amplification strategy will enable high performance-to-cost ratio for the detection of asymptomatic malaria on paper substrates in resource limited settings.

Funding Source (If applicable):

US National Institute of Allergy and Infectious Diseases (award number R01-AI-143809)

70

A meta-analysis model approach to identifying metabolites in untargeted metabolomics studies

Amanda O. Shaver[1,2], Zihao Liu[3], Brianna M. Garcia[2,4], Ricardo M. Borges[5], Goncalo J. Gouviea[2,6], Carter K. Asef[7], Franklin E. Leach III[2,8], Facundo M. Fernández[7], Alison M. Morse[3], Arthur S. Edison[1,2,6], and Lauren M. McIntyre[3]

[1]Department of Genetics, University of Georgia, USA
[2]Complex Carbohydrate Research Center, University of Georgia, USA
[3]Department of Molecular Genetics and Microbiology, University of Florida, USA
[4]Department of Chemistry, University of Georgia, USA
[5]Walter Mors Institute of Research on Natural Products, Federal University of Rio de Janeiro, BR
[6]Department of Biochemistry, University of Georgia, USA
[7]School of Chemistry and Biochemistry, Georgia Institute of Technology, USA
[8]Department of Environment Health Science, University of Georgia, USA
Amanda.shaver@uga.edu

Abstract

The field of metabolomics can measure the underlying biochemical activity and physiological state of an organism yielding some of the richest datasets in omics studies. While rich in information, metabolomics datasets are chemically complex and filled with unknown metabolites that create a dilemma in how to discern which features are biologically relevant and 'valuable' enough to spend the considerable effort it takes to translate an unknown feature into chemical formula and structure. When lacking reliable metabolite identification, metabolomic analyses are biologically and chemically indecipherable. This problem is closely followed by challenges in batch variation, making identification and tracking of unknown peaks across experiments a challenge in large studies. Using *Caenorhabditis elegans* (*C. elegans*) as a model system, we show how the use of control samples, genetically diverse strains, careful experimental design, combing phenotypic datasets, and meta-analysis can aid in compound identification and batch correction that can lead to critical biological insights in a complex and imperfect experimental world.

Meta-analysis presents a promising approach to address the complicated variance across experiments in large metabolomics studies and focuses on the biological variation of relevance. We performed a meta-analysis on rank transformed chemical data for each of the pathways and study groups of interest to detect differentially expressed metabolites between our *C. elegans* reference strain and test strains that include a combination of evolutionarily diverse and genetically mutated worms. Using a meta-analysis approach, we could manage the complicated variance and structure in a large metabolomics study across six batches, 104 independent samples, and their chemical data collected from NMR and UPLC-MS. Furthermore, we were able to confidently pull out a list of metabolites to further explore and validate.

Funding Sources (if applicable):

NIH Grant #: 5U2CES030167-02

71

MetaLINCSR: An intuitive R-Shiny application for metabolomics processing, biomarker discovery, and analysis

Zyad Shehadeh[1], Fadhl Alakwaa[2]

[1]Cellular and Molecular Biomedical Science, University of Michigan, USA
[2]Neurology, University of Michigan, USA
zyads@umich.edu

Abstract

Data analysis tools utilizing advanced prediction algorithms and databases have proven crucial in understanding novel disease, expediting diagnosis, and improving therapeutics. Most current tools, however, require familiarity with unconventional packages and programming skills, and lead to time consuming code writing and troubleshooting. To address these problems, we developed an easy-to-use interactive R Shiny web application that analyzes metabolomics data, called “MetaLINCSR”. It includes a variety of streamlined functions, starting with quality control, missing imputation, normalization, and scaling. The user then has the option for biomarker discovery which was implemented using a variety of methods (such as LIMMA, T-test, Elastic Net, and linear regression). Furthermore, pathway enrichment analysis and classification of biomarkers is made possible using machine learning algorithms. Lastly, integration with the LINCS (Library of Integrated Network-Based Cellular Signatures) database provides for drug repurposing by mapping changes due to an ever-expanding database of perturbing agents and gives the chance for expandability of the app. For demonstration, we added sample data from metabolome profiles of patients that are lean, obese, and obese patients with neuropathy. Researchers can use the full functionality of our tool and can access our code from the GitHub page. “MetaLINCSR” facilitates metabolomic biomarker and pathway research, and we hope it will speed ongoing research efforts in the field.

Funding Sources (If applicable):

72

Metabolomics unveils the complexity of lipid signatures in bacteria exposed to functional chitosan nanoparticles and its membrane active mechanism

Sarangapani Sreelatha[1*], & Anand Asundi [2]

[1]School of Mechanical & Aerospace Engineering, Nanyang Technological University, SG
[2] d'Optron Pte Ltd, SG.

Abstract

Bacterial biofilms are responsible for several chronic diseases, which has seriously jeopardized the use of antibiotics causing the spread of microbes that are resistant to first line drugs. In the current scenario of high antibiotic resistance, the search for new therapeutic measures along with robust tools are of high importance. In this regard, chitosan nanoparticle system prepared were characterized with various physico chemical techniques. The antibacterial activity of the nanoparticle system was evaluated with minimum inhibitory concentration (MIC). The mode of action defining the membrane permeability detecting bacterial cell ruptures suggested improved action of the nanoparticle system against the pathogens. In order to obtain quantitative information on the complex growth of bacterial cells Metabolomics information on the bacterial colonies becomes a promising approach. The outcomes demonstrate the rapid changes in bacterial biomass identifying potential therapeutics for the control of infectious disease.

Funding Sources (if applicable):

Nil

73

Untargeted metabolomics and controlled progeny tests reveal that green ash (*Fraxinus pennsylvanica*) presents a multigenic metabolite-based resistance phenotype against the invasive emerald ash borer (*Agrilus planipennis*)

Robert Stanley[1], David Carey[2], Aletta Doran[2], Julia Wolf[2], Mary Mason[2], Jennifer Koch[2], A. Daniel Jones[3], Jeanne Romero-Severson[1].
[1]Department of Biological Sciences, University of Notre Dame, USA
[2]USDA Forest Service, Delaware Research Station, USA
[3]Department of Biochemistry and Molecular Biology, Michigan State University, USA
Rstanle3@nd.edu

Abstract

North American Forests are under threat by non native insects and diseases without a shared evolutionary history. Here we report that select native green ash (*Fraxinus pennsylvanica*) present a multigenic metabolite based resistance phenotype against the invasive Emerald Ash Borer (*Agrilus planipennis,* EAB) the most destructive and economically devastating forest tree insect in North American history. Using parents with known phenotypes, and 99 two year old progeny, we demonstrate that the progeny of high performing parents performed significantly better ($p < 0.01$) than those of low performing parents in a manner consistent with a multigenic trait. Using untargeted metabolomics, we can distinguish family pedigree and phenotype response to EAB in green ash progeny. We show that discriminating the larval kill phenotypes can be best achieved through multivariate discriminate analysis of the concentration of groups of metabolites, primarily phenylpropenoids and terpenoids. Based on these results, we postulate that the defensive mechanism is based on regulatory systems which enable individual trees to respond appropriately to EAB, providing support that many native species may have resistance potential against invasive species. Our study is the first to employ progeny tests combined with untargeted metabolomics to investigate the basis for defensive responses to EAB in North American *Fraxinus*.

Funding Sources (if applicable):

USDA APHIS

75

NMR-based metabolomics for olive oil cultivar classification: a comparison with standard targeted analysis of fatty acids and triglycerides.

Fenfen Tang[1], Juan J. Polari[2], Hilary S. Green[2], Selina C. Wang[2,3], Emmanuel Hatzakis[1,4]

[1]Department of Food Science and Technology, The Ohio State University, USA
[2]Department of Food Science and Technology, University of California Davis, USA
[3]Olive Center, University of California Davis, USA
[4]Foods for Health Discovery Theme, The Ohio State University, USA
tang.1263@osu.edu

Abstract

Chromatographic techniques such as GC and HPLC are traditionally used for the analysis and classification of olive oil, while NMR is another powerful technique. In this study we used NMR for the first time to classify olive oil produced by four cultivars, namely 'Arbequina', 'Arbosana', 'Koroneiki', and 'Sikitita' planted in super-high-density frames in California, United States; we compared the results with those obtained by GC-FID and UHPLC-CAD. All three techniques were able to distinguish between cultivars, with GC-FID generated the best models. GC-FID and UHPLC-CAD analysis are time-consuming and require different sample preparation and protocols for different classes of compounds, while NMR is rapid and able to conduct a simultaneous broad-spectrum analysis of compounds, which led to the discovery of more biomarkers. In addition, NMR was in a good agreement with GC-FID for the determination of fatty acids, as found by Bland-Altman analysis, although in several cases it is not able to distinguish among individual fatty acids.

Funding Sources (if applicable):

76

Understanding the role of the substrate on laser desorption/ionization and primary ion beam analysis of lipids through molecular modeling

Michael J. Taylor[1], Will Kew[1], Amity Andersen[1], Christopher R. Anderton[1]

[1]Environmental Molecular Sciences Laboratory and Biological Sciences Division, Pacific Northwest National Laboratory, USA
Michael.Taylor@pnnl.gov

Abstract

Laser and ion beams are routinely employed to desorb and ionize molecules from surfaces, especially in mass spectrometry based spatial metabolomics analyses. Many factors play a role in beam-based ionization of molecules, and a significant contributor to the mechanism of desorption/ionization is the environment surrounding a molecule (i.e., 'matrix'). To gain a mechanistic understanding of how matrix changes influence biomolecule desorption/ionization at surfaces, we used Molecular Dynamics (MD) simulations to model the interactions of N-palmitoylsphingomyelin (SM 34:1) on gold and mica surfaces and combined this information with beam ionization analysis empirical data. From our MD approach, we observed a preference for surface adsorption of the phosphocholine headgroup on both substrates, which was consistent with the theory of a strong charge-based surface interaction of the polar headgroup. Simulations showed that increasing the number of molecules on the surface revealed changes in lipid orientation, where increased surface density resulted in more molecules interacting via the terminal trimethylamine headgroup compared to whole headgroup. Corresponding beam analysis identified distinct spectral differences between the two surfaces. Secondary ion mass spectrometry (IONTOF V) analysis of SM 34:1 on gold produced abundant high m/z fragments [M - CH_3, M - N-$(CH_3)_3$, M – $C_2H_4N(CH_3)_3$] combined with low m/z fragments. None of these species were observed on mica, only low m/z fragments, with the most abundant corresponding to the fragmented phosphocholine headgroup ($C_5H_{17}PNO_4^+$, $C_5H_{15}PNO_4^+$, $C_5H_{14}NO^+$). This finding presents evidence of substrate dependent changes in in-source fragmentation. Laser desorption/ionization (15 Tesla FTICR-MS, Bruker Daltonics) measurements on gold produced cation adducts (M + Na, M + K), in combination with the fragmented headgroup ($C_5H_{15}PNO_4^+$) as major species. However, the mica surface suppressed lipid desorption/ionization, preventing detection of phosphocholine head group, ceramide tail group, or any cation adducts by LDI.

Funding Sources (if applicable):

77

Characterization of Zwitterionic HILIC Columns Based on Hybrid Particles

Jonathan E. Turner*, Bonnie A. Alden, Kenneth Berthelette, Jessica A. Field, Nicole L. Lawrence, Stephen Shiner, Thomas H. Walter
Chemistry Operations, Waters Corporation, USA

Abstract

Hydrophilic Interaction Chromatography (HILIC) is one of the most effective approaches for separating mixtures of polar metabolites. Of the stationary phases used for HILIC, zwitterionic chemistries have been among the most popular due to their good retention for a wide range of polar compounds, including neutrals, anions and cations. We have developed a zwitterionic stationary phase based on 95 Å ethylene-bridged hybrid (BEH) particles, with particle sizes ranging from 1.7 to 5 µm. The stability of columns packed with this stationary phase to both acidic and basic mobile phases has been assessed using accelerated tests, with the results demonstrating good stability from pH 2 - 10. We have also characterized the retention and selectivity of columns packed with this new stationary phase and compared the results to those for several existing zwitterionic columns. The new stationary phase exhibits excellent retention for a wide range of compounds. An investigation of batch-to-batch reproducibility was also carried out, and the results show reproducibility comparable to that of reversed-phase materials based on BEH particles. Using column hardware with inert surfaces, columns packed with the new stationary phase were found to give symmetrical peaks, even for analytes known to interact with metal surfaces. The columns are compatible with mass spectrometry detection, exhibiting minimal column bleed. Applications of the columns for separating several important classes of polar analytes will be shown.

Funding Sources (if applicable):

78

Extending the MassBank.us natural product reference mass spectral library with more than 4,400 new reference standards

Arpana Vaniya[1], Uri Keshet[1], Jie Tang[1], Briana Le[1], Eesha Gupte[1], Oliver Fiehn[1]

[1]University of California Davis, West Coast Metabolomics Center, USA
avaniya@ucdavis.edu

Abstract

In metabolomics, matching experimental MS/MS spectra with reference spectra from mass spectral libraries is the gold standard in annotating or identifying a compound. To date, there are many highly curated open-source ESI-MS libraries, such as MassBank of North America (MoNA) or mzCloud, plus licensed libraries like NIST20. However, natural products are still a small fraction of reference spectra that are freely available. Here, we extended the MassBank.us Natural Products Library with more than 4,400 new reference standards. We expanded the library with MS/MS spectra from three different collision energies, different adduct types in both positive and negative modes, and collected retention time information on a Phenomenex Kinetex PFP column. Previously, we have acquired data for more than 2,500 standards and published these at MassBank.us. The library has been extended with more than 4,400 new reference standards. There are 222 unique compound classes of these standards for example carboxylic acids and derivatives, organooxygen compounds, fatty acyls, prenol lipids, and steroids and steroid derivatives, with the largest contributing class being benzenoids with more than 600 compounds. Data were acquired for molecular adducts, such as protonated, sodiated, ammoniated in positive mode and deprotonated, formiated, and chlorinated in negative mode. This study design yielded more than 70,000 spectra of authentic natural product chemicals, adding to the already existing library containing more than 44,000 MS/MS spectra from Q Exactive HF, Agilent Q-TOF, and linear ion trap mass spectrometers. Importantly, this MassBank.us natural products library also contains retention times to be used for machine learning to predict novel compounds or to improve confidence in compound identification. Our existing PFP polyphenol retention time library contains more than 1,540 metabolites with MS/MS spectra in positive mode and 1,327 metabolites with MS/MS spectra in negative mode. Spectra and retention times are accessible and freely downloadable in MassBank.us.

Funding Sources (if applicable):

79

Effect of diet and probiotics on plasma oxylipin concentrations during the development of atherosclerosis in ApoE-knockout mice

Dajana Vuckovic[1], Lise Cougnaud[1], Antoine St-Amant[2], Andreas Bergdahl[2]

[1]Department of Chemistry and Biochemistry, Concordia University, CA
[2]Department of Health, Kinesiology and Applied Physiology, Concordia University, CA
Dajana.vuckovic@concordia.ca

Abstract

Low carbohydrate – high protein diets (LCHP) have recently been shown to have adverse cardiovascular effects in both animal models and human studies. In ApoE-knockout mice, LCHP diet promotes the increased formation of atherosclerotic plaques in comparison to both control and western-type diets. The differences in LCHP and Western diet are not cholesterol-driven, and the mechanism of increased atherogenicity of LCHP diets still remains unknown. Our previous research implicated PUFA-containing lipids as one of the key differences between LCHP and Western diets in both plasma and aortic tissue. The objective of current study was to examine PUFAs and related oxylipin mediator pathways in response to diet and modulation using anti-inflammatory probiotics and to assess whether probiotic administration can reduce the atherogenicity of LCHP diet. The study was carried out for 6 weeks in male ApoE-knockout mice (n=12 per group) receiving control, Western or LCHP diet with or without administration of low or high dose of selected probiotic. Blood samples were collected using tail bleed at 0, 1, 3 and 5 weeks and the resulting plasma was analyzed using targeted oxylipin method which combined C18 solid-phase extraction and LC-HRMS profiling. The method can successfully quantitate up to 73 oxylipins from 15 uL of plasma. Several oxylipins in linoleic acid pathway showed significant changes between control diet and LCHP/Western diets including 9-HODE and 13-HODE both of which have previously been implicated in plaque development. Arachidonic acid-derived oxylipins did not show significant changes during the early time points. However, full oxylipin profiles from the 1-week time point could already confidently distinguish control and other diet groups, before any visible signs of atherosclerotic plaque formation. In conclusion, our results provide an invaluable and detailed map of how oxylipin pathways change with time, diet and probiotic supplementation during the early stages of the development of atherosclerosis.

Funding Sources (if applicable):

MITACS

80

Synthesis of deoxynivalenol metabolites for use as analytical standards

Jacob P. Walsh[1,2], Ken K.-C. Yeung[2,3], Mark W. Sumarah[1,2]

[1]London Research and Development Center, Agriculture and Agri-food Canada, CA

[2]Department of Chemistry, University of Western Ontario, CA

[3] Department of Biochemistry, University of Western Ontario, CA

jwalsh69@uwo.ca

Abstract

Fusarium graminearum is fungal species capable of infecting maize and wheat throughout the world; it is responsible for producing a few known mycotoxins, including a particular toxin class of concern is deoxynivalenol (DON). DON is commonly detected as 3-acetyldeoxynivalenol (3-ADON) and 15-acetyldeoxynivalenol (15-ADON) in grain and maize, and it has been linked to health concerns in mammalian systems such as diarrhea, vomiting, gastro-intestinal inflation, and intestinal necrosis. As such its detection and consequent monitoring is of growing importance. DON can also exist in other conjugated forms, which are produced as part of the host plants defense mechanism, namely 15-ADON-3-glucoside, and DON-3-glucoside. These conjugated products are difficult to monitor for and can lead to an underreporting of the mycotoxin exposure in maize and wheat products. After consumption of DON, mammalian metabolism converts it to the less toxic DON-3-glucuronide, which is the key biomarker of DON exposure. Mass spectrometry is an effective tool to quantity these DON derivatives; however, chemical standards of these compounds are not commercially available, especially those labelled with stable isotopes. Our group therefore pursued the synthesis of both labelled and unlabelled versions of 15-ADON3G and DON3-glucuronide to facilitate the mass spectral analysis of DON in crops and for human exposure studies. We used recombinant enzyme catalysed semi-synthesis and a modified könings-knorr reaction to produce the glucoside and glucuronide standards respectively. DON-glucuronide standards were produced and validated for the liquid chromatography high-resolution mass spectrometry monitoring of DON exposure in a cohort of Rwandan women. The recombinant enzyme Os79 derived from *oryza sativa* was used to conjugate DON and 15ADON to produce DON-3-glucoside and 15ADON-3-glucoside. These conjugated standards will allow for the accurate quantification of total DON concentrations in crops and exposures in human populations.

Funding Sources (if applicable):

82

Quantifying amine metabolites in single cells by integrating isobaric labeling, nanoPOTS sample preparation, and LC-MS

Sarah M Williams[1], Aivett Bilbao Pena[1], Jongmin Woo[1], Chia-Feng Tsai[1], Isaac K Attah[1], Daniel J Orton[1], Ronald J Moore[1], Ljiljana Pasa-Tolic[1], Richard D Smith[1], Ying Zhu[1]

[1]Environmental Molecular Sciences Laboratory, Pacific Northwest National Laboratory, USA
sarah.williams@pnnl.gov

Abstract

Single-cell metabolomics could provide insight into functional heterogeneity of different cell populations in response to external perturbation. Amine-containing metabolites are a large and important group of molecules that play critical roles in physiological and pathological environments. However, the measurements of these nucleophilic amines with mass spectrometry are a challenge due to low retention in RPLC columns and poor ionization efficiency. Here, we presented an integrated solution to allow sensitive quantification of amine metabolites in single cells. We employed our recently developed nanoPOTS platform to isolate single cells, and perform cell lysis and metabolite extraction. To increase the LC retention and ionization efficiency, we labeled the released amines with tandem mass tags (TMT), followed by LC-MS analysis.

We used Influx II cell sorter to deposit single A549 cells into nanoPOTS chip. Intracellular metabolites were extracted from cells with a solution containing 0.1% (w/v) DDM, 0.5× PBS, and 50 mM TEAB. Next, TMT10 plex was used to label the released amine-containing metabolites. After reaction quench and acidification, the nanoPOTS chip were loaded on a home-built autosampler to directly inject sample into a nanoflow LC system (i.d. 50 µm) operated at 100 nL/min. A Lumos Orbitrap MS operated at data dependent or data-independent mode was used to collect data. LC-MS data were analyzed using the MS-DIAL, Skyline and Freestyle. MS2 peaks from the DIA data were using MONA public database, with a tolerance of 5 ppm.

We first employ amino acid standards to evaluate the performance of TMT labeling on amine identification and quantification. Without TMT labeling, we were unable to confidently assign all the amines to detected LC-MS features. This is likely due to the hydrophilic nature of amino acids, which leads to low retention during LC separation and poor ionization efficiency in the nanoelectrospray. For the TMT-labeled amino acid standards, we were able to identify all the 20 amino acids with the peak intensities in proportion to the concentrations. In a sensitivity evaluation with an Exactive MS, we were able to identify the amino acid standards with input amounts as low as 1 fmol. The TMT labeling workflow also allows identifying isobaric molecules, such as L-isoleucine and L-leucine, which could be separated by LC and fragmented to generate distinguishable MS/MS spectra. Therefore, by labeling amine with TMT, we can confidently identify and quantify low amount of amine metabolites based LC retention time, predicted accurate mass, and HCD fragments.

Next, we applied the established workflow to analyze FACS-sorted single A549 cells. Using amino acid standards, we can assign an average of 19 amino acids in single A549 cells. To validate our result, we also analyze pools of 50 A549 cells, which showed an increase in peak intensities for these identified amine metabolites. To access the reproducibility, we performed pair-wise correlation between all the identified amino acids. The median of Pearson correlation coefficient is 0.98 across the 20 single cells. The median of amine coefficient of variation (CV) is 35%, indicating these amino metabolites are stable in single cells. The low CV and high correlation coefficient validate the high reproducibility of the developed workflow.

The coupling of isobaric labeling with nanoPOTS can allow to precisely quantify amine-containing metabolites in single cells with high sensitivity

Funding Sources (if applicable):

85

Global effects of arbuscular mycorrhizal and ectomycorrhizal associations on root metabolomes feature alterations on carbon partitioning and flavonoid profiles

Mengxue Xia[1], Vidya Suseela[1], M. Luke McCormack[2], Peter Kennedy[3], Nishanth Tharayil[1]

[1]Clemson University, USA
[2]The Morton Arboretum, USA
[3]University of Minnesota, USA
mxia@clemson.edu

Abstract

- The broad type of mycorrhizal associations, *i.e.,* Arbuscular mycorrhizal (AM) *vs.* ectomycorrhizal fungi (EM), has been increasingly recognized as a key functional trait to generalize mycorrhizal-associated plant and ecosystem functioning. However, comparison between the effects of these two mycorrhizal types on tissue metabolites that underpin plant functions is still lacking. Moreover, our current knowledge on mycorrhizal-associated chemical alterations is largely based on studies that focused on a small subset of metabolites. Such limited range of responses may not necessarily reflect major chemical events involved in root-mycorrhizal interactions, limiting our ability to establish a comprehensive understanding of mycorrhizal symbiosis.
- Here, we examined root metabolomes in eight plant-fungus combinations across AM and EM associations. We characterized the impacts of AM and EM associations with a broad coverage of metabolites by combining untargeted metabolomics, GNPS and a multiple-level *in silico* chemical classification.
- Both AM and EM induced significant alterations in root metabolomes, while AM exhibited a stronger perturbation. Although the responses in soluble sugars were variable depending on plant-fungus combinations, we observed a more consistent shift on sugar alcohols, where sugar alcohols tended to accumulate in colonized roots, but in a manner specific to AM *vs.* EM, indicating unique carbon partitioning strategies. Symbiosis also induced extensive modification on secondary metabolites, featuring a sharp reconfiguration of flavonoids; flavan-3-ols tended to increase by symbiosis in most plant-fungus combinations across AM and EM, highlighting the essential role of this chemical family in root-mycorrhizal interactions.
- Using a metabolome-level approach, this study uncovered novel chemical processes that were frequently associated with mycorrhizal symbiosis, with implication for carbon partitioning and tissue protection unique or common to AM and EM associations.

Funding Sources (if applicable):

DEB 1754679

86

Response of metabolic and lipid synthesis gene expression changes in *Camellia oleifera* to mulched ecological mat under drought conditions

Honglian Ye[1,2], Jacob Folz [2], Chao Li [1], Ying Zhang [2], Zhixia Hou [1], Lingyun Zhang [1], Shuchai Su [1] *

[1]Key Laboratory for Silviculture and Conservation, Ministry of Education, Beijing Forestry University, PR CN
[2]West Coast Metabolomics Center, University of California-Davis, USA

Abstract

Plants respond to adverse conditions by activating defense mechanisms that alter metabolism and impact agricultural crop yield. Organic mulching of *Camellia olei*fera leads to increased oil yield compared to control. In this study, multi-platform untargeted metabolomics and qRT-PCR were used to measure the effects of organic mulching on seed kernel metabolism. Metabolomics analysis revealed that tyrosine, tryptophan, and several flavonoids and polyphenol metabolites were significantly lower in the mulched treatment compared to the control, indicating lower stress levels with mulching. The qRT-PCR analysis showed that EAR, SAD, and CoHCD were up-regulated by mulching, while CT, FAD7, FAD8, CoATS1, SQS, SQE, FATB, and β-AS were down-regulated. Correlation network analysis was used to integrate data from this multi-omics investigation to analyze the relationships between differentially expressed genes, metabolites, and fruit and soil indicators concerning mulch treatment of *C. oleifera*.

Funding Sources (if applicable):

This work was supported by National Key Research and Development Program [Grant No. 2019YFD1002401].

87

An evaluation of the National Institutes of Health Grants Portfolio: identifying opportunities and challenges for multi-omics research that leverage metabolomics data

Catherine T. Yu[1], Brittany N. Chao[2], Rolando Barajas[1], Majda Haznadar[3], Padma Maruvada[4], Holly L. Nicastro[5], Sharon A. Ross[6], Mukesh Verma[1], Scott Rogers[1], Krista A. Zanetti[1]

[1]Division of Cancer Control and Population Sciences, National Cancer Institute, USA
[2]Office of Workforce Planning and Development, National Cancer Institute, USA
[3]Laboratory of Human Carcinogenesis, Center for Cancer Research, National Cancer Institute, USA
[4]Division of Digestive Diseases and Nutrition, National Institute of Diabetes and Digestive and Kidney Diseases, USA
[5]Office of Nutrition Research, National Institutes of Health, USA
[6]Division of Cancer Prevention, National Cancer Institute, USA
Catherine.yu@nih.gov

Abstract – **Maximum 300 words**

Through the systematic profiling of metabolites, metabolomics provides a highly informative biochemical profile of diseases and other physiological endpoints. Integration of metabolomics data with other -omics data through multi-omics analyses has the potential to advance understanding of human disease development and treatment. To understand the current funding and potential research opportunities for when metabolomics is used in human multi-omics studies, we cross-sectionally evaluated National Institutes of Health (NIH)-funded grants to examine the use of metabolomics when collected with at least one other -omics type. First, we aimed to determine what types of multi-omics studies included metabolomics data collections. Then, we looked at those multi-omics studies to examine how often grants employed an integrative analysis approach using metabolomics data. The abstracts and specific aims of all grants were examined for grant mechanism, disease phenotype, biospecimen type, -omics data types collected, and whether metabolomics data were integrated with other -omics data types, including genomics, epigenomics, transcriptomics, proteomics, and microbiomics. We identified 330 relevant grants to include in the portfolio analysis, 68% of which were Research Grants. Majority of grants focused on diabetes and other metabolic diseases (16%), cancer (14%), cardiovascular disease (12%), and child and human development (10%); while the most commonly used biospecimen type varied by -omics technology employed. Only 121 (37%) grants that met our inclusion criteria integrated metabolomics data with other -omics data. The majority integrated metabolomics data with only one other -omics data type, more frequently pairing metabolomics with genomics data. In grants where data was integrated, the primary outcomes studied were diabetes and other metabolic disease, cardiovascular disease, child and human development, and lung disease. We observed that multi-omics studies in the NIH grants portfolio have limited data integration with metabolomics data. Some opportunities to improve data integration may include addressing variability between -omics approach requirements and -omics data incompatibility.

Funding Sources (if applicable):

89

Lipid isomer separation and C=C double bond localization using ozone-induced dissociation and ion mobility spectrometry

Joon-Yong Lee[1], Aivett Bilbao[1], Xueyun Zheng[1]

[1]Biological Sciences Division, Pacific Northwest National Laboratory, USA
xueyun.zheng@pnnl.gov

Abstract – **Maximum 300 words**

Lipids play essential roles in many biological processes and disease pathology, yet it is still challenging to unambiguously identify identify lipids and distinguish numerous isomeric species which can result from different fatty acyl chain lengths, fatty acyl positions and carbon-carbon (C=C) double bond orientations and locations. Therefore, developing a lipidomics workflow that enables comprehensive isomer separation and structural elucidation is of significance for confident identification of lipids in complex samples and for better understanding the roles of lipids in biological processes and disease pathology. In this work, we demonstrate a comprehensive workflow integrating ozone-induced dissociation and ion mobility spectrometry-mass spectrometry (OzID-IMS-MS) approaches and robust bioinformatics tools developed to identify lipid double bond locations and distinguish isomers. Novel in-house bioinformatics tools are developed to analyze OzID data and assign double bond positions. In specific, we have developed a Python-based tool to effectively extract the chromatographic and IMS information for specific lipid targets. The OzID data of a lipid standard mixture has been tested and the results showed that both the precursor ion and the OzID fragment ions of each lipid were accurately identified with matched retention times. For complex sample, a target list of lipids was created from the LC-MS/MS data without OzID. Scores for OzID spectra arising from each possible double bond location were calculated and used to screen for unsaturated lipids. A machine learning model is being developed to predict OzID fragments and identify double bond locations using these true identifications of double bonds as training sets. A library of simulated OzID fragment spectra is also being developed to increase the size of the training sets for machine learning. These tools will be integrated to allow robust analysis for complex lipidomic data, and our progress will be presented.

Funding Sources (If applicable):

The research described in this presentation is part of the *m/q* Initiative at Pacific Northwest National Laboratory. It was conducted under the Laboratory Directed Research and Development Program at PNNL, a multiprogram national laboratory operated by Battelle for the U.S. Department of Energy.

91

Bacille Calmette-Guérin vaccine reprograms human neonatal lipid metabolism *in vitro* and *in vivo*

Joann Diray-Arce[1,2,*], Kristoffer Jarlov Jensen[3,4,5], Asimenia Angelidou[1,2,6], Maria Giulia Conti[1,7], Rachel S. Kelly[8], Matthew A. Pettengill[1,9], Mark Liu[1], Simon D. van Haren[1,2], Scott McCulloch[10], Greg Michelloti[10], the EPIC Consortium[#], Tobias Kollmann[11], Beate Kampmann[12], Hanno Steen[1,2,13], Al Ozonoff[1,2], Jessica Lasky- Su[8], Christine Stabell Benn[3,4,14], Ofer Levy[1,2,15,*]

[1]Precision Vaccines Program, Division of Infectious Diseases, Boston Children's Hospital, USA
[2]Department of Pediatrics, Harvard Medical School, USA
[3]Research Center for Vitamins and Vaccines (CVIVA), Bandim Health Project, University of Southern Denmark, DK
[4] Bandim Health Project, IN-DEPTH Network, 1004, Bissau, GW
[5]Experimental and Translational Immunology, Department of Health Technology, Technical University of Denmark, DK
[6]Division of Newborn Medicine, Boston Children's Hospital, USA
[7]Department of Maternal and Child Health, Sapienza University of Rome, IT
[8]Channing Division of Network Medicine, Brigham and Women's Hospital and Harvard Medical School, USA
[9]Department of Pathology, Anatomy and Cell Biology, Thomas Jefferson University, USA
[10]Metabolon Inc. Morrisville, NC, USA
[11]Telethon Kids Institute, University of Western Australia, AU
[12]The Vaccine Centre, Faculty of Infectious and Tropical Diseases, London School of Hygiene and Tropical Medicine, UK
[13]Department of Pathology, Boston Children's Hospital, USA
[14]OPEN, Institute of Clinical Research, University of Southern Denmark, DK
[15]Broad Institute of MIT & Harvard, USA
[#]The Expanded Program on Immunization (EPIC)
joann.arce@childrens.harvard.edu

Abstract

Vaccines have generally been developed with limited insight into their molecular impact. While systems vaccinology, including metabolomics, enables fresh characterization of vaccine action, these tools have yet to be applied to infants, who are at high risk of infection and receive the most vaccines. Bacille Calmette-Guérin (BCG) protects infants against disseminated tuberculosis (TB) as well as TB-unrelated infections via mechanisms that are incompletely understood. We employed mass spectrometry-based metabolomics of blood plasma to profile BCG-induced infant responses in Guinea Bissau *in vivo* and in the U.S. *in vitro*. BCG selectively altered plasma lipid pathways, including lysophospholipids *in vivo* and *in vitro.* BCG-induced lysophosphatidylcholines (LPCs) correlated with both TLR agonist- and mycobacterial antigen (PPD)-induced blood cytokine production *in vitro*, raising the possibility that LPCs contribute to BCG immunogenicity. Analysis of an independent newborn cohort from The Gambia, demonstrated shared significant vaccine-induced metabolites such as phospholipids and sphingolipids. BCG-induced changes to the plasma lipidome, and in particular to LPCs, may contribute to its immunogenicity and inform discovery and development of early life vaccines.

Funding Sources (if applicable):

92

The impact of placebo or multivitamin supplementation on the plasma metabolome in ADHD youth

Djawed Bennouna[1], Madeline Stern[1,2], Leanna Perez[1], Haley Chatelaine[1,3], Fawn Yan[1], Jeanette Johnstone[4,5], Barbara Gracious[6], Brenda Leung[7], Gabriella Tost[5], Eugene Arnold[8], Irene Hatsu[1], Rachel E. Kopec[1,9]*

[1]Department of Human Sciences, Human Nutrition Program, The Ohio State University, USA
[2]Nationwide Children's Hospital, USA
[3]National Center for Advanced Translational Sciences, National Institute of Health, USA
[4]National University of Natural Medicine (NUNM), Helfgott Research Institute, USA
[5]Department of Child & Adolescent Psychiatry, Oregon Health & Science University, USA
[6]Orange Park Medical Center, USA
[7]Faculty of Health Sciences, University of Lethbridge, CA
[8]Department of Psychiatry & Behavioral Health, Ohio State University, USA
[9]Foods for Health Discovery Theme, The Ohio State University, USA
bennouna.1@osu.edu

Abstract

Attention-deficit/hyperactivity disorder (ADHD) is a neuropsychiatric condition commonly diagnosed in children, and characterized by hyperactivity, impulsivity and inattention. Because a range of nutrient deficiencies have been linked to altered brain development and functioning, multivitamin use has been considered as a potential treatment to ameliorate ADHD symptoms in children. However, the short-term effects on nutrient status and the underlying metabolome interactions have not been characterized. Blood samples were collected from medication-free children with ADHD (n = 76) enrolled in a double–blind randomized placebo-controlled multivitamin trial at baseline and 8 weeks post-multivitamin intervention. Polar extracts were analyzed using targeted LC-MS metabolomics with a triple-quadrupole mass spectrometer (Thermo Quantiva), with multiple reaction monitoring, to quantitate tyrosine, phenylalanine and tryptophan. Polar and lipophilic extracts were also analyzed using untargeted LC-MS metabolomics, with separation using a HILIC, C8 and C18 column and detection with a quadrupole time-of-flight mass spectrometer (Agilent 6545). Following data cleanup and normalization, a two-sample t-test was used to compare 8 week placebo vs. multivitamin plasma extracts. No significant differences in the plasma concentrations of tyrosine, phenylalanine, or tryptophan were observed. Preliminary results from the C8 lipidomic analyses revealed 26 metabolites which were significantly different between the 8 week placebo vs. multivitamin plasma extracts. Metabolites of difference included phosphatidylcholines, sterols, and phosphatidylethanolamines. Fluctuations in the concentration of these metabolic pathways have not been previously associated with ADHD, and merit further investigation. Continuing analyses include the identification of significantly different metabolites following the C18 and HILIC analyses, as well as the utilization of mixed models to elucidate the relative influence of multivitamin treatment and measures of anxiety, depression, and inattention on plasma metabolite concentration.

Funding Sources (if applicable):

The human study was made possible by a grant from the Foundation for the Center of Excellence in Mental Health, Canada; The Ohio State University Department of Human Sciences, College of Education and Human Ecology; The Ohio State University Wexner Medical Center, Clinical Research Center. The sample analyses were partially supported by an NIH Grant P30 CA016058, OSU, and OSUCCC.

93

Metabolomic dysregulation in myalgic encephalomyelitis/chronic fatigue syndrome: a multi-study Bayesian analysis

Christopher Brydges[1], Oliver Fiehn[1]

[1]NIH West Coast Metabolomics Center, University of California Davis, USA
crbrydges@ucdavis.edu

Abstract

Myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS) is a debilitating disease that is characterized by unexplained physical fatigue that is unrelieved by rest, and its pathogenesis is poorly understood. Symptoms also frequently include gastrointestinal problems, orthostatic intolerance, sleep disturbances, and cognitive and sensory dysfunctions. Previous research has suggested a decrease in phospholipids and sphingomyelins, and an increase in triglycerides. However, these studies exclusively used traditional frequentist statistics, which are limited in that findings from previous studies cannot be incorporated into the analyses of further studies. The current study addressed this shortcoming by conducting Bayesian analyses. Three existing ME/CFS untargeted metabolomics datasets were used. 663 compounds were observed in the first two datasets, and 95 metabolites were found to be common to all three datasets. For the first dataset, a weakly informative prior distribution was used to model the effects of ME/CFS on each compound. The posterior distributions of each compound in the first and second datasets were used as the prior distributions of the second and third data sets, respectively. Bayesian parameter estimates were used as estimates of the true effect size, and compounds were considered to be altered if 95% credible intervals did not overlap with zero. Results after the second dataset showed that plasmalogens were downregulated in ME/CFS patients, although unsaturated phosphatidylethanolamines were unaffected. Several drug compounds were upregulated in the ME/CFS group, while food exposomes were downregulated. With the third dataset, vitamins B4 and B5, and eicosapentaenoic acid were upgregulated, while cholesterol was downregulated. Bayesian statistics allow researchers to incorporate knowledge from previous research into their own studies through the use of informed prior distributions. By allowing for results from previous research to be incorporated into a study, researchers can add to knowledge rather than having to assume that no effect exists and having to demonstrate otherwise.

Funding Sources (if applicable):

NIH U2C ES030158

94

Using KBase and PickAxe to build a metabolic model and predict novel metabolic pathways in Bacillus subtilis

Daniel Cardenas[1], Sara Greenfield[1], Jacob Folz[1] , Christopher Henry[2], Oliver Fiehn[1]

[1]University of California Davis, West Coast Metabolomics Center, USA
dancard@ucdavis.edu

Abstract

Introduction: Metabolic models are mathematical constructs to understand how metabolic pathways are integrated within organisms. Such models are usually built by subsequent drafts of genomic evidence for pathways, gap-filling to add missing enzymes within and between pathways, and experimental data in labor intensive, slow ways. Metabolic models are useful to understand the pathway constraints for flux balance analysis, utilization and dependency for external nutrients, growth restrictions caused by potential gene dysfunctions, and for discovering new pathways. Yet, metabolic models are rarely used in metabolomics. We here showcase how the DoE-funded KBase ('knowledgebase') tool environment can be used to build metabolic networks to predict novel pathways in Bacillus subtilis.

Methods: All software tools within KBase are interoperable in a seamless way, opening new ways to assemble information into studies called 'narratives'. Metabolic modeling in KBase starts by importing a genome. We used the Bacillus subtilis genome from NCBI and annotated genes by using the RASTtk annotation tool within KBase, ran the metabolic modeling tool, gap-filled missing enzymes and achieved a genome-scale metabolic network. To predict novel pathways, we added a metabolomics dataset into the new KBase tool 'PickAxe' and applied both spontaneous and enzymatic reaction rules.

Preliminary Data: Previous E. coli models in KBase predicted a total of 1582 reactions and 1466 compounds. Gap-filling did not change the total number of identified reactions or compounds in our model, but it is viewed as an integral part of the greater workflow nonetheless. After following the aforementioned workflow, we observe a total of 163 pathways which can be viewed as a list or individual diagrams.

Novel Aspect: Using KBase, we can both model and predict metabolism within an organism using an extremely streamlined and optimized workflow

Funding Sources (if applicable):

National Science Foundation MCB 1611846 ; National Institutes of Health U19 AG023122

95

A multi-modal metabolomics platform in zebrafish reveals organ-specific drug effects

Casey A. Chamberlain[1], Madelyn M. Jackstadt[1], Steven R. Doonan[1], Gary J. Patti[1,2]

[1]Department of Chemistry, Washington University in St. Louis, USA
[2]Department of Medicine, Washington University in St. Louis, USA
chamberlain.c@wustl.edu

Abstract

Characterizing the pharmacology of drugs is critical to understanding the scope of their biochemical effects, intended or otherwise, on human health. Two major questions to be answered in such studies are: 1) where does the drug accumulate, and 2) what metabolic alterations (off-target effects) are induced by the drug? Oftentimes, drugs exhibit organ-specific pharmacodynamics, whether due to different accessibility to certain tissues (determining accumulation), or to organ-specific properties influencing biochemical response. To investigate these fundamental, yet elusive, pharmacological questions, we developed a multi-dimensional metabolomics workflow for drug analysis in adult zebrafish, a model organism which has emerged as a powerful tool due to key advantages over other systems. Our integrated platform, demonstrated using hydroxychloroquine (HCQ) sulfate as a representative pharmaceutical, combines four unique metabolomics technologies — absolute quantitation, MS imaging, unbiased profiling, and isotope tracing — into a comprehensive assessment of drug movement and behavior *in vivo*. Absolute quantitation, shown in the context of our novel matrix-matched approach using a pooled organ extract calibrator matrix for streamlined, multi-organ quantitation, revealed tissue-specific pharmacokinetics which were corroborated and visualized using DESI-MS imaging. Untargeted profiling was performed on all tissues to generate a pharmacodynamic panel of organ-specific off-target effects, which revealed increased N-acetylaspartate (NAA) pools in the brain. Isotope tracing revealed increased M+3 labeling in NAA, suggesting increased pyruvate carboxylase (PC) activity behind elevated NAA biosynthesis. Moreover, M+3 labeling was found to be increased in TCA cycle metabolites and amino acids in the brain and eye, but not other organs, suggesting that HCQ induces organ-specific PC stimulation. While the biological implications of this enzymatic stimulation require further investigation, this finding represents the type of unique biochemical insight that can be revealed using our zebrafish metabolomics workflow. We believe this platform will serve the (zebrafish) metabolomics community by providing an integrated, multi-dimensional approach to pharmacological studies.

Funding Sources (if applicable):

NIH awards R35ES028365 (G.J.P.), U01CA235482 (G.J.P.), and R24OD024624 (G.J.P.).

96

Metabolomics on a Chip: Development of an Impedance-Based Metabolite Biosensor for Early Diagnosis of Colon Cancer

Ya-Chun Chan[1], Payton LeBlanc[2], Yeganeh Khaniani[3], Prashanthi Kovur[4], Scott MacKay[5], David Wishart[6]

[1]Department of Biological Sciences, Faculty of Science, University of Alberta, Canada
ychan@ualberta.ca

Colon cancer is the third most common cancer in both United States and Canada and. Colon cancer is also known as the silent killer as it is often diagnosed at a very late stage, at which point it is very aggressive and lethal. Yet, when diagnosed early, the survival rate is higher than 90% and can be readily treated. Therefore, early-stage diagnosis is necessary to reduce the mortality rate of colon cancer! The available screening tests right now expensive and uncomfortable contributing to a low compliance rate. Using MS-based metabolomics we have discovered a couple of urinary metabolite markers that are very effective in diagnosing early stage colon cancer. When combined with some simple-to-measure clinical features, the test is 90% accurate. To bring the assay into practice, something that is to be done in a doctor's office or at home, Aasmaller, chip-based, colorimetric device has been developed by our team and it was released just two months ago. This chip-based system uses microfluidics, optical sensors and enzymatically or chemically driven color changes to detect and quantify creatinine, diacetylspermine and Hippurate simultaneously.

Last year we started working on a smaller, quicker point-of-care biosensor that detects metabolites for early-stage colon cancer diagnosis based on electrical impedance spectroscopy. Rather than using enzymes or compound-specific reactions, this sensor device uses antibodies. This impedance-based system has a number of potential advantages in that it has increased sensitivity and specificity through the use of antibodies and it allows for greater degree of miniaturization and multiplexing.

As mentioned, we chose electrochemical impedance detection for our detection method. To maximize the sensitivity of the system we designed and developed a special set of interdigitated electrodes.

This hand-held, metabolomics-on-a-chip device utilizes specially prepared nanomaterials (gold nanoparticles and liposomes), which not only recognize the metabolite biomarkers but also enhance the sensitivity of detection. The metabolite biosensor, as it currently exists, consists of an impedance reader, a solution handling system, and a sensor chip with interdigitated electrodes that are functionalized with the antibodies for detecting the target metabolites. The principle of detection is based on using electrical impedance to measure the competitive binding between nanoparticle-metabolite conjugates and free metabolites found in urine. Because the free metabolites in the urine samples have higher binding affinity to the antibodies, the free metabolites will compete and remove the nanoparticle-metabolite conjugates. As a consequence, the impedance signal will change, which can be used to quantify metabolite levels. The use of signal-enhancing nanomaterials in our sensor design is vital to detect the specific metabolites at lower concentrations for accurate and sensitive diagnosis for colon cancer. Designs, preliminary results and a general assessment of this sensor system will be presented. We believe this work could open the door to measuring even more metabolites and making "metabolomics-on-a-chip" a reality.

In this presentation I will describe our progress on the development of a metabolomic-based test for colon cancer and the creation of two different metabolomics-on-a-chip systems.

We are now developing a fast, inexpensive point-of-care metabolomic device for detecting these three urinary metabolites.

Funding Sources (if applicable):

Nanomedicines Innovation Network

97

Lipidomics analysis elucidates postprandial metabolic excursions following milk protein ingestion in individuals with prediabetes

Li Chen[1,2], Rui Xu[1], Joshua D. McDonald[1], Richard S. Bruno[1], Shiqi Zhang[1], Fouad Choueiry[1], Jiangjiang Zhu[1,2]

[1] Human Nutrition Program, Department of Human Sciences, The Ohio State University, USA
[2] James Comprehensive Cancer Center, The Ohio State University, USA
chen.10289@osu.edu

Abstract

Dietary intervention is an effective approach to attenuate postprandial hyperglycemia (PPH) and lower cardiovascular disease (CVD) risk. Among many healthy choices, diary milk and its protein contents have been suggested to attenuate PPH-induced oxidative stress, however, how these protein contents alter the metabolic excursions in at risk population is unclear. Therefore, in this study, our objective is to examine the differential plasma lipidomic responses to whey or casein proteins in a group of twenty-three adults (17 males and 6 females) with prediabetes. In this randomized cross-over study, the participants consumed glucose alone or with dairy proteins (whey or casein proteins), and 207 plasma samples were collected at multiple time points post-consumption (0, 90 and 180 mins) for an untargeted lipidomics analysis. The UPLC-HRMS analysis was performed to identify a total of 764 lipids from 7 lipid categories in all samples. Linear and logistic regression models were used to assess the lipidomes and their relationships to other health outcomes after adjusted for BMI, age and gender. Our analyses revealed that milk proteins could significantly modulate the lipid profiles to potentially reduce the CVD risks of the participants with prediabetes, and casein protein have a quicker and different impact to the lipidome after ingestion than whey protein. Additionally, multiple significant correlations between lipidomics-based biomarkers and participant characteristics were identified. For instance, PE (36:1), a lipid that was associated with increased risk of diabetes, was significant decrease in our study and positively correlated with both the fasting plasma insulin and HOMA-IR in the casein group at 180 min. Collectively, our study suggested that milk proteins could protect against lipid changes induced by PPH and mediate CVD risks, and different milk proteins (whey vs. casein) could impact the lipid metabolism from different pathways in adults with prediabetes.

Funding Sources (if applicable):

The study was partially supported by the National Institute of General Medical Sciences of the National Institutes of Health under Award Number R35GM133510 (to JZ), and by the National Dairy Council (to RSB).

98

Trace cloud: an online resource for detecting low-intensity signal peaks in CE/LC/GC/-MS data using neural networks

Jerry Chen[1], Aleksandr Smirnov[1], Eric Sun[1], Jie Li[2], Peter Nemes[2*], and Xiuxia Du[1*]

[1]Department of bioinformatics and genomics, University of North Carolina at Charlotte, USA
[2]Department of Chemistry and Biochemistry, University of Maryland, USA
jerry.w.chen.official@gmail.com

Abstract
A key challenge in ultrasensitive high-resolution mass spectrometry (HRMS)-based biological research is analyzing vast amounts (terabytes) of the resulting data that contains signals from compounds of trace-level concentrations. This limitation has been bioinformatical in nature. Current commercial and open-source software packages anticipate high signal abundance and Gaussian chromatographic peak shapes, but neither of which necessarily applies to new-generation HRMS datasets. As a result, current peak finding algorithms return a substantial number of noises rather than true signals. To address this challenge, we developed Trace a few years ago, a desktop software tool that uses deep learning to distinguish true signals from false ones. Here, we develop Trace Cloud an online resource that enables users to use Trace with ease.

Trace takes in profiled or centroided HRMS data in mzML format. It first picks out candidate peaks from the centroided data. For each resulting peak, an image is then rendered in the space of separation time vs. m/z vs. abundance based on the profile data. Finally, it predicts which images are true peaks using a neural network-based classification approach. This Trace peak detection workflow is at the core of Trace Cloud. As an online resource, Trace Cloud is also equipped with a user-friendly graphical user interface (GUI) that allows users to select input files, specify analysis parameters, and visualize and download analysis results.

In Trace Cloud, data is passed through a centralized GUI with fill-in boxes for parameters and a preview to guide researchers to make informed decisions on setting parameters. The preview executes an analysis on a small portion of the data so users can tune parameters before running the entire program. Cloud computing runs the program, so users do not need to supply processing power or storage for files from their end. Furthermore, users do not have to execute the program from a scripting environment or manual code in any of the parameters spread out among several script files in the original Trace.

Funding Sources (if applicable):

U01CA235507

99

Metabolomics and nutritional biomarkers of motor chemotherapy-induced peripheral neuropathy

Ciao-Sin Chen[1], Kathleen A. Stringer[1,2], N. Lynn Henry[3], Daniel L. Hertz[1,3]

[1]Department of Clinical Pharmacy, College of Pharmacy, University of Michigan, USA
[2]NMR Metabolomics Laboratory, College of Pharmacy, University of Michigan, USA
[3]Rogel Cancer Center, Medical School, University of Michigan, USA
cscchen@umich.edu

Abstract

Background: Chemotherapy-induced peripheral neuropathy (CIPN) is the major treatment-limiting and debilitating toxicity that presents as sensory and/or motor symptoms in patients receiving several anti-cancer agents, including paclitaxel. Our prior work has identified potential predictive biomarkers of sensory CIPN, including higher systemic paclitaxel exposure and lower pre-treatment levels of histidine, phenylalanine, threonine, or vitamin D. The objective of this study was to identify candidate metabolomics and nutritional biomarkers of motor CIPN.
Methods: Patients scheduled to receive paclitaxel 80 mg/m2 weekly for 12 weeks for stage I-III or oligometastatic breast cancer were enrolled at the University of Michigan Rogel Cancer Center. Pre-treatment blood samples were collected for whole blood 1D-1H-NMR metabolomic profiling and clinical laboratory measurement of vitamin B12, vitamin D, homocysteine, and folate. Patients completed the European Organisation for Research and Treatment of Cancer Quality of Life Questionnaire CIPN20 at baseline and weekly until the end of treatment. Motor CIPN was quantified as the maximum change from baseline of the CIPN20 motor subscale (ΔCIPN-M), which was scaled to 0-100. Metabolomic and nutritional biomarkers of motor CIPN were identified using linear regression adjusted for clinical covariates including age, race, paclitaxel systemic exposure, number of doses received, weeks of treatment delay, and relative dose intensity. Significance was defined using α=0.05 and false discovery rates (FDR) <25%.
Results: Patients who completed CIPN20 (N=60; ΔCIPN-M mean=10.8, std=15.2) and had whole blood metabolomics profiles (N=47) and/or nutrient measurements (N=38) were included in this study. A total of 35 metabolites were detected and identified, but none were significantly associated with motor CIPN (histidine p=0.036, FDR=94%). Vitamin D insufficiency (≤20ng/mL) was associated with worse motor CIPN (p=0.015, FDR=12%).
Conclusions: Similar to our prior work, these findings suggest that vitamin D insufficiency may be a potential biomarker of motor CIPN.

Funding Sources (if applicable):

100

Fecal microbial transplantation as a potential therapeutic strategy to mitigate the debilitating effects of the neurodegenerative disease, Familial dysautonomia

Alexandra Cheney[1], Stephanann Costello[1], Nicholas Pinkham[2], Heather Walk[2], Seth Walk[2], Frances Lefcort[2], & Valérie Copié[1]

[1]Department of Chemistry & Biochemistry, Montana State University, USA
[2]Department of Microbiology & Immunology, Montana State University, USA
AlexandraTownsend@montana.edu

Abstract

Familial dysautonomia (FD) is a progressive developmental neurodegenerative disease resulting from a genetic mutation in the *Elongator protein-1 (ELP-1)* gene that leads to reduced tissue-specific expression of the ELP-1 protein. It has been previously shown that reduced ELP-1 activity results in mitochondrial dysfunction, neuronal cell death, and alterations in protein translation/synthesis. Although FD is rare, this study will further support efforts in address the incapacitating symptoms that patients endure. In addition, this disease presents similarities to more common multi-factorial neurological disorders characterized by mitochondrial dysfunction and is thus a valuable genetic model to investigate neurodegenerative patterns and phenotypes shared across these disorders. Of particular interest is the relationship between neurological impairments and the gut microbiome, and their influence on metabolic functions. We have discovered that gut microbiome dysbiosis and metabolic impairments accompany neurodegeneration in FD patients, with phenotypes that are recapitulated in FD mouse models. With this knowledge, we are now exploring potential therapeutic strategies such as gut microbial fecal transplants and/or metabolic supplementation to attenuate FD disease phenotypes. We have first started such studies by cohousing FD mice with sibling control mice, with the objective that their coprophagic behavior might simulate a self-administered oral fecal microbial transplant (FMT). Stool samples were collected across the animals' lifespan and analyzed for gut microbe composition via 16S rRNA gene sequencing, and metabolic profiles via nuclear magnetic resonance (NMR) analyses. In addition to observed improvement of the overall FD mouse phenotypes and gut function when cohoused, our analyses of cohoused mouse stool samples revealed alterations of the gut microbiome and stool metabolome profiles. Overall, our findings demonstrate that FMT strategies may be of significant value as an intervention approach or future therapeutic strategy, to mitigate the debilitating effects associated with Familial dysautonomia and other neurological disorders that share disease hallmarks with FD.

Funding Sources (if applicable):

This research is supported in part by the National Institutes of Health (NIH 1 R01 DK11747473-01A1). Some funding for graduate research/tuition is also in part by the Molecular Biosciences Fellowship of Montana State University.

101

Screening human lung cancer with predictive models of serum magnetic resonance spectroscopy metabolomics

Tjada A. Schult[1,2,†], Mara J. Lauer[1,3,†], Yannick Berker[4], Marcella R. Cardoso[1], Lindsey A. Vandergrift[1], Piet Habbel[2], Johannes Nowak[5], Matthias Taupitz[2], Martin Aryee[1], Mari A. Mino-Kenudson[1], David C. Christiani[1,6,] and Leo L. Cheng[1]

[1]Massachusetts General Hospital and Harvard Medical School, USA
[2]Charité Universitätsmedizin Berlin, corporate member of Freie Universität Berlin, Humboldt-Universität zu Berlin, and Berlin Institute of Health, DE
[3]University of Würzburg (JMU), DE
[4]German Cancer Research Center (DKFZ), DE
[5]SRH Poliklinik Gera GmbH, Radiological Practice Gotha, DE
[6]Harvard T.H. Chan School of Public Health, USA

Abstract

The current high mortality of human lung cancer stems largely from the lack of feasible, early disease detection tools. An effective test with serum metabolomics predictive models able to suggest patients harboring disease could expedite triage patient to specialized imaging assessment. Here, using a training-validation-testing-cohort design, we establish our High Resolution Magic Angle Spinning (HRMAS) magnetic resonance spectroscopy (MRS)-based metabolomics predictive models to indicate lung cancer presence and patient survival using serum samples collected prior to their disease diagnoses. Studied serum samples were collected from 79 patients *before* (within 5.0 years) and *at* lung cancer diagnosis. Disease predictive models were established by comparing serum metabolomic patterns between our *training cohort's*: patients with lung cancer at time of diagnosis, and matched healthy controls. These predictive models were then applied to evaluate serum samples of our *validation and testing cohorts'*, all collected from patients *before* their lung cancer diagnosis. Our study found that the predictive model yielded values for prior-to-detection serum samples to be intermediate between values for patients at time-of-diagnosis and for *healthy controls*; these intermediate values significantly differed from both groups, with an F1 score = 0.628 for cancer prediction. Furthermore, values from metabolomics predictive model measured from prior-to-diagnosis sera could significantly predict five-year survival for patients with localized disease.

Funding Sources (if applicable):

NIH grants CA141139, CA243255, AG070257, OD023406 and the MGH A.A. Martinos Center for Biomedical Imaging.

102

Why measuring choline and related metabolites matters in nutrition research

Rachel Coble[1], David Kirchner[1], Susan Sumner[1]

[1]Nutrition Research Institute, University of North Carolina at Chapel Hill, USA
rachel_coble@unc.edu

Abstract

Choline is a vitamin-like compound that is an essential nutrient for humans and other animals. It is needed to synthesize phospholipids and sphingomyelin, which are necessary for the structure and integrity of cell membranes, and to produce the neurotransmitter acetylcholine, which is known to be important in nervous system function, muscle control, and brain development. It is also needed to produce the universal methyl donor, S-adenosylmethionine, and has a role in modulating gene expression, cell signaling, and lipid transport. Low levels of choline have been associated with cognition and memory disorders, mood disorders, liver disease, pregnancy complications, fertility, and eye disease. The quantitative targeted analysis of choline and its related metabolites can be conducted using liquid chromatography stable isotope dilution multiple reactions monitoring mass spectrometry (LC-SID-MRM/MS). Our method has been applied to a diverse range of biospecimens (e.g., plasma, serum, urine, stool, breast milk, food, and seminal plasma) to detect the levels of choline and other metabolites in on carbon metabolism.
While humans and other animals can synthesize choline, the amount produced is often not sufficient. Thus, choline must be obtained from the diet in the form of choline or choline phospholipids. Many factors influence the amount of dietary choline that individuals need, including several common genetic polymorphisms that have a substantial impact on choline metabolism and bioavailability. Because dietary intake alone is not a good predictor of physiological choline status, direct measurement of choline (and its metabolites) in biospecimens is critical to detect and diagnosis choline deficiency. This presentation will describe the method for measurement of choline and related metabolites and recent studies.

Funding Sources (if applicable):

grant#P30DK056350

103

Development of a knowledgebase of environmentally relevant compounds for exposomics

Ciara Conway[1,4], Aleksandr Smirnov[1,4], Yuanyuan Li[3,4], Blake Rushing[3,4]
Susan McRitchie[3,4], Timothy Fennell[2,4], Susan Sumner[3,4], Xiuxia Du[1,4]

[1]Department of Bioinformatics and Genomics, University of North Carolina at Charlotte, USA
[2]RTI International, USA
[3]Nutrition Research Institute, University of North Carolina at Chapel Hill, USA
[4]NIEHS North Carolina Human Exposure Analysis Resource (HHEAR) Hub, USA
cconwa10@uncc.edu

Abstract
The field of exposomics has become more and more relevant due to the rapidly increasing manufacturing of a vast array of chemicals, insufficient regulation of their production, and very limited or nonexistent knowledge of their impact on human health. Furthermore, with the completion of the Human Genome Project showing that genetic features seem to only accounting for approximately ten percent of disease progression, leaving about 90% to external causes, such as exposomes, creating a gap in metabolic understanding and the unknown molecules that are possibly contributing to disease progression and interfering with metabolic pathways. For this reason, it is imperative to develop an extensive and comprehensive knowledgebase of these environmentally relevant exposomes by assembling relevant information that are currently scattered in a myriad of databases and publications.

Toward this end, we have mined a number of acclaimed and reputable open-source databases and developed python scripts for filling any identifier gaps. The knowledgebase currently contains more than 4,000 compounds from Toxcast, drugs and their metabolites from DrugBank, and multi-class panels of environmentally relevant compounds. Each compound record includes monoisotopic mass and relevant identifiers such as InChIKey, InChI, and SMILES. These information makes it possible for users to query experimental data from mass spectrometry against the knowledgebase as a first-step screening for environmentally relevant compounds. Currently, development is ongoing to leverage the cloud infrastructure and informatics capabilities of ADAP-KDB for building a web portal for users to visualize and search against this exposomics knowledgebase.

Funding Sources (If applicable):

U2CES030857

104

Investigating the gut microbiome-brain-metabolism axis in Familial dysautonomia; Implications of choline and taurine metabolism in disease phenotype and progression.

Stephanann Costello[1], Alexandra Cheney[1], Nicholas Pinkham[2], Brian Tripet[1], Marta Chaverra[2], Maria Cotrina-Vidal[3], Horacio Kaufmann[3], Lucy Norcliffe-Kaufmann[3], Seth Walk[2], Valerie Copie[1] and Frances Lefcort[2]

[1]Chemistry and Biochemistry, Montana State University, USA
[2]Microbiology and Immunology, Montana State University, USA
[3]Langone Health, New York University, USA
Stephanann.costello@student.msu.edu

Abstract

Familial dysautonomia (FD) is a rare monogenic developmental and progressive neurodegenerative disease characterized by a systemic reduction in the Elongator complex protein subunit 1 (ELP1), with neuronal tissues being the most impacted. The reduction in Elp1 gives rise to numerous clinical hallmarks, many of which stem from autonomic dysfunction. Presently, the gut microbiome's influence in neurodegeneration is becoming increasingly acknowledged, however, few studies have investigated the extensive methods by which central metabolism regulates the gut-brain axis.
An untargeted Nuclear Magnetic Resonance (NMR) spectroscopy metabolomics method was used to identify polar metabolites in FD patient's and control relative's fecal samples. This method provided a snapshot of the systemic and gut-microbial metabolic states and a paired statistical model enabled the interrogation of metabolic disturbances between FD and control relatives.
Choline and taurine, two crucial dietary metabolites, were identified as being elevated in the FD patient's samples. Choline functions as a precursor for three metabolic pathways: phosphatidylcholine, acetylcholine and betaine synthesis. Therefore, disturbances in choline levels could negatively impact cellular membrane integrity, neuronal signaling and methylation, respectively. Taurine is an inhibitory neurotransmitter, an antioxidant, and is a constituent of bile acids which are necessary for the absorption of dietary nutrients. Dietary deficits of taurine have been linked to many functional complications that are also hallmarks in FD, such as progressive blindness and mitochondrial dysfunction.
These data, partnered with other -omics data, provide an avenue for further exploration to determine whether choline and/or taurine metabolism promote disease progression. Additionally, due to the numerous clinical hallmarks shared between FD and other neurodegenerative diseases, and the developing understanding of the critical role the gut-brain-metabolism axis plays in neuronal health, knowledge gained regarding the role of metabolism in FD disease progression could be transferrable to understanding the disease progression of Alzheimer's, Parkinson's and other neurodegenerative diseases.

Funding Sources (if applicable):

National Institutes of Health (NIH 1 R01 DK11747473-01A1 and Supplement 3R01DK117473-02S1)

105

Hierarchical clustering analysis of plasma metabolomics identifies biologically distinct subtypes of head and neck cancer associated with overall survival

Ronald C. Eldridge[1], PhD, MPH, Zhaohui S. Qin[2], PhD, Nabil F. Saba[3], MD, D. Neil Hayes[4], MD, MPH, Deborah W. Bruner[1], PhD, RN, FAAN, Dean P. Jones[5], PhD, Canhua Xiao[1], PhD, RN, FAAN

[1]Nell Hodgson Woodruff School of Nursing, Emory University, USA
[2]Biostatistics and Bioinformatics, Rollins School of Public Health, Emory University, USA,
[3]Winship Cancer Institute, Emory University, USA,
[4]Department of Medicine, UT/West Institute for Cancer Research, University of Tennessee Health Science Center, USA,
[5]Division of Pulmonary, Allergy and Critical Care Medicine, Emory University, USA
ronald.eldridge@emory.edu

Abstract

Background: Head and neck cancer (HNC) needs new biomarkers to continue progress towards precision medicine. Metabolomics is gaining prominence as a new source of HNC biomarkers, yet we know almost nothing about metabolic subtypes. Here we cluster 209 HNC patients using plasma metabolomics to discover metabolic subtypes that present with distinct overall survival outcomes.

Methods: High-resolution liquid chromatography-mass spectrometry of pre-treatment patient blood extracted 186 laboratory confirmed metabolites. After standardization, we performed hierarchical clustering using Bayesian information criterion (lowest) and silhouette scores (no negatives) to determine the optimal number of clusters. We estimated overall survival by cluster group and annotated each cluster via pathway enrichment analysis.

Results: The clustering identified two metabolically distinct HNC subtypes A (n=86) and B (n=123). There were no subtype differences by HPV status (50% positive vs 47%), smoking history (62% ever smokers vs 61%), sex (76% male vs 74%), or race (85% white vs 79%), and a minimal difference by average age (61.7 years vs 57.6). There was a prominent difference in overall survival, as patients in subtype A had a 3-year survival of 69%, versus 88% for patients in subtype B (P=0.007). The survival difference remained after adjustment for the above factors and tumor site, stage, ECOG status, and feeding tube (Cox model HR=2.39, 95% CI: 1.19-4.78). Subtype A showed prominent levels of amino acid metabolism (e.g., betaine, arginine, aspartate, urea cycle), the Warburg effect, and fatty acid biosynthesis. Subtype B showed higher levels of steroidogenesis, selenoamino acids, glutathione, and bile acid metabolism.

Conclusions: HNC may have distinct metabolic clusters that may be useful clinically given the independence of the clusters with known HNSCC risk factors. These results should be replicated with tumor samples, but the reported metabolic pathways should be further investigated as targets for biomarker and treatment modalities in HNC.

Funding Sources (if applicable):

R.C. Eldridge would like to thank the Georgia Clinical and Translational Science Alliance (UL1TR002378 and KL2TR002381), and C. Xiao would like to thank the National Institute of Nursing Research (K99/R00NR014587 and R01NR015783) and the Winship Cancer Institute via the National Cancer Institute (P30CA138292) for funding the conducted research.

106

Application of the goodness-of-fit test to prioritizing unknown metabolites

Rasmus Erlemann[1], Aleksandr Smirnov[1], Ciara Conway[1] and Xiuxia Du[1]

[1]Department of bioinformatics and genomics, University of North Carolina at Charlotte, USA
rasmus.erlemann@uncc.edu

Abstract
Untargeted metabolomics where analyses are performed with GC-MS and LC-MS/MS result in vast number of unknown signals that cannot be matched to known compounds. From a biological perspective it is imperative to elucidate what these unknowns could possibly be for the purpose of understanding metabolic pathways and the molecular mechanisms within the living organism. However, identifying unknowns, including both known unknowns and unknown unknowns, is very costly and time-consuming and therefore unknowns need to be prioritized for subsequent compound identification. ADAP-KDB spectral knowledgebase is developed to address this issue by providing researchers with reliable information that allows them to make an informed decision in selecting unknowns for compound identification. This information is extracted from the vast volume of publicly available metabolomics data using big data analytics. One of the computational steps in this extraction process is to compare two categorical distributions and provide a statistical measure of the difference between the two distributions. The goodness-of-fit test is carried out in this context and is used for deciding whether two samples came from the same distribution. For the test statistic choice, chi-square test for independence fits the categorical data. For calculating the p-values, permutation test was used to facilitate small sample sizes. Power study was also done to determine the appropriate sample size in clusters. After calculating the p-values for all the metabolites in ADAP-KDB, the significant cases are analyzed in more detail. Distributional difference between a cluster and the database for an attribute, indicates that there is a biological cause worth investigating further.

Funding Sources (if applicable):

NIH U01CA235507

107

Inter-laboratory comparison of known and unknown features in untargeted lipidomics data

Tong Shen[1], Jennifer E. Kyle[2], Thomas O. Metz [2], David A. Gaul [3], Facundo M. Fernández[3], Hani Habra[4] ,Nathanial Chase Stevens[1], Uri Keshet[1], Raquel Cumeras[1,5], Oliver Fiehn[1], Charles R. Evans[4]

[1]University of California-Davis, USA
[2]Biological Sciences Division, Pacific Northwest National Laboratory, USA
[3]School of Chemistry and Biochemistry, Georgia Institute of Technology, USA
[4]University of Michigan, USA
[5]Institut d'Investigació Sanitària Pere Virgili, URV, CIBERDEM-ISCIII, CERCA, ES
chevans@umich.edu

Abstract

The number of compounds that can be identified using untargeted lipidomics has steadily improved with the development of high-quality spectral databases. However, many detectable features in lipidomics data remain unidentified. The extent to which different laboratories observe the same unidentified features has not been studied. Evaluating consistency in unknown features detected could help prioritize routinely observed unknowns for more study, ultimately improving the biological insight that can be gained from lipidomics data. To this end, laboratories participating in the National Institutes of Health Metabolomics Consortium analyzed a common set of lipid extracts from several animal and human sources using a shared high-resolution reversed-phase liquid chromatography method. Data acquisition was performed on different mass spectrometers including quadrupole-time of flight and orbital ion trap instruments. Data were processed using MSDIAL software, which was used to align known and unknown features across labs.

Data generated from the experiment demonstrated reproducible chromatography between labs; r-squared coefficients for quadratic fit of retention time alignment between labs were above 0.99 for all datasets. Slight differences in identified features were noted between different instrument types. QTOF instruments identified slightly more ceramides and sphingomyelins while orbital ion trap instruments identified more PC, ether-linked PE, and N-acetylethanolamine species. Processing data from all labs yielded 21,884 aligned features (1951 identified by MS/MS match against LipidBlast spectral libraries). Of the ~19,000 unknown features detected in the data, approximately 45% of the unknowns were detected by only one lab or analytical platform, while approximately 55% were observed by at least two labs. Efforts are currently underway to better identify and annotate unknown features of interest in the data. Our findings highlight the advantages of shared analytical methods and enhanced inter-laboratory collaboration to improve the consistency of untargeted lipidomics data and to facilitate unknown compound identification.

Funding Sources (if applicable):

108

2,3,7,8-tetrachlorodibenzo-p-dioxin elicits changes in gut microbiome consistent with progression of non-alcoholic fatty liver disease

Russell Fling[1,2,3], Tim Zacharewski[1,2]

[1]Department of Biochemistry & Molecular Biology, Michigan State University, USA
[2]Institute for Integrative Toxicology, Michigan State University, USA
[3]Department of Microbiology and Molecular Genetics, Michigan State University, USA
Flingrus@msu.edu

Abstract

Gut dysbiosis and disrupted bile acid metabolism, commonly associated with non-alcoholic liver disease (NAFLD), are recapitulated in male mice exposed to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). Mediated through activation of the aryl hydrocarbon receptor, TCDD causes increases serum levels of taurolithocholic acid and deoxycholic acid. These secondary bile acids produced by microbial metabolism are ligands for farnesoid x receptor (FXR) and G-protein-coupled bile acid receptor (Gpbar1) involved in bile acid, glucose, and lipid homeostasis. To investigate TCDD elicited effects on the gut microbiota, the cecum contents of male C57BL/6 mice orally gavaged with sesame oil vehicle control or 0.3, 3 or 30 µg/kg TCDD every 4 days for 28 days were subjected to shotgun metagenomic sequencing. Taxonomic analysis identified dose-dependent increases in *Turcibacter sanguinis* and lactobacilli i.e. *Limosilactobacillus reuteri* (formerly known as *Lactobacillus reuteri*). Enriched species were also associated with the dose-dependent increases in bile salt hydrolases which perform the first deconjugation step in secondary bile acid metabolism. Increased L. reuteri levels were associated with the mevalonate-dependent isopentenyl diphosphate [IPP] biosynthesis, a key intermediate that feeds into menaquinone (aka Vitamin K12) and peptidoglycan biosynthesis. The mevalonate-dependent IPP pathway was also enriched in Streptococcus and Lactobacillus species in patients with hepatic fibrosis. These results expand on the association of lactobacilli with AhR/intestinal axis and how the AhR can impact microbial/host signaling pathways relevant to NAFLD development.

Funding Sources (if applicable):

Russell Fling is supported by the NIEHS Multidisciplinary Training in Environmental Toxicology (NIEHS EHS T32ES007255). This work was funded by the NIEHS Superfund Research Program (NIEHS SRP P42ES04911), and NIEHS R01ES029541.

109

Lipidomics of generation 1 and 2 FASN inhibitors in p53 and KRAS mutant colorectal cancer tumor spheroids

Brian Fries, Fernando Tobias, Amanda B. Hummon[1]

[1]Department of Chemistry and Biochemistry, The Ohio State University, USA
fries.94@osu.edu

Abstract

Cancer cells have increased proliferation, resistance to apoptosis, and poorly differentiated character. Three-dimensional (3D) cell culture technologies can better mimic the tumor microenvironment than traditional monolayer (2D) culturing platforms. Therefore, it provides a useful platform to conduct preclinical drug screening. Lipids have a large role to play in cancer biology, dictating processes such as how well a cancer cell will metastasize to the cancer's ability to become resistant to chemotherapy. Understanding the molecular underpinning of the altered lipidome may better help identify how the cancer is progressing. Previously, we evaluated the lipidome of 2D and 3D cultures after conducting serial trypsinization to obtain cell population representing the proliferating, quiescent, and necrotic regions. We have identified triacylglycerols (TG) to be highly enriched in the quiescent and necrotic regions of spheroids. Additionally, the TG species found in spheroids consisted of fatty acyls with high degree of unsaturation. At the same time, 2D cultures consisted of lipid species with saturated and mono-unsaturated fatty acyls, further suggesting the differences in the lipid metabolism occurring between 2D and 3D cultures. Fatty Acid Synthase (FASN) has recently become a druggable target of interest in treating colorectal cancer. In this study, two generations of FASN inhibitors have been investigated, the antibiotic Cerulenin (generation 1) and TVB-2640 (generation 2), which has recently entered a Phase 1 clinical trial. We conducted a lipidomic profiling study on colorectal cancer tumor spheroids from HCT-116 and HT-29 cell lines to investigate the change in the lipid profile after FASN inhibition. In order to provide a comprehensive identification and reproducible quantification of lipids, we also evaluated the performance of a C18 and a C30 reversed-phase column for chromatographic separation prior to introduction to mass spectrometry analysis on a quadrupole time-of-flight instrument.

Funding Sources (if applicable):

Funded by the National Cancer Institute 1R01CA247863 to ABH

110

Integrated metabolic flux and pool size analysis of plant central metabolism under varying photorespiratory conditions

Xinyu Fu[1], Berkley Walker[1]

[1]Department of Energy-Plant Research Laboratory, Michigan State University, USA
fuxinyu2@msu.edu

Abstract

Photorespiration is a high flux pathway that interacts with many other metabolic pathways (e.g., the Calvin-Benson cycle, respiration, and amino acid metabolism) across multiple subcellular compartments. Measuring carbon fluxes through central metabolic network in intact illuminated leaves remains difficult due to the analytical challenges associated with the interconnectedness and compartmentalization of metabolic pathways. To examine how photorespiration is linked to other processes of central metabolism, we performed targeted metabolic profiling of central carbon metabolites and generated metabolic network flux maps in leaves of tobacco (*Nicotiana tabacum*) acclimated at high (40% O_2), normal (21% O_2), and low photorespiratory (2% O_2) conditions. Metabolic models were solved to estimate ~100 network-wide fluxes based on an isotopically nonstationary metabolic flux analysis (INST-MFA) using isotopic labeling patterns of 40 central metabolites during $^{13}CO_2$ labeling time course, gas exchange measurements, starch and sucrose partitioning determined by $^{14}CO_2$ labeling, and the levels of vascular sucrose and amino acids. The integrated analysis of INST-MFA and metabolic profiling clearly highlighted a network-wide shift of metabolic flux distribution, with fewer changes in metabolite pool sizes. These findings demonstrate that INST-MFA is a promising tool for quantitatively investigating the dynamics of central metabolic network, complementing the metabolomic approaches.

Funding Sources (if applicable):

111

Digging deeper into the root metabolome of Sorghum accessions to evaluate the mechanisms of phosphorus acquisition

Jasmine Gill[1], Vidya Suseela[1]

[1]Department of Plant and Environmental Sciences, Clemson University, USA
gill8@clemson.edu

Abstract

Phosphorus(P) is an essential macronutrient for plants. Due to the strong sorption and low mobility in soils, P is often apparently unavailable for the plant. Therefore, plants use various phosphorus acquisition strategies to mine the soluble and insoluble P from the soil. These strategies include soil exploration by roots, root exudate production, and forming a symbiotic association with various beneficial soil microbes, including Arbuscular Mycorrhizal fungi. Ideally, plants can adopt all the strategies mentioned above under low phosphorus conditions, but generally plants tend to tradeoff one strategy over another to reduce carbon costs of P acquisition. We hypothesized that the quantity and composition of root metabolites will significantly differ in P efficient and P inefficient accessions under no phosphorus treatment. We also hypothesized that the root exudate mediated P acquisition by an accession will be inversely related to its capacity to form AMF symbiosis.

We tested the first hypothesis using a greenhouse hydroponics experiment with eight genotypes (four P-efficient and four P-inefficient) of Sorghum, two P treatments- no P and full P. The P treatments were applied 96 hours before harvesting to observe the change in metabolite profiles of genotypes in response to P stress. We chose these accessions based on the reported P-efficiency trait. Plants were harvested after a month, and the hydroponic solution was collected and analyzed for primary metabolites using GC-MS and secondary metabolites using LC-MS/MS analysis. Our results revealed a unique metabolic signature in secondary metabolites between P-efficient and P-inefficient accessions under no Phosphorus treatment. There was differential upregulation of flavonoid compound classes in both accession groups in response to no phosphorus. These results will be further discussed with regard to the second hypothesis to understand the root exudate profile of P-efficient and P-inefficient accessions in response to AMF and phosphorus treatments.

Funding Sources (if applicable):

112

Comprehensive and quantitative metabolomics platform for characterizing the human exposome

Raúl González-Domínguez[1,2], Cristina Andres-Lacueva[1,2]

[1]Biomarkers and Nutrimetabolomics Laboratory; Department of Nutrition, Food Sciences and Gastronomy; Food Technology Reference Net (XaRTA); Nutrition and Food Safety Research Institute (INSA); Faculty of Pharmacy and Food Sciences; University of Barcelona, ES

[2]CIBER Fragilidad y Envejecimiento Saludable (CIBERfes), Instituto de Salud Carlos III, ES

raul.gonzalez@ub.edu

Abstract

Metabolomics is nowadays one of the most powerful technologies for holistically deciphering the molecular mechanisms driving the final phenotype, which is influenced by multiple biological and lifestyle conditions (e.g., disease, genetic background, xenobiotics). In this vein, emerging evidence highlights the prominent involvement of environmental factors in health status, including the diet, drug consumption and exposure to pollutants, i.e. the exposome. However, to address the metabolome complexity, novel tools are needed for comprehensive metabolomics analysis, preferably in a quantitative manner to allow for cross-cohort comparisons.

We have developed a large scale quantitative platform for comprehensive and rapid metabolomics fingerprinting of common biological samples (i.e., urine, serum/plasma).1 The methodology is based on the application of simple and robust in-plate extraction protocols (solid phase extraction and dilution for urine, protein precipitation for serum/plasma) and subsequent analysis by reversed-phase ultra-high performance liquid chromatography coupled to tandem mass spectrometry (RP-UHPLC-MS/MS).2-3 The method enables the simultaneous quantitation of more than 1000 metabolites in very short run times and using low volumes of biological sample. The coverage of this method comprises about 450 food-derived metabolites, 40 common pollutants (e.g., pesticides, phthalates, parabens), 40 pharmaceuticals, and some other biomarkers related to lifestyle habits (e.g., smoking, alcohol consumption), thus providing a comprehensive and accurate characterization of the human exposome. This metabolomics approach also includes around 500 endogenous metabolites involved in central biochemical pathways, covering a broad range of chemical classes: amino acids and derivatives, organic acids, biogenic amines, carbohydrates, vitamins, lipids (e.g. fatty acids, acylcarnitines, steroids, phospholipids). To construct this metabolite library, we also considered the central role of microbiota on detoxification processes, metabolism and health.

To sum up, this novel multi-targeted approach represents one-step further towards comprehensive metabolomics-based exposome research. The rapidity and low sample requirements of this methodology facilitate its implementation in large epidemiological studies.

[1] González-Domínguez et al. Characterization of the Human Exposome by a Comprehensive and Quantitative Large-Scale Multianalyte Metabolomics Platform. Anal. Chem. 2020 92(20):13767-13775.
[2] González-Domínguez et al. Quantitative Dietary Fingerprinting (QDF) - A Novel Tool for Comprehensive Dietary Assessment Based on Urinary Nutrimetabolomics. J. Agric. Food Chem. 2020 68(7):1851-1861.
[3] González-Domínguez et al. Quantifying the human diet in the crosstalk between nutrition and health by multi-targeted metabolomics of food and microbiota-derived metabolites. Int. J. Obesity 2020 44:2372–2381.

Funding Sources (if applicable):
The Spanish Ministry of Economy and Competitiveness (PCIN-2015-229, PCIN-2015-238, PCIN-2017-076), CIBERFES and ISCIII projects AC19/00111 and AC19/00096 (co-funded by the FEDER Program from EU, "A way to make Europe"), and the Generalitat de Catalunya's Agency AGAUR (2017SGR1546). Raúl González-Domínguez thanks the "Juan de la Cierva" program from MINECO (FJCI-2015-26590 & IJC2019-041867-I).

113

Analyzing the diversity of *in vitro* microbially conjugated bile acid products

Douglas Guzior[1,2], Robert Quinn[2]

[1]Department of Microbiology and Molecular Genetics, Michigan State University, USA
[2]Department of Biochemistry and Molecular Biology, Michigan State University, USA
guziordo@msu.edu

Abstract

Bile acids, key molecules responsible for the digestion of fats and homeostatic regulation, circulate through our gastrointestinal tract in an efficient mechanism known as enterohepatic circulation. These bile acids also function as antimicrobials, actively shaping the structure of our gut microbiome. Because of this, members of our gut microbiome have developed methods of modifying bile acids. Traditionally this has been done in four distinct ways: deconjugation, dehydroxylation, oxidation, and epimerization. However, a fifth transformation was recently described: reconjugation. Originally, only three microbially conjugated bile acids were described (leucocholate, phenylalanocholate, tyrosocholate). *Enterocloster bolteae* was the original organism associated with the production of microbially conjugated bile acids and so we focused our screen on members of the family *Lachnospiraceae* and other nearby, related members of our gut microbiota. Strains were grown in the presence of cholate, overnight, followed by cold methanol extraction prior to analysis via untargeted liquid chromatography-tandem mass spectrometry on a Q Exactive Hybrid Quadrupole Mass Spectrometer. Data was submitted to the Global Natural Products Social Molecular Networking Database to match MS^2 spectra to those annotated within the database. Here, we identify novel amino acid-cholic acid conjugates with amino acids containing charged, polar uncharged, and hydrophobic side chains. In total, 12 novel conjugates were identified among 10 strains screened. However, approximately half of the metabolites with related MS^2 spectra remain unidentified within GNPS and remain to be identified. Interestingly, the most abundant amino acid used in microbial conjugation was glycine resulting in the production of glycocholate, originally thought to only be produced in the host liver. This is the first description of members of our gut microbiota contributing to the host primary bile acid pool.

Funding Sources (if applicable):

114

Towards inferring absolute concentrations from relative abundances in metabolomics data

Justin Lee[1], Yue Han[1], Mark Styczynski[1]

[1]School of Chemical & Biomolecular Engineering, Georgia Institute of Technology, USA
yhan309@gatech.edu

Abstract
Metabolomics provides a direct readout of metabolic state and has the potential to be integrated into metabolic modeling frameworks to better understand how cellular systems function. Analytical chemistry techniques used for metabolomics such as liquid and gas chromatography-mass spectrometry (LC/GC-MS) provide relative abundances of metabolites, which do not allow fully quantitative analysis across samples and experiments. These relative values also cannot be directly integrated into metabolic models, which often use absolute concentrations of metabolites. Although chemical standards can be used to quantify a few metabolites at a time, they can be costly or infeasible to scale to many metabolites, or even unavailable for some. As a result, a computational method for determining absolute concentrations without the use of chemical standards would be beneficial to the metabolomics community.

Our group is developing Metabolomics Prediction of Absolute Concentrations (MetaboPAC), a tool for inferring absolute concentrations from relative abundance metabolomics data for cellular metabolism. MetaboPAC attempts to avoid the need for chemical standards by leveraging the mass balances of a metabolic system and any known kinetic reaction equations to determine the most biologically likely metabolic profiles. We have demonstrated that MetaboPAC can infer absolute concentrations with high accuracy for systems with known kinetics, and we are pursuing improvement in prediction accuracy for systems without *a priori* kinetics information.

Funding Sources (if applicable):

National Institutes of Health (NIH) GM119701

115

The cannabis compound database and novel cannabis metabolite assays

Mickel Hiebert[1], Zaid H. Maayah[3], Gozal Inchehborouni[1], Xuan Cao[1], An Chi Guo[1], Marcia LeVatte[1], Jaanus Liigand[1,4], Fei Wang[2], Sudarshana Bhumireddy[1], Yilin Wang[1], Jiamin Zheng[1], Rupasri Mandal[1], Jason Dyck[3], David S. Wishart[1,2]

[1]Biological Sciences Department, Faculty of Science, University of Alberta, CA
[2]Computing Science Department, Faculty of Science, University of Alberta, CA
[3]Cardiovascular Research Centre, Departments of Pediatrics, Faculty of Medicine and Dentistry, University of Alberta, CA
[4]Institute of Chemistry, University of Tartu, EE

Abstract

Cannabis sativa (marijuana or hemp) has been used by different cultures throughout human history for a wide variety of applications. The cannabis market (both legal and illegal) is estimated to be worth $340 billion/yr and in recent decades, many national, provincial, and state governments have changed the legal status of *C. sativa*, allowing Cannabis to be used for both medicinal and recreational purposes. In order to centralize the available information regarding the chemistry of Cannabis and to help fill the knowledge gaps of its physiological effects we have assembled a web-accessible database called the Cannabis Compound Database (www.cannabisdatabase.ca). This database contains detailed information about small molecules found in *C. sativa* as well as *C. indica* (plant and smoke compounds) that were measured from in-house developed assays, extracted from extensive literature reviews and derived via genomic-based metabolite inference. The CCDB covers a total of 6,329 chemicals and 2,336 concentrations for 115 *C. sativa* and *C. indica* cultivars. Additionally, 5,660 human protein targets, 50,817 MS/MS, 1,529 NMR and 5,929 GC-MS spectra are currently in the database to facilitate compound identification and characterization of *C. sativa/indica* cultivars and products. Using this information, we have developed several in-house GC-MS and LC-MS assays for measuring: 1) primary and secondary plant/Cannabis metabolites; 2) cannabinoids and terpenes in Cannabis; 3) cannabinoids in serum/plasma; and 4) herbicides/pesticides in Cannabis.

Funding Sources (if applicable):

Canadian Institute for Health Research, Canada Foundation for Innovation

116

Characterization of NIST standard teference material 1950 — metabolites in frozen human plasma

Jiri Hricko[1], Tomas Cajka[1], Michaela Novakova[1], Lucie Rudl Kulhava[1], Michaela Paucova[1]

[1]Institute of Physiology of the Czech Academy of Sciences, CZ
jiri.hricko@fgu.cas.cz

Abstract

Liquid chromatography−mass spectrometry (LC−MS) is the preferred technique in metabolomics and lipidomics permitting effective compound separation and detection. However, the true breadth of a metabolome and lipidome cannot be captured by a single extraction method or instrumental platform. Hence, the main task is to cover polar metabolites and lipids using as few platforms as possible while maintaining the requisite precision and accuracy for the metabolite classes detected by the chosen platforms. Recently, the use of NIST SRM 1950 as a control material for small molecule-based omics studies has been supported by the metabolomics and lipidomics community. It is recommended that this certified reference material be used as a material to aid in standardization and quality assessment across time and laboratories.
For human plasma NIST SRM 1950 characterization we used an LC−MS workflow LIMeX for the simultaneous extraction of complex LIpids, polar Metabolites, and eXposome compounds that combines untargeted and targeted analysis. The sub-groups of compounds were isolated using an 'all-in-one' extraction with a methanol/methyl *tert*-butyl ether mixture and water. These extraction solvents contained over 60 internal standards covering main lipid classes, selected polar metabolites, and exposome compounds (drugs and food components). Analysis of complex lipids was conducted using reversed-phase LC (RPLC) in positive and negative electrospray (ESI) mode while polar metabolites were separated using hydrophilic interaction chromatography (HILIC) in ESI(+) and RPLC in ESI(–). Simultaneous acquisition of MS1 and MS/MS spectra in data-dependent mode was used for each platform. The acquired raw files were processed using user-friendly MS-DIAL software including also MS/MS library search. For NIST SRM 1950 human plasma we annotated 500+ complex lipids and polar metabolites.

Funding Sources (if applicable):

Czech Science Foundation (20-21114S), Ministry of Education, Youth and Sports (LTAUSA19124), Czech Health Research Council (NU20-01-00186)

117

Application of hybrid surface technology for improving sensitivity and peak shape of phosphorylated and carboxylate lipids

Giorgis Isaac[1], Ian Wilson[2], and Robert S. Plumb[1]

[1]Biomedical Research, Waters Corporation, MA, USA
[2]Computational & Systems Medicine, Imperial College, UK
giorgis_isaac@waters.com

Abstract

The phosphate group of the phosphatidic acids (PA and LPA) and the carboxylate group of the phosphatidylserines (PS and LPS) bind to metal ions to form chelation complexes that causes peak tailing and reduced intensity. To address the issue, we have developed the use of a hybrid organic/inorganic barrier surface applied to the metal substrates in the ACQUITY Premier System and column. A serial dilution of LPA, PA, LPS and PS was analyzed using standard column with stainless steel surface ACQUITY UPLC system and Premier System. The Premier System significantly improved the peak tailing and sensitivity of both phosphorylated and carboxylate lipids compared to standard system. A 25-30 times increase in signal intensity was observed for all investigated phosphorylated and carboxylate lipid classes. It also improved peak shape and reduced tailing by minimizing analyte-surface interaction. Premier System reduced peak tailing by 65-80% and increased lipidomics coverage by simultaneous analysis of phosphorylated and carboxylate lipids in addition to other lipid classes. This new methodology was applied to the analysis of egg (chicken) PA and brain (porcine) PS extracts and compared to that obtained with the conventional system/column. The results clearly show that the PA lipid species at m/z 671.47 (16:1_18:1 and 16:0_18:2) and 673.48 (16:0_18:1) eluted with very broad tailing peaks using the conventional system and column. This can be compared to the data acquired with the Premier System, where the peaks are significantly improved in shape and intensity. Similarly, the method was applied for the analysis of brain porcine PS extract. The Premier System not only provided reduced peak tailing but also allowed the visualization of three additional "new" peaks. These additional peaks were identified as PS(38:2) m/z 814.55, PS(40:3) m/z 840.577 and PS(44:2) m/z 898.654. In contrast, these minor PS species were not detected using the conventional system.

Funding Sources (if applicable):

118

Analysis of geosmin consumed by bacterial cultures by HSPME-GC-MS/MS

Trevor Johnson[1], A. Paulina de la Mata[1], Camilla Nesbo[2], James J. Harynuk[1]

[1]Departmant of Chemistry, University of Alberta, CA
[2]Department of Biological Sciences, University of Alberta, CA
taj2@ualberta.ca

Abstract

A common culprit to the earthy-musty smell emitted by *Streptomyces Spirulina* is the compound known as geosmin. This compound, though non-toxic, has been of concern for perception of quality in food, drink, and other sensory-based industries. Geosmin is commonly associated with the smell of rain and has a very low threshold of detection by the human nose at <10 ng/L. There are few methods for the removal of geosmin from water, and the process often involves extensive filtration and pretreatment.
An alternative way for geosmin removal is biodegradation by microorganisms such as bacteria and fungi. By growing microbial communities or selected species in a culture with geosmin as a carbon source and analyzing the amount of the compounds remaining, an idea can be gained of the effectiveness and viability of biological removal methods.
In this study, geosmin levels in a number of enrichment cultures established using aquaculture water were analyzed at different time points to understand how effective they were at geosmin removal. Analysis was done using headspace SPME extraction followed by GC-MS/MS analysis. The method presented is capable of detecting geosmin at a concentration below that of typical human sensory capability, and the use of headspace SPME operates in a way that is relatively similar to human olfactory senses. A number of challenges that arose during analysis are discussed, including the microbial communities themselves interfering with internal standard concentration before analysis, as well as issues with using headspace SPME and the method presented. Results have shown a variety of differences between cultures and conditions when considering their potential to break down geosmin in water over time.

Funding Sources (if applicable):

Mermaid sea systems, Norquest

119

Tocotrienols improve the metabolomic profile of maintenance hemodialysis (MHD) patients

Hansani Karunarathne[1], Tilakavati Karupaiah[2], Pramod Khosla[1] and Smiti V. Gupta[1]

[1]Department of Nutrition and Food Science, Wayne State University, USA
[2]School of BioSciences, Taylor's University Lakeside Campus, MY
gd1395@wayne.edu

Abstract

Markers of inflammation, oxidative stress and dyslipidemia have found to be novel uremia related risk factors observed in MHD patients. An increase in these markers is associated with higher rates of cardiovascular diseases (CVD) among MHD patients. It is believed that supplementation with Vitamin E tocotrienol rich fraction (TRF) could potentially overcome these non-traditional risk factors associated with CVD among hemodialysis population. Here, we report the effect of TRF supplements on the metabolomic profile of African American (n = 85) and Malaysian (n = 80) maintenance hemodialysis patients (MHD) who participated in Palm Tocotrienols in Chronic Hemodialysis study. Changes in plasma metabolomic profile of the MHD patients with or without TRF supplements for 12 months period were investigated using ^{1}H-NMR based metabolomics approach. A distinct variation between the TRF supplemented group and the placebo group was observed during 12 months period in both cohorts, using partial linear square discriminate analysis. Metabolite identification and quantification followed by pathway analysis showed that the key metabolites linked with arginine and proline metabolism and synthesis and degradation of ketone bodies pathways were significantly ($p < 0.05$) altered in the placebo group due to disease progression over 12 months period. These key metabolites include proline, 4-hydroxyproline, ornithine, glutamate, acetoacetate and 3-hydroxybutyrate. TRF supplementation modulated these metabolites in a favorable direction, indicating the potential beneficial impact of TRF supplements in MHD patients. In addition, the key metabolites could serve as biomarkers for kidney disease progression and/ or for evaluating efficacy of TRF/drug supplementation in MHD patients.

Funding Sources (if applicable):

120

MetaboFood-KDB: A cloud knowledgebase for searching metabolomics data for nutritionally relevant compounds

Colin Kay,[1,5] Aleksandr Smirnov,[2,5] Yuanyuan Li,[4,5] Blake Rushing,[4,5] Zhaocong Yang,[3]
Ciara Conway,[2,5] Jing Yang,[3] Susan Sumner,[4,5] Xiuxia Du[2,5]

[1]Plants for Human Health Institute, North Carolina State University, USA
[2]Department of Bioinformatics and Genomics, University of North Carolina at Charlotte, USA
[3]Department of Computer Science, University of North Carolina at Charlotte, USA
[4]Nutrition Research Institute, University of North Carolina at Chapel Hill, USA
[5]NIEHS North Carolina Human Exposure Analysis Resource (HHEAR) Hub, USA
cdkay@ncsu.edu

Abstract

Untargeted mass spectrometry-based metabolomics is becoming increasingly utilized in nutrition cohort and biomarker studies. LC-MS(/MS) signals arising from metabolomic datasets require assignment for researchers to interpret the data. This assignment relies on the availability of databases of nutritionally relevant compounds and their metabolites. Unfortunately, dietary phytochemical metabolites are poorly represented in metabolomics databases. To address this research gap, we are building a cloud knowledgebase named MetaboFood-KDB, which currently features a database (P-MetDB, registered) of nutritionally relevant phytochemicals derived from systematic literature reviews of 17 commonly consumed phytochemical-rich foods, with careful curation of compound synonyms matched to InChI Key, chemical (mass, formula etc.) and database identifiers (i.e., PubChem, HMDB, PhytoHub, KEGG etc.).

In building this MetaboFood-KDB, we aim to fill gaps in missing metabolomic datasets associated with phytochemical metabolites, and overcome the reported issue of inaccurate nomenclature and structural assignment in present food and metabolite databases. We are also developing libraries of LC-MS/MS spectra on a SCIEX 6500+QTRAP and HRMS accurate mass and adduct spectra on a UPLC-Orbitrap. Users will be able to search by synonym, mass, formula, chemical identifier or LC-MS(/MS) signals against the database, following upload of features that have been generated by software tools that preprocess raw LC-MS(/MS) data. After completion of the database, researchers will be able to interactively explore MetaboFood-KDB using built-in visual analytics capabilities. Notably, linkages between food and disease using a self-organizing map, and similarities among different foods, their metabolites, biochemical and disease pathway using an interactive multi-level Sankey diagram. In addition, users will be visually guided to interactively build diets as well as intuitively compare and analyze diets which link to certain disease pathways. Continued development of our MetaboFood-KDB includes expansion to other fruits, vegetables, supplements, and processed foods most common in the US diet.

Funding Sources (if applicable):

NIH U01CA235507, U2CES030857

121

Combining a suite of rapid profiling LC-MS/MS methods and ion mobility workflows to investigate the metabolome of prostate cancer patients

Adam King[1,2], Jade Talbot[3], Ian Wilson[4], Robert Plumb[5], Garth Maker[1], Lee Gethings[2], Tony Whetton[3], Paul Townsend[6]

[1]Medical, Molecular and Forensic Sciences, Murdoch University, AU
[2]SciOps, Waters Corporation, UK
[3]Division of Cancer Sciences, The University of Manchester, Manchester, UK
[4]Imperial College London, UK
[5]SciOps, Waters Corporation, USA
[6]Faculty of Health and Medical Sciences, University of Surrey, UK
adam_king@waters.com

Abstract

Prostate cancer is ranked as the 6th leading cause of cancer related deaths globally and the second most common cancer in men. Currently, early detection of prostate cancer is determined using a PSA (prostate specific antigen) assay where increased concentration can be indicative of prostate cancer. Although, elevated PSA levels can also present in benign cases and inflammation. Therefore, further investigation is required to determine metabolic markers to reduce false positives.
Identification of small molecule markers of disease statistically require analysis of large cohorts of samples from populations. Analysis of these can be time consuming and can impact on resource availability. This can be compounded when trying to obtain as much information as possible using complimentary separation techniques.
Serum samples (n=350) from prostate cancer patients (disease and benign) and control sample donors were aliquoted (20 µL) into two 96 deep well plates for lipid and small molecule analysis. A pooled QC and phenotypic group pools were created from each sample. The lipid samples were extracted with isopropanol and small molecules with Water:MeCN. LC-MS data were collected by HDMS^E data independent acquisition (DIA) mode in both positive and negative ESI with ion mobility separation with chromatographic separation comprised of rapid reversed-phase, HILIC and reversed-phase lipid chromatography using columns with 1 mm i.d.
Each batch consisted of 1157 injections for a single method and polarity. Using conventional methods (~10-minute gradient), the batch took ~8 days to complete. Using the rapid profiling methods, the batch was analysed within 3 days showing >60% reduction in acquisition time. With ion mobility, the number of detected peaks when compared to non-mobility data doubled. Furthermore, ion mobility generated collisional cross section (CCS) values for each compound, thereby improving the confidence of identifications when queried against in-house CCS libraries, covering both polar molecules and lipids.

Funding Sources (if applicable):

122

Metabolomics of secreted specialized metabolites from isolated *Medicago truncatula* border cells

Clayton Kranawetter[1,2,3,4] and Lloyd W. Sumner[1,2,3,4]

[1]Depart. of Biochemistry, University of Missouri-Columbia, USA
[2]Metabolomics Center, University of Missouri-Columbia, USA
[3]Christopher S. Bond Life Sciences Center, University of Missouri-Columbia, USA
[4]Interdisciplinary Plant Group (IPG), University of Missouri-Columbia, USA
CDK374@mail.missouri.edu

Abstract

Border cells are a terminal tissue encasing the root apical meristematic region by means of a complex, water-soluble secreted matrix. These cells can detach after pectin methylesterase activity breaks down the cell wall at the root cap outer epidermal layer. After their release, border cells remain poised at the interface between the root and surrounding soil, secreting mucilage, DNA, proteins, and metabolites. Previous studies on isolated border cells have shown that their secretions are involved in mediation of both biotic and abiotic stressors. Our lab has also shown that there is differential localization of metabolites between border cells, individual root tissues, and rhizosphere secretions. However, the origin of rhizosphere secreted metabolites (i.e. the root or border cells) remains relatively unknown. As such, there is a need to isolate border cells and study their specific secretions and, thus, contributions to rhizosphere chemistry. In our study, border cells were harvested from roots of *Medicago truncatula* and isolated in a custom bioreactor. A syringe pump containing basal salts medium was attached upstream of this bioreactor to maintain a proper osmotic environment. A custom inline Solid Phase Extraction (SPE) column was attached downstream of the bioreactor for trapping of secreted metabolites. Trapped metabolites were harvested and analyzed using Ultra High Pressure Liquid Chromatography-Quadrupole Time of Flight-Mass Spectrometry (UHPLC-QTOF-MS). Data were analyzed using Bruker Data Analysis and Metaboscape 2021. Results indicated the presence of new metabolites being secreted by isolated border cells. Of particular interest was the diverse metabolite profiles and the implications of this data in manipulation of rhizosphere chemistry and mediation of plant-microbe interactions. Specific metabolite identifications and their roles/implications in rhizosphere manipulation will be discussed.

Funding Sources (if applicable):

Wayne L. Ryan Graduate Fellowship, The Ryan Foundation
University of Missouri-Columbia

123

Comparing MS-FINDER, NIST MS Interpreter and CFM-ID to evaluate substructure annotation capabilities

Sharie Kwok[1], Arpana Vaniya[1], Shunyang Wang[1], and Oliver Fiehn[1]

[1]University of California Davis, West Coast Metabolomics Center, USA
sskwok@ucdavis.edu

Abstract

In metabolomics, the use of MS/MS spectral matching to identify or annotate compounds is the gold standard. MS/MS provides fragmentation data information such as neutral loss and *m/z* difference. Determining the structure of metabolites depends on interpretation of MS/MS spectra however, manual interpretation is time consuming. There are many in silico software available for compound identification and predicting and annotating the fragment ions with substructure information, all aiming to aid in the interpretation of MS/MS spectra. Having substructure information is an important process of structure elucidation. We have compared the predicted substructures of three different software to understand the accuracy and capabilities of these tools.

MS/MS spectra from CASMI 2012, containing 26 total compounds with a total number of 12 unique compounds, was used as a benchmark dataset. Substructures were generated for this dataset using CFM-ID 3.0, NIST MS-Interpreter 2.3, and MS-FINDER 3.50. Substructures were selected by using a threshold of 10% intensity of the base peak. Substructures were compared using ChemMine Tools which calculates AP Tanimoto and MCS Tanimoto scores. Tanimoto scores are used to measure similarity between chemical structures. These substructures were also compared to those published in literature which uses the same benchmark dataset.

As the term 'substructure' may be defined differently, each software generated different numbers and types of substructures per compound. MS-FINDER yielded a total of 160 substructure annotations, MS-Interpreter found 66, and CFM-ID resulted 153 total substructure annotations. Not all substructures that were produced were deemed plausible per classic physical-organic chemistry rules. Overall, only 3 substructures matched across the three software programs. Evaluation of the accuracy of tools is difficult if no accepted ground truth is developed, based on general criteria and definitions. Although each algorithm produced different substructure annotations, use of such information is highly useful in compound annotation of unknowns.

Funding Sources (if applicable): NIH U2C ES030158

124

Exploring dose-response in glyphosate-resistant and -susceptible Palmeri amaranth by UHPLC-HRMS based untargeted metabolomics

Jisun H.J Lee[1,2], Pawanjit K. Sandhu[1], and Nishanth Tharayil[1,2,*]

[1]Department of Plant & Environmental Sciences, Clemson University, USA
[2]Multi-User Analytical Lab (MUAL) and Metabolomic Core, Clemson University, USA
jslee.cu@gmail.com

Abstract

Glyphosate, a broad-spectrum herbicide, controls weeds by specifically targeting enzyme EPSPs in shikimate pathway, which in turn kills plants through the depletion of aromatic amino acids. However, the literature available in non-target toxicity which could compromise the efficiency of the herbicide caused by glyphosate in Planta is scanty. Here we used a global metabolomics followed by the TOXcms to uncover the potential secondary toxicity caused by glyphosate in *Amaranthus palmeri*, a troublesome weed species that have developed resistance to glyphosate. In this study, we aimed to elucidate the effect of different doses of glyphosate on the globe metabolic signature of three-week-old resistant (CoR: 0, 0.8, 1.6, and 3.2 Kg a.e. ha^{-1}) and susceptible (CoS: 0, 0.025, 0.1, 0.4, and 1.6 Kg a.e. ha^{-1}) *palmeri amaranth* using UHPLC-HRMS. Samples were harvested after 8 hours of treatment, and 80% methanolic extracts were analyzed by Thermo Orbitrap Fusion™ Tribrid™ mass spectrometer using data-dependent acquisition (DDA) mode. The acquired dataset was preprocessed by XCMS and submitted to TOXcms, a learning-based dose-response metabolomics platform. Lethal dose values (LD_{50}) were 1.12 and 0.03 kg a.e. ha-1 for CoR and CoS, respectively. TOXcms resulted in 676 (CoR) and 602 (CoS) features with monotonic changes. Annotation and chemical classification were conducted, and 2 (CoR) and 11 (CoS) metabolites were confirmed with monotonic changes (ED_{50} < half point of the highest concentration). Systematic class annotation of unknown metabolites was further performed using a computational tool, Canopus, and 31 and 64 features were classified into 5 and 6 superclasses in CoR and CoS, respectively. Our study implies that among features with low ED_{50} values, the metabolism of the CoS was more significantly affected in response to dose-response than the CoR, and TOXcms can be an efficient tool for dose-response metabolomics to understand the biochemical mechanisms toxicity of herbicides.

Funding Sources (if applicable):

125

Metabolomics analysis of American lobster (*Homarus americanus*) larvae after exposure to conventional heavy crude oil

Elena Legrand[1], Tahereh Boloori[2], Rebecca Eldridge[2], Benjamin de Jourdan[2], Andree Gravel[3], Sanjoy Kumas Das[3], Jessica Head[1]

[1] Faculty of Agricultural and Environmental Sciences, McGill University, CA
[2] Huntsman Marine Science Center, St Andrews, NB, CA
[3] Research Institute of the McGill University Health Centre, CA
elena.legrand@mcgill.ca

Abstract

American lobster (*Homarus americanus*) is the most valuable Canadian fishery, producing $1.3 B in 2016. Lobster larvae may be vulnerable to oil spills, particularly during planktonic stages. While it has been reported that water-accommodated fractions (WAF) of oil alters fish metabolome and in particular amino acids metabolism, it is not clear whether similar impacts would be observed in lobster larvae. This study aims to improve our knowledge of the molecular response of the American lobster larvae after exposure to conventional heavy crude oil and a cold lake crude oil. Stage I lobster larvae were exposed to one dose of each WAF (32%) and a negative control (0.22 µm filtered seawater) for 24 hours. In order to reach 50mg wet weight required for the analysis, 8 to 10 lobsters were pooled per sample and 7 pooled samples were analyzed per treatment group. The polar fraction of the lobster larvae metabolome was extracted using a chloroform methanol water technique and characterized using nuclear magnetic resonance (NMR) spectroscopy. Multivariate statistical analysis will be performed using MetaboAnalyst to identify the changes in the lobster larvae metabolome between treatment groups. A pathway impact analysis will be realized based on the metabolites that are contributing to the differences between exposed and control groups. This work will contribute to understanding of the potential impacts of oil spills on American lobster populations and will facilitate future studies on metabolomics in lobster larva. Considering that oil spills are common in Canada and the United States, this is a very valuable study for the protection and management of lobster populations.

Funding Sources (if applicable):

126

Ultrahigh resolution imaging mass spectrometry unveils lipidome alterations in mild traumatic brain injury

Dmitry Leontyev[1], Manvitha Manyam[2], Alexis Pulliam[2], Michelle LaPlaca[2], Facundo M. Fernández[1]

[1] School of Chemistry and Biochemistry, Georgia Institute of Technology, USA
[2] Coulter Department of Biomedical Engineering, Georgia Institute of Technology/Emory University, USA
dmitry.leontyev@gatech.edu

Abstract

Traumatic brain injury (TBI), caused by a physical loading of the head, is a significant cause of disability and death. Despite its widespread occurrence, currently there are no effective approaches to diagnose or treat TBI. Lipids are promising TBI biomarkers that could help with both diagnosis and treatment. A non-targeted spatial metabolomics workflow utilizing ultrahigh resolution mass spectrometry imaging was developed to determine and localize lipid changes in rats following a repeat closed head injury.

Six male Sprague-Dawley rats were used for the study. Three of the animals were subjected to three impacts with a controlled piston to the dorsal head surface, with the remaining three treated as SHAM controls. Brains were embedded in gelatin and sectioned sagittally at 20 µm. Slides were sprayed with a 5 mg/mL 1,5-diaminonapthalene solution. MALDI imaging data was collected on a Bruker solariX 12T FTICR mass spectrometer from 147-1500 *m/z* at 50 µm raster width. The laser was set to 100 shots, small focus, 12% power and 1000 Hz.

Averaged mass spectra across the images showed that the highest abundance peaks were in the 700-850 m/z range, rich in phosphatidylcholines (PCs). Three of the most abundant peaks were at m/z 760.58417, 734.56982 and 788.617, and tentatively identified as PC(34:1), PC(32:0) and PC(36:1) with mass errors of -0.25, 0.39 and 0.67 ppm, respectively. MSiReader was used to compare ion abundances between injured and SHAM tissue sections in the superior part of the brain where the injury was localized. Twenty four ions had noteworthy distributions, including higher or lower abundances in the injured section. PCA biplots showed significant separation between injured and SHAM sections. Furthermore, many important ions were identified from the loading vectors that differentiated the two datasets. Data analysis efforts are currently underway to build supervised and unsupervised models to better classify injured sections.

Funding Sources (if applicable):

The authors acknowledge support from NSF MRI CHE-1726528 grant for the Acquisition of an ultra-high resolution Fourier transform ion cyclotron resonance (FTICR) mass spectrometer for the Georgia Institute of Technology core facilities and NIH grant R01NS101909-01.

127

The dietary exposome matters for developing nutritional interventions

Yuan Li[1], Blake Rushing[1], Madison Schroder[1], Colin Kay[2], Xiuxia Du[3], Timothy Fennell[4], and Susan Sumner[1*]

[1]NIEHS Human Exposure Analysis Resource (HHEAR) Hub, Nutrition Research Institute, University of North Carolina at Chapel Hill, USA
[2]Department of Food Bioprocessing and Nutrition Sciences, Plants for Human Health Institute, North Carolina State University, USA
[3]Department of Bioinformatics and Genomics, University of North Carolina at Charlotte, USA
[4]Discovery Sciences, RTI International, Research Triangle Park, USA
yuanyli4@unc.edu

Abstract

Exposome research is key to informing the development of nutritional intervention strategies. The North Carolina HHEAR Hub is using quantitative targeted and untargeted metabolomics methods to determine constituents in biospecimens that are derived from ingestion of foods. In addition to using annotations against public databases, an in-house Dietary Exposome Physical Standard Library (DEPSL) was established to support identification of signals. There were three considerations for compounds included in the DEPSL. First, biospecimens that had been previously obtained from feeding trials in which humans ingested commonly consumed foods (e.g., blueberries and cocoa) were assessed. Signals in the biospecimens from subjects ingesting the commonly consumed foods were characterized by UPLC-MS(n). Compounds that were quantified in biospecimens included phytoestrogens, aromatic ketones, benzoic acids, elegiac acids, flavonoids, caffeoylquinic acids, catecholamines, coumarins, hippuric acid, hydroxytoluenes, phenylamines, stilbenes, urolithins, valerolactones, and xanthonoids. The identified compounds were then analyzed using a UPLC-Q-Exactive HFx-MS untargeted metabolomics platform to aid in the identification of unknown signals derived from the diet. A second important consideration was to include analytes related to essential nutrients, vitamin-like compounds, and vitamins which serve as cofactors for hundreds of biochemical reactions important in the production and utilization of acetyl-CoA, ATP, NADPH, fatty acids and lipids, amino acids, and neurotransmitters. A third aspect of the Dietary Exposome includes the assessment of environmentally relevant chemicals and their metabolites, that can be consumed during the ingestion of foods and beverages, including compounds such as parabens, phthalates, and pesticides. This presentation will provide an overview of the metabolites we have detected in human subject biospecimens that are derived from naturally occurring compounds that are in common foods, vitamins, essential nutrients, and vitamin-like compounds, as well as additives or contaminants of foods and beverages.

Funding Sources (if applicable):

Funded in part by the NIEHS HHEAR Program 1U2CES030857

128

A prescreening algorithm to speed up spectra search in the ADAP-KDB spectral knowledgebase

Yunfei Liao[1], Aleksandr Smirnov[1], Xiuxia Du[1]

[1]Department of Bioinformatics and Genomics, University of North Carolina at Charlotte, USA
yliao13@uncc.edu

Abstract

ADAP-KDB, a cloud-based mass spectra knowledgebase, has been built for clustering and classifying mass spectra from many metabolomics studies. Users can search their own mass spectra against spectra in ADAP-KDB to discover studies where similar spectra have been observed for prioritizing known and unknown compounds for further investigation. As ADAP-KDB grows rapidly with more and more spectra, searching against ADAP-KDB is becoming very time-consuming. To address this issue, a prescreening algorithm has been developed.

The prescreening algorithm speeds up spectra search by pre-selecting candidate library spectra that will most-likely produce a high similarity score when matched to the user's spectrum. Specifically, m/z values of the top N most intense peaks in the user's spectrum are compared to the top M most intense peaks in the library spectra. All of the library spectra are then sorted by the number of the m/z values in common with the m/z values of the user spectrum. Finally, high ranking spectra are returned from the pre-screening algorithm as candidates. After this prescreening, the spectra similarity score between each candidate spectrum and the query spectrum is calculated. Because the number of candidate spectra is much smaller than the total number of spectra in ADAP-KDB, search speed is improved substantially.

The performance of the library search with prescreening has been evaluated against the original search algorithm that is not equipped with the prescreening capability. A test file with a total of 1,110 spectra has been used to search against ADAP-KDB library and a total of 16 different prescreening settings have been studied to select the optimal setting. Overall, the prescreen algorithm speeds up the search time by more than 10 times while preserving the spectra as candidates that are very likely good matches to the query spectra.

Funding Sources:

NIH U01CA235507

129

Characterization of Withania somnifera extracts using LC-HRMS/MS in combination with GNPS networking analysis

Armando Alcazar Magana[1,2,3], Jaewoo Choi[1,3], Md Nure Alam[1], Mikah Brandes[1,4], Christine McClure[1,4], Amala Soumyanath[1,4], Jan F. Stevens[1,3,5], and Claudia S. Maier[1,2,3]

[1]BENFRA Botanical Dietary Supplements Research Center, Oregon Health and Science University, USA
[2]Department of Chemistry, Oregon State University, USA
[3]Linus Pauling Institute, Oregon State University, USA
[4]Department of Neurology, Oregon Health and Science University, USA
[5]Department of Pharmaceutical Sciences, Oregon State University, USA
alcazara@oregonstate.edu

Abstract

Withania somnifera (WS) is a medicinal plant used to support resilience to neurological changes associated with aging. WS produces a complex group of steroidal lactones known as withanolides that are considered as the main active compounds. However, non-standardized plant extraction procedures create several challenges in confirming botanical integrity, achieving batch-to-batch reproducibility. The phytochemical composition of a plant extract will govern not only the bioactivity and potential health benefits but also the reproducibility of clinical trials. Knowledge of chemical profiles and quantification of marker compounds for standardization purposes is necessary to minimize variability in biological response.

In this study, WS water and ethanolic extracts were analyzed by combining liquid chromatography with high-resolution mass spectral data acquisition. In brief, samples were resuspended in aqueous methanol 70% v/v (5 mg/mL), spun and analyzed by UPLC-HRMS. The chromatographic separation was achieved using an Inertsil phenyl-3 column (2 micron 150 X 2.1mm, GL Sciences) and the gradient elution was performed using solvent A, water containing formic acid 0.1% v/v and 10 mM of ammonium formate and B, acetonitrile containing 0.1% v/v formic acid in a 12 minutes chromatographic run.

More than 4,000 *m/z* molecular features (deconvoluted detected ions) containing MS/MS data were recorded using positive electrospray ion mode acquisition. Using the same raw data, we quantified nine phytochemicals by external calibration with authentic standards, including six withanolides. We uploaded the WS spectral data onto the Global Natural Products Social Molecular Networking (GNPS) algorithm. The GNPS algorithm creates a network of structurally related compounds whose nodes are connected by similarity in their mass fragmentation patterns. This procedure adds in dereplication and may guide prioritization of further structure elucidation efforts by orthogonal techniques such as NMR spectroscopy and testing of bioactivity.

Funding Sources (if applicable):

National Center for Complementary and Integrative Health (NCCIH) and the Office of Dietary Supplements (ODS)

130

Effects of food processing on the fatty acids composition of ultra-processed foods

Lisaura Maldonado-Pereira[1,2], Nama Naseem[2], Ashley Xu[2], Lisa Zou[2], Grant Gmitter[2], Ilce Medina-Meza [1,2]

[1]Department of Chemical Engineering, Michigan State University, USA
[2]Department of Biosystems and Agricultural Engineering, Michigan State University, USA,
maldon60@msu.edu

Abstract

Ultra-processed foods (UPFs), which comprise up to 60% of the calories in the American's daily diet, have been recently studied because of their relationship with several chronic diseases because of their poor nutrient content and their high unhealthy ingredient loads. Even though different processing techniques are employed to secure food safety standards and federal regulations, over-processing may impact their nutritional quality. Fatty acid profile of 69 UPFs (39 Ready to Eat (RTE) items and 23 Fast Foods (FF) meals) was completed by means of GC-FID. SFA percentage was higher in RTE ($p<0.05$) while MUFA percentage was higher in FF ($p<0.05$). A higher percentage of PUFA was observed for FF compared to RTE, however, it was not statistically significant. Seven SFA (C10, C11, C14, C15, C16, C17, and C18) and 2 MUFA (C18:1trans, and C20:1) were significantly higher in RTE ($p<0.05$). Meanwhile, C18:1cis and C20:2 were significantly higher in FF. SFA content was higher in dairy products, while MUFA contents were similar in meat and poultry, and baby foods groups. PUFA content was higher in egg and eggs derivatives group. Even though, these fatty acids are common in every animal derived product, the difference in FAME percentages between RTE and FF suggests a potential effect of the over-processing methods employed during their confection. UPFs are rich on sodium, sugar, and unhealthy fats which have adverse health effects if consumed in a regularly basis. Type and levels of processing during food manufacturing is an additional factor that must be considered in terms of food quality. This highlights a potential health risk in long-term consumption of these products. Thus, the FAME profiling of UPFs will provide more insight to the actual FAME load of these compounds because of UPFs processing.

Funding Sources (if applicable):

131

Targeted metabolomics identifies high performing diagnostic and prognostic biomarkers for COVID-19

Rupasri Mandal[1], Jiamin Zheng[1], Lun Zhang[1], Douglas D Fraser[2], Yamilé López-Hernández[3] and David S Wishart[1]

[1]Department of Biological Sciences, University of Alberta, CA
[2]Lawson Health Research Institute, CA
[3]Cátedras-CONACyT, National Council of Science and Technology, MX
rmandal@ualberta.ca

Abstract

Coronavirus disease 2019 continues to spread rapidly with high mortality. Research exploring the development and outcome of COVID-19 infections has led to the need to find better diagnostic and prognostic biomarkers. We performed serum metabolomics profiling of critically ill coronavirus disease 2019 patients to understand better the underlying pathologic processes and pathways, and to identify potential diagnostic/prognostic biomarkers. Here we studied two cohorts from Canada and Mexico. For the Canadian cohorts blood samples were collected from patients until either testing was confirmed negative on ICU day 3 (COVID negative) or until ICU day 10 if the patient tested positive (COVID positive). For the Mexican cohort, blood specimens were collected within two days after admission on average. Targeted quantitative metabolomics was used to identify and determine the concentration of different endogenous metabolites including amino acids, biogenic amines and derivatives, acylcarnitines, lipids and organic acids using a reverse-phase liquid chromatography-mass spectrometry (LC-MS)/MS custom assay. For both cohorts, metabolomics profiling with feature classification easily distinguished both healthy control subjects and COVID negative patients from Covid positive patients. Arginine/kynurenine ratio and kynurenine: tryptophan ratio accurately identified coronavirus disease status, whereas creatinine/arginine ratio accurately predicted COVID associated death. Administration of tryptophan (kynurenine precursor), arginine, sarcosine, and/or lysophosphatidylcholines may be considered as potential adjunctive therapies. Through our analysis we were able to propose a COVID-19 diagnostic panel consisting of three metabolites including the kynurenine: tryptophan ratio, LysoPC a C26:0, and pyruvic acid to discriminate controls from not hospitalized with a high AUC (0.947 (0.931–0.962).

Funding Sources (if applicable):

- CONACyT grant 311880 and Autonomous University of Zacatecas, Mexico
- Funding from Western University (Research), the Department of Medicine and Department of Pediatrics at Western University, Canada
- Genome Canada, Canada Foundation for Innovation

132

Investigating the mechanistic roles of oligofructose-enriched inulin in reducing body fat of overweight and obese children

Salma OA Omardeen[1], Shyamchand Mayengbam[1]

[1]Department of Biochemistry, Memorial University of Newfoundland, Canada
smayengbam@mun.ca

Abstract

Prebiotics, a type of fermentable dietary fiber, are known to mitigate several metabolic diseases, including obesity. They stimulate the growth of beneficial gut microbiota and normalize metabolic reactions. A specific prebiotic known as oligofructose-enriched inulin demonstrated reduced body weight and improved fat metabolism in overweight and obese children. In this study, we aimed to investigate novel mechanisms by which oligofructose-enriched inulin improved fat metabolism. Serum and fecal samples of overweight and obese children treated with oligofructose-enriched inulin (prebiotic, n=22) or maltodextrin (placebo, n=20), from a previous clinical trial study, underwent a mechanistic investigation by the use of metabolomics and gut microbiota approaches. For serum metabolomics, we used a Partial Lease Square Discriminant Analysis to identify ten significant metabolites that were altered in the presence of oligofructose-enriched inulin. Metabolites such as D-glucose, L-Lysine, Aspartate, L-Glutamine, and L-Glutamic acid were significantly reduced. Furthermore, pathway analysis revealed modulation of six metabolic pathways due to oligofructose-enriched inulin, such as, Aminoacyl-tRNA biosynthesis; Alanine, Aspartate and Glutamate metabolism; and D-glutamine and D-Glutamate metabolism. For fecal gut microbiota profiling, we used Linear discriminant analysis Size Effect and identified seven significant bacteria belonging to the Oscillospiraceae and Lachnospiraceae families. The relative abundance of these bacteria significantly increased with the consumption of oligofructose-enriched inulin. Lastly, a correlation analysis revealed negative associations between the identified metabolites and gut microbiota. Our data provide evidence that a gut microbiota-linked modulation of amino acid absorption and metabolism could play a significant role in downregulating the lipid synthesis in overweight and obese children.

Funding Sources (if applicable):

Memorial University of Newfoundland Start-up fund

133

How exposome research is informing precision medicine and precision nutrition

Susan McRitchie[1,2], Xiuxia Du[1,4], Colin Kay[1,3], Yuan Li[1,2], Wimal Pathmasiri[1,2], Blake Rushing[1,2], Madison Schroder[1,2], Aleksandr Smirnov[1,4], Susan Sumner[1,2], Timothy Fennell[1,5]

[1]NIEHS North Carolina Human Exposure Analysis Resource (HHEAR) Hub, USA
[2]Nutrition Research Institute, University of North Carolina at Chapel Hill, USA
[3]Plants for Human Health Institute, North Carolina State University, USA
[4]University of North Carolina at Charlotte, USA
[5]RTI International, USA
susan_mcritchie@unc.edu

Abstract

The NIEHS North Carolina Human Exposure Analysis Resource (HHEAR) Hub brings together 3 UNC System research groups located on the North Carolina Research Campus (NCRC), and RTI International, a nonprofit research organization located in the Research Triangle Park. Our team uses untargeted metabolomics to detect tens of thousands of signals for molecules that are present in human biospecimens (such as urine and blood) to define an individual's exposome, which differs depending on their genetics, diet, use of supplements or natural products, medications, drugs of abuse, tobacco products or e-cigarettes, stress, and exposures to chemicals in our foods and beverages, in cosmetics and personal hygiene products as well as exposures from cleaning and agricultural products used in the workplace, home and garden, and environmental pollution. Examples will be shown for how exposome research is informing both precision medicine, which focuses on how well some individuals respond to a treatment, and precision nutrition, which focuses on individual differences in nutrition requirements and responses to nutrient intake.

Funding Sources (if applicable):

The NIH NC HHEAR Hub is funded by grant 1U2CES030857 (Sumner, Fennell, Du, MPIs)

134

Metabolomics analysis of asthma phenotypes across multiple cohorts

Kevin M. Mendez[1], Mengna Huang[1], Rachel S. Kelly[1], Priyadarshini Kachroo[1], Su H. Chu[1], Djøra I. Soeteman[1,2], Stacey N. Reinke[3], Craig E. Wheelock[4], Clary B. Clish[5], Scott T. Weiss[1], Jessica A. Lasky-Su[1]

[1]Channing Division of Network Medicine, Brigham and Women's Hospital and Harvard Medical School, USA
[2] Center for Health Decision Science, Harvard T.H. Chan School of Public Health, USA
[3]Centre for Metabolomics & Computational Biology, School of Science, Edith Cowan University, AU
[4]Department of Medical Biochemistry and Biophysics, Karolinska Institutet, SE
[5] Broad Institute of MIT and Harvard, Cambridge, Massachusetts, USA
kevin.mendez@channing.harvard.edu (K.M.M.)

Abstract

Childhood asthma is a heterogeneous disease characterized by traits of lung function, allergy, airways hyperresponsiveness, eosinophils, and neutrophils, amongst others. In the development of more personalized approaches for treatment, the identification of metabolites relevant both broadly across a spectrum of disease phenotypes and specifically for particular disease characteristics will likely prove important. In this study, we utilized the plasma metabolomic profiling of two TOPMed childhood asthma cohorts; the Childhood Asthma Management Program (CAMP) clinical trial (n = 865) and the genetic epidemiology of the Costa Rica cohort (CRA) (n = 1,155). Using a meta-analysis approach, we identified multiple metabolomic pathways that were associated both within and across the asthma phenotypes, including metabolites of histidine, taurine, and tryptophan metabolism.

We also utilized 5 external cohorts (n=3,226) to assess the generalizability of the findings to adult asthma populations, broader adult populations, and broader childhood. For example, taurine was significantly increased in children with eosinophil count ≥ 300 in CAMP (p-value = 0.04) and CRA (p-value = 1.8e-5), and this finding was replicated in the Unbiased Biomarkers in Prediction of Respiratory Disease Outcomes cohort (U-BIOPRED) (p-value = 5.2e-5).

Funding Sources (if applicable):

135

Solvent extraction method optimization for mycorrhizal fungal metabolome analysis

Chathuri U.G. Mohottige[1], Todd Mlsna[1], Richard Baird[2]

[1]Department of Chemistry, Mississippi State University, USA
[2]Department of Biochemistry, Molecular biology, Entomology and plant pathology, Mississippi State University, USA
Cug3@msstate.edu

Abstract

Mycorrhizae are essential fungi on earth, forming symbiotic mutual relationships with their host plant's root system. This symbiosis is vital to plant nutrition, and some studies estimate that 90% to 95% of land plants support mycorrhizal associations. This symbiotic relationship is hundreds of millions of years old, but there is not a comprehensive understanding of arbuscular mycorrhiza metabolome up to date. Furthermore, most fungal metabolomics extraction methods have been developed using yeast (S*accharomyces ceravicea*) as the model organism. Therefore, this study focused on optimizing a metabolite extraction method for mycorrhizal fungi. We used glass house-grown pot cultures with two separate compartments (hyphal and root compartment) to harvest mycorrhizal fungi. This presentation will present the comparison of the metabolite extraction efficiency and the protein precipitation efficiency of the different solvent extraction systems

Funding Sources (if applicable):

136

Enzymatic synthesis assisted discovery of proline-rich macrocyclic peptides in marine sponges

Sam Moore[1], Ipsita Mohanty[1], Nguyet A. Nguyen[1], Jason Biggs[2], Neha Garg[1], Vinayak Agarwal[1], David A. Gaul[1]

[1]School of Chemistry and Biochemistry, Georgia Institute of Technology, Atlanta, GA 30332
[2] University of Guam Marine Laboratory, UOG Station, Mangilao, Guam 96923

Abstract
Proline-rich macrocyclic peptides (PRMPs) are natural products present in geographically and phylogenetically dispersed marine sponges. PRMPs have been identified as potential future therapeutics and scaffolds for novel drug discovery processes. However, common natural product isolation and structural elucidation strategies cannot be utilized effectively due to the large diversity and low abundance of PRMPs in sponge biomass. Here, we present results of a liquid chromatography – mass spectrometry (LCMS) method to separate and sequence native PRMPs in marine sponges containing one to three Leu/Ile residues.

Sponge extracts were generated from *Stylissa sp.* and *Axinella sp.*, while PRMP standards were enzymatically generated by cyclizing linear substrates with PCY1. Samples and standards were separated using ultra high performance liquid chromatography equipped with a Thermo Scientific Accucore C8, C30, or Phenyl-X column. A Thermo Scientific ID-X Tribrid Mass Spectrometer collected MS^n spectra for sequencing analysis of PRMPs. A combination of HCD and CID fragmentation methods were required during the MS^n workflow to appropriately produce and activate sequential product ions containing Leu/Ile residues of interest. Once the 86 *m/z* Leu/Ile immonium ion is generated from a precursor ion, the immonium ion can be activated with a lower HCD energy of 15% and discriminate between Leu and Ile.

Developed LC-MS^n methods useful in separating and sequencing the cyclic pepetides. Optimized methods were used to analyze standard peptides generated by PCY1 cyclization with single, double, or triple isomeric residues (Leu/Ile). At least twelve unique PRMPs were detected in the sponge biomass, where seven were identified to be novel sequences. The LCMS workflow demonstrates a streamlined and more sensitive approach to cyclopeptide sequencing compared to other methods for structural elucidation.

Funding Sources (if applicable):

137

NMR based metabolic profiling distinguishes the differential impact of capture techniques on wild bighorn sheep

Galen O'Shea[1], Rashell Lambert[2], Brian Tripet[1], James Berardinelli[2], Jennifer Thomson[2], Robert Garrott[2], and Valérie Copié[1]

[1]Department of Chemistry and Biochemistry, Montana State University, USA
[2]Department of Animal and Range Sciences, Montana State University, USA
[3]Fish and Wildlife Ecology and Management Program, Department of Ecology, Montana State University, USA
galenoshea@gmail.com

Abstract
Wild ruminants are fundamental elements of healthy ecosystems and of these, bighorn sheep (*O. canadensis)* are iconic in Western North America. Limited tools are currently available to wildlife scientists to assess the nutritional health and disease status of wild bighorn sheep. The subjective and qualitative assessment tools currently used by wildlife biologists provide limited understanding of wildlife-habitat relationships and the etiology of respiratory disease, which are major contributing factors to the health and demographic vigor of wild bighorn sheep. Herein, ^{1}H NMR metabolomics has been employed to determine the impact of several capture practices on the serum metabolic profiles of wild bighorn sheep. An overarching goal is to provide a suite of more quantitative analytical tools that could be used to more closely monitor the physiological status of this wildlife species.

As free ranging, wild animals must be captured to obtain biological samples for analysis, this study aimed to examine the impact of different capture techniques (helicopter, dart, or net) on the serum metabolomes of wild bighorn sheep over the course of three years. Untargeted ^{1}H NMR metabolomics was employed to characterize polar metabolite serum profiles of 543 individual animals. These analyses enabled to identify characteristic metabolite patterns that separated distinct groups of wild animals based on capture techniques. Our findings suggest that when designing such studies that require the capture of wild animals, it may be prudent to employ a single capture technique, if possible, to reduce confounding factors that may alter serum metabolome profiles. This is supported by our analysis which shows differences in capture techniques appears to significantly impact the serum metabolomes of these animals. Furthermore, our data demonstrate the utility of employing metabolomics in wildlife ecology and conservation, providing a first example of its applications to the study of wild ungulates.

Funding Sources (if applicable):

Funding and scholarships were provided by Montana State University, the Montana Agricultural Experiment Station, and the National Institute of Food and Agriculture, U.S. Department of Agriculture, Hatch Project under Accession No 1009680. V.C and G.O.S were supported in part by the NSF research program (NSF Grant No. MCB-1714556). Funding for NMR instrument acquisition and upgrades were provided by the NIH SIG program (Grant No. 1S10RR13878 and 1S10RR026659). Financial support for the NMR Center at MSU and its recent NMR equipment was provided by the National Science Foundation (Grant No. NSF-MRI:DBI-1532078), the Murdock Charitable Trust Foundation (Grant No. 2015066:MNL), and the Vice President for Research and Economic Development's office at MSU.

138

Expanding the tropane alkaloid metabolic network: metabolite discovery in *Atropa belladonna*

Hannah M. Parks[1], Maris A. Cinelli[1], Josh M. Grabar[2], A. Daniel Jones[1], Cornelius S. Barry[2]

[1]Department of Biochemistry and Molecular Biology, Michigan State University, USA
[2]Department of Horticulture, Michigan State University, USA
parksha2@msu.edu

Abstract

Understanding how metabolic pathways emerge and networks evolve is a key challenge in biology. Advances in high resolution mass spectrometry, coupled with multivariate statistical analyses, provides a powerful platform for metabolite discovery and we are combining these approaches with gene-silencing to reveal novel insight into plant specialized metabolism. Tropane alkaloids are a pharmacologically important class of plant specialized metabolites synthesized by just a few plant families. *Atropa belladonna* (Solanaceae) synthesizes the anticholinergics hyoscyamine and scopolamine through a multistep pathway that was recently elucidated. However, little is known about the alternative metabolic fates of tropane pathway intermediates or their role in generating novel metabolic diversity. Virus-induced gene silencing (VIGS) was utilized to silence key tropane pathway genes in *A. belladonna* roots and the metabolite profiles of these plants were captured using liquid chromatography coupled with mass spectrometry (LC-MS). Orthogonal projections to latent structures discriminant analysis (OPLS-DA) was used to identify metabolites that differ between control and silenced lines, revealing the existence of many novel metabolites that arise from the repurposing of tropane alkaloid pathway intermediates. These metabolites were targeted for downstream analyses, including liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) experiments, compound purification, structural characterization by nuclear magnetic resonance (NMR) spectroscopy, and pathway reconstruction in *Nicotiana benthamiana*. These data highlight the potential of utilizing non-targeted metabolite profiling of silenced lines for advancing the discovery of novel compounds and improving understanding of metabolic networks.

Funding Sources (if applicable):

NSF IOS-1546617
NIH Grant Number T32-GM110523

139

Assessment of metabolism and microbiome of infants at 6 weeks of age and the relationship to delivery mode and feeding type

Wimal Pathmasiri [1], Anne Hoen [2, 3], Modupe Coker [4], Juliette Madan [2, 3, 5], Susan McRitchie [1], Erika Dade [2], Brett Doherty [2], Susan Sumner [1], Margaret Karagas [2, 3]

[1]Department of Nutrition, Nutrition Research Institute, University of North Carolina at Chapel Hill, USA
[2]Department of Epidemiology, The Geisel School of Medicine at Dartmouth, USA
[3]Center for Molecular Epidemiology, The Geisel School of Medicine at Dartmouth, USA
[4] Department of Oral Biology, Rutgers School of Dental Medicine, USA
[5]Departments of Pediatrics and Psychiatry, Children's Hospital at Dartmouth, USA
wimal_pathmasiri@unc.edu

Abstract

Background: The intestinal microbiome has a critical role in infant development, with vital functions for nutrient metabolism and maturation of the immune system. We assessed the metabolism and microbiome of infants at 6 weeks of age and the relationship to delivery mode (e.g., vaginal, c-section) and feeding type (e.g., breast-feeding, formula, mixed).

Methods: Subjects were selected from the NHBCS mother-infant dyads. Delivery mode information and feeding practices during the first six weeks of life were obtained from delivery medical records and telephone survey data, respectively. 1H NMR metabolomic profiling was conducted on infant stool samples collected at six weeks of age. Select microbe-host co-metabolites were identified and their relative concentrations were determined. Multivariate data analyses were applied to examine discrimination of study groups defined by delivery mode and feeding type, and differences in individual metabolite concentration and pathway abundances were evaluated.

Results: We found that formate and lactate levels were significantly lower while maltose level was significantly higher in stool samples from Cesarean section delivered infants compared to vaginally delivered infants and linked to perturbations in pyruvate metabolism and glyoxylate and dicarboxylate metabolism, starch, and sucrose metabolism. Propionate, malonate, butyrate, and lysine were among the metabolites that were significantly lower in formula fed infants compared to exclusively breast-fed infants and linked to perturbations in amino acid, sugar, lipid, vitamin, purine and pyridine metabolic pathways.

Conclusion: Microbial communities colonizing the gastrointestinal tracts of infants are not only taxonomically, but also functionally distinct when compared according to delivery mode and feeding type groups. Furthermore, different sets of metabolites and metabolic pathways defined delivery mode and feeding type metabotypes.

Funding Sources (if applicable):

This research was funded by the National Institutes of Health National Institute of Environmental Health Sciences, grant numbers P01ES022832 and P20ES018175, the National Institute of General Medical Sciences, grant numbers R01GM123014 and P20GM104416, the National Library of Medicine, grant numbers K01LM011985 and R01LM012723, the National Institute of Diabetes and Digestive and Kidney Diseases, grant number U24DK097193, NIEHS HHEAR Program 1U2CES030857, and the Office of the Director, grant number UH3OD023275, and by the US Environmental Protection Agency, grant numbers RD-83544201 and RD-83459901. The APC was funded by the National Institutes of Health.

140

MetaboSPAN: an R package for network-based pathway analysis of metabolite data

Andrew Patt[1*], Kevin Coombes[2], Ewy A. Mathé[1]

[1]Division of Preclinical Innovation, National Center for Advancing Translational Sciences, USA
[2]Biomedical Informatics Department, The Ohio State University, USA
andrew.patt@mail.nih.gov

Abstract

While there are many existing pathway analysis strategies, most tests do not account for differences in metabolite coverage between experiments, which can lead to poor reproducibility of findings. To address this gap, we developed a novel network method for pathway/chemical enrichment analysis of a list of metabolites/lipids of interest called MetaboSPAN.
In our method, metabolites/lipids that share pathway annotations with the set of interest are extracted from our RaMP 2.0 database, which harmonizes metabolic pathway annotations from multiple metabolic pathway databases. We then quantify the proportion of shared pathway annotations the database metabolites share with the list of interest and with each other, represented as similarity network models. To identify subnetworks enriched in the metabolites of interest ("seed" set), we run a Markov random walks with restarts algorithm that uses the seed set as starting points in the network to generate a linear measure of distance between each node in the network and the seed set. Lastly, we extract metabolites that were highly proximal to the seed set, generating a list of metabolites that are subsequently evaluated for enrichment analysis.
We performed several simulation experiments for validation. First, we compared the number of significant pathways returned by each test in randomized data, which serve as negative controls. Second, we compared the recall rate of significant pathways that had been purposely seeded in simulated data. Third, we compared the ability of each test to recapitulate findings in an unaltered dataset when samples were removed. Lastly, we ran each test on two independent datasets generated from the same cohort on different platforms, and compared the similarity in findings between sets. Overall, we found that MetaboSPAN was able to improve the sensitivity of the Fisher's exact test in some contexts, while maintaining a lower false positive rate than other tests.

Funding Sources (if applicable):

141

Lipidomic profiling of COVID-19 patients' plasma by timsTOF Pro PASEF

Jun Yang[1], Aaron Peng[1], Xuejun Peng[2], Lucy Woods[2]

[1]University of California-Davis, USA
[2]Bruker Scientific LLC, San Jose, CA, USA
Xuejun.peng@bruker.com

Abstract

One of the key pathways regulating the immune response to COVID-19 infection is the release of regulatory lipid mediator which leads the dysregulation of lipid metabolism and causes the alteration of blood cholesterol and lipoprotein homeostasis. All suggested lipids can be involved in SARS-CoV-2 pathogenesis. In this work, a non-targeted Elute UHPLC-timsTOF Pro PASEF lipidomics workflow was performed on both ESI positive and negative modes to profile lipids and their changes within 30 plasma samples from six COVID-19 patients over five days. Plasma samples were prepared with methanol/MTBE extraction method and the dried extracts were resuspended with 100µL of methanol/dichloromethane (9:1) prior to injection (n=5). Data analysis was conducted in DataAnalysis 5.3 and MetaboScape 2022 (Bruker).

PASEF provides fast MS/MS acquisition speeds at full sensitivity following ion mobility separation to deeply profile low abundant lipids with DDA. Peak findings of plasma data were performed in MetaboScape with the T-ReX®4D algorithm applied for automatic feature extraction and alignment. The ion mobility $1/K_0$ and CCS of each ion were calculated. Lipids were annotated with rule-based lipid class annotation and LipidBlast database searching based on mass accuracy, isotope pattern matching, MS/MS, and CCS. Lipid annotation quality was further evaluated by mass error, mSigma of isotope pattern matching value, MS/MS score and ΔCCS error to exclude redundant and false results. Lipid Kendrick MassDefect Plot of DG, TG, LPC/PC, CE, Cer and SM etc. were analyzed for the deep analysis of annotated lipid classes and lipids. PCA and clustering analysis were conducted to identify potential lipid biomarkers between patients and from same patient over 5-days. Some lipid mediators exhibited irregular levels in COVID-19 patients, such as linoleic acid, oxylipins, epoxy-octadecenoic acids and dihydroxy-octadecenoic acid. Free fatty acids such as omega-3 fatty acids, EPA and DHA were also investigated.

Funding Sources (if applicable):

142

Blood metabolite profile and risk of estrogen receptor negative breast cancer: A meta-analysis

Mary C. Playdon [1,2,3], Benedikt Hauner [2,3], Jennifer Sinnott [3,4], Ying Wang [5], Marji McCullough [5], Mia Gaudet [5], Ph.D., Danxia Yu [6,7], Xiao Ou Shu [6,7], Steven C. Moore [1]

[1]Metabolic Epidemiology Branch, Division of Cancer Epidemiology and Genetics, National Cancer Institute, MD, USA
[2]Department of Nutrition and Integrative Physiology, University of Utah, USA
[3]Cancer Control and Population Sciences, Huntsman Cancer Institute, UT, USA
[4]Department of Statistics, Ohio State University, USA
[5]Epidemiology Research, American Cancer Society, GA, USA
[6]Vanderbilt Ingram Cancer Center, USA
[7]School of Medicine, Vanderbilt University, USA

Abstract

Background
Metabolite profiles can unveil important risk exposures and the mechanisms underlying breast cancer etiology, yet few studies have characterized metabolites associated with estrogen receptor (ER)-negative breast cancer.
Methods
We meta-analyzed associations of 827 blood metabolites with risk of ER-negative breast cancer in three nested case control studies from the Prostate, Lung, Colorectal and Ovarian cancer cohort, Cancer Prevention Study II and Shanghai Women's Health Study.
Results
In an analysis of 314 cases and 326 controls, blood metabolites were not associated with ER-negative breast cancer at a false discovery rate <0.05. Forty-two metabolites were nominally associated at p<0.05. Odd ratios ranged from 0.24-3.06. The strongest named metabolite association was for glycerophosphoethanolamine (OR=0.32, 95% CI=0.16, 0.64), a plasmalogen cell membrane antioxidant. In addition to other metabolites involved in oxidative stress response (e.g., cysteine-glutathione disulfide, OR=3.06, 95% CI=1.43, 6.57), top associated metabolites were derived from diet: dairy fat (caprate (10:0), OR=1.96, 95% CI=1.07, 3.57), fish (3-Carboxy-4-methyl-5-propyl-2-furanpropionic acid, OR=0.51, 95% CI=0.28, 0.93), microbial metabolism of meat (o-cresol-sulfate, OR=0.49, 95% CI=0.28, 0.85), and other foods of known relevance to cancer. Other biologically plausible associations implicated gut microbial metabolism of tryptophan (e.g., indoleacetylglutamine (OR=0.39, 95% CI=0.19, 0.79), 4-allylphenol sulfate (OR=0.43, 95% CI=0.21, 0.91)), inflammation (1-linolenoyl-GPC (18:3) (OR=0.44, 95% CI=0.23, 0.87)), and nucleotide salvage (uridine (OR=0.52, 95% CI=0.29, 0.93), urate (OR=0.54, 95% CI=0.30, 0.95)). Evidence for heterogeneity was not strong (32 metabolites had I2 <0.25; five 0.25 to <0.50; four 0.50 to 0.75; and 2 >0.75).
Conclusions
Blood metabolites were not associated with ER-negative postmenopausal breast cancer in this population of postmenopausal white and Asian women after statistical adjustment for multiple testing. Some nominal associations do have etiological and biological plausibility, however. Larger, consortium-based analyses may be needed to reliably detect such associations.

Funding Sources (if applicable):

1K99CA218694-01 /CA/NCI NIH HHS/United States (M.C. Playdon)

143

A complete solution for targeted lipid analysis using a smart compact LC-ToF

Giorgis Issac[1], Lisa Reid[2], Lee Gethings[2], Jayne Kirk[2]

[1]Waters Corporation, Milford, MA, USA
[2]Waters Corporation, Wilmslow, UK
lisa_reid@waters.com

Abstract

Routine lipid analysis of blood and tissue samples in biomedical research requires a robust, rapid and simple analysis of identified lipids of interest. Once a potential biological marker of disease has been hypothesized and identified a focused analysis for specific lipids of interest, or class changes, can be performed to test the validity of the biomedical hypothesis. This can be achieved using simple sample preparation, non-expert instrument usage and easy to use semi-automated data processing.

To demonstrate: cancer vs healthy human plasma samples, NIST plasma and Avanti SPLASH lipidomic standards were protein precipitated, separated using reversed-phase UPLC and analyzed in ESI^+ on the ACQUITY RDa™ Detector. Data were acquired and processed using UNIFI™ software with targets identified against a curated lipid library. The workflow generated sensitive, accurate and robust data, and was processed simply and rapidly with the commercially available peak identification and data comparison software packages.

The robustness, mass accuracy, dynamic range and limit of detection (LOD) of the workflow was assessed with a continuous acquisition totaling >6 days. Over the entire analysis this workflow demonstrated excellent chromatographic retention consistency, with peaks of interest varying by a maximum of 0.81%. The signal intensity and peak area showed a <10% deviance in both signal count and calculated analyte response, the mass accuracy for the 9 standards averaged ±6.5 ppm. Data comparison processing simplicity and efficacy was demonstrated using plasma taken from healthy donors and cancer patients.

This workflow: delivered by the ACQUITY RDa MS platform, has great potential to fulfill a range of basic lipid screening requirements. Combining the complete instrument set-up, acquisition and processing package allows for greater efficiencies and a more automated approach, improving the accessibility of mass spectrometry analysis, thus enabling a wider range of scientists and organizations to utilize these techniques.

Funding Sources (if applicable):

144

Elucidation of unknown metabolites using a new NMR/MS combinatorial approach

Nicholas Rigel[1], Abigail Leggett[2], Lei Bruschweiler-Li[3], Rafael Brüschweiler[4]

[1]Department of Chemistry and Biochemistry, The Ohio State University, USA
[2]Ohio State Biochemistry Program, The Ohio State University, USA
[3]Campus Chemical Instrument Center, The Ohio State University, USA
[4]Department of Chemistry and Biochemistry, Campus Chemical Instrument Center, and Department of Biological Chemistry and Pharmacology, The Ohio State University, USA
Rigel.17@osu.edu

Abstract

Metabolomics research is severely limited by the "dark metabolome", which are metabolites that have not been characterized previously or that are available in databases but without experimental spectra. Consequently, they cannot be included in biological analysis. In 2D ^{13}C-^{1}H HSQC NMR spectra, such "unknown" metabolites are manifested as cross-peaks that are not contained in metabolomics NMR databases. In order to characterize unknown metabolites, we designed the Motif Builder software, which constructs "molecular motifs", which are sub-structures representing an ^{1}H spin system using sub-motifs as building blocks taken from the COLMAR metabolomics database. Building motifs from COLMAR sub-motifs allows for the creation of existing and novel motifs described by experimental ^{13}C and ^{1}H chemical shifts. These motifs are then searched against a wider database, such as the HMDB, for putative metabolites that are a potential match for the unknown spin system. We will demonstrate the approach for a complex mouse urine mixture and show how true motifs can be reconstructed with high confidence using known metabolites before the Motif Builder is applied to unknown metabolites whose identity is confirmed by spiking experiments.

Funding Sources (if applicable):

145

Using UHPLC High Resolution Mass Spectrometry to Analyze Stool and Seminal Plasma

Blake Rushing[1], Yuan Li[1], Madison Schroder[1], Rachel Coble[1], Susan Sumner[1]

[1]Nutrition Research Institute, Department of Nutrition, University of North Carolina-Chapel Hill, USA
blake_rushing@unc.edu

Abstract

Untargeted metabolomics is a powerful tool to understand how metabolites and exposures are associated with states of wellness or disease. To accomplish this, methods must be developed to analyze multiple biospecimen types because exposure related compounds as well as metabolites of host and microbial metabolism may be localized within specific biological compartments under different states of health. This presentation will describe the identification and annotation of endogenous metabolites and exposure-related compounds in stool and seminal plasma matrices using a UHPLC Q-Exactive HFx High Resolution Orbitrap Mass Spectrometer (UHPLC-HR-MS). Pooled stool samples from vegan and omnivore participants were provided by NIST, and seminal plasma was provided by Dr. Richard Pilsner for untargeted exposome analysis. Over 10,000 signals were detected in each biospecimen using UHPLC-HR-MS. Signals were matched using in-house physical standards retention time, exact mass, and fragmentation library of over 2,200 compounds, as well as using public databases. These identifications and annotations revealed a diverse range of host and microbial metabolites, as well as multiple classes of exposure-related compounds including those from dietary sources, medications, drugs, and environmentally relevant exposures. Multivariate statistics and pathway analysis was performed to reveal biological differences between phenotypic groups for both matrices. Microbial-related pathways such as tryptophan and butanoate metabolism were significantly altered between vegan and omnivore diets. Additional perturbations were seen in many amino acid pathways such as aspartate, asparagine, arginine, and proline metabolism. Fatty acid/lipid metabolism as well as hormone metabolism were significantly perturbed in seminal plasma based on fertility status. This method development was funded by the NIEHS HHEAR Program 1U2CES030857.

Funding Sources (if applicable):

1U2CES030857.

146

Impact of microbially conjugated bile acids on the cultured murine gut microbiome

Madison M. Rzepka[1], Douglas V. Guzior[1], Kerri A. Neugebauer[1], Robert A. Quinn[1]

[1]Department of Biochemistry and Molecular Biology, Michigan State University, USA

Abstract

Bile acids (BAs) are compounds present in the gut that aid in digestion and absorption of nutrients and shape the gut microbiota. Primary BAs cholic acid and chenodeoxycholic acid are produced from cholesterol by the liver and are then conjugated by the enzyme bile acid-CoA:amino acid N-acyltransferase (BAAT) with either glycine or taurine as the last step in BA synthesis. Upon entry into the large intestine these conjugated BAs undergo transformations, such as deconjugation, by microbes to produce secondary bile acids. This balance between primary (conjugated) and secondary bile acids are important for outbreaks of gastrointestinal infection by pathogens such as *Clostridioides difficile*. It was recently discovered that gut microbes can also conjugate bile acids with different amino acids similar to BAAT resulting in the production of microbially conjugated bile acids (MCBAs), but is unclear if MCBAs can also be deconjugated. Here we explored the effects of MCBAs on microbiome structure in a cultured gut microcosm. In this experiment, murine fecal samples from wildtype and *BAAT* knockout mice were exposed to primary BAs and MCBAs in fecal culture medium to observe shifts in the microbiome community structure. 16S sequencing was used to identify differences in the presence and abundance of bacterial species following BA exposure. LCMS was performed on fecal extracts to identify if these fecal cultures can deconjugate BAs. This work will give insights into the role MCBAs play in the development of a dysbiotic gut as well as how our gut microbiota may transform them.

Funding Sources (if applicable):

147

Glucuronidation of metabolites and triglyceride accumulation as specific biomarker for alcohol induced liver disease in rat hepatocytes

IT Sakallioglu,[1] PG Thomes,[2,3] JL Kubik,[2,3] R Powers[1,4] and CA Casey[2,3]

[1]University of Nebraska-Lincoln, Department of Chemistry, USA
[2]University of Nebraska Medical Center, Department of Internal Medicine, USA
[3]Department of Veterans' Affairs, Research Service, Omaha, NE, USA
[4]University of Nebraska-Lincoln, Nebraska Center for Integrated Biomolecular Communication, USA
isin-tuna.sakallioglu@huskers.unl.edu

Abstract

Background: Fatty liver (steatosis) is an important component of the alcohol-induced liver disease (AILD) progression. We have previously reported results from our laboratories where we combined metabolomics with lipidomics, and were able to enhance the coverage of metabolic alterations to better define major metabolic and lipidomics changes that occur during ethanol administration which contribute to the development of liver steatosis. In the current studies we have extended our initial findings to provide more focus on two potential biomarkers (glucuronidation and triglycerides) for ALD. **Results**: Principal component analysis of three different data sets showed that there is significant difference between ethanol treatment and controls. Furthermore combined multivariate statistical analysis of the LC-MS^E and NMR data set indicates significant differences between the metabolomes of ethanol treated rat hepatocytes and untreated controls. A tentative network analysis identified five significantly changing metabolic pathways: glucuronidation of the metabolites, bile acid biosynthesis, squalene and cholesterol biosynthesis, linoleate metabolism and fatty acid metabolism. Combined univariate and multivariate statistical analysis on metabolomics agreed that the fatty acid metabolism and the glucuronidation of metabolites are the most altered metabolic pathways. These distinct findings in hepatocytes will aid our examination of alcohol's effect on the liver itself, and also provide for future examination of different cell types in the liver. **Conclusions and future work:** Combined analysis showed that glucuronidation of metabolites and triglyceride accumulation could be one of the specific biomarkers of the alcoholic liver disease in rat hepatocytes. Using a combined NMR and LC-MS^E approach, we can better characterize the metabolic impact of alcohol induced steatosis and identify the specific cell types where changes occurred. Our on-going studies with the identification of the significant metabolites and lipids will enhance the coverage of all the polar and non-polar metabolites and this will provide a comprehensive view of metabolic alterations due to ethanol exposure.

Funding Sources (if applicable):

148

Accurate mass spectral and retention index library for metabolomics based on quadrupole time-of-flight gas chromatography/mass spectrometry

Wasim Sandhu[1], Shunyang Wang[1], Luis Valdiviez[1], Tobias Kind[1], Honglian Ye[1] and Oliver Fiehn[1]

[1]West Coast Metabolomics Center, University of California-Davis, USA
wasandhu@ucdavis.edu

Abstract

Reference mass spectral libraries play an important role in compound identification of small molecules and metabolites. Databases for metabolic profiling commonly use derivatization techniques and retention time matching for GC/MS based studies. The use of high-resolution and accurate mass quadrupole-time-of-flight mass spectrometers makes it possible to use accurate mass spectral data to improve the confidence in compound annotation. Chemical ionization (CI) spectra obtained on the same instrument can be compared to commonly used electron ionization (EI). Chemical ionization as a soft ionization method allows for improved detection of molecular ions and subsequent elemental formula calculations. Here we present a new accurate mass spectral and retention index library with both electron ionization (EI) and chemical ionization (CI) mass spectra.

Metabolite reference standards were selected according to their frequency of occurrence from over 450 published GC-MS based studies at the Metabolomics Workbench website. Compounds were chemically derivatized before measurement including a methoximation and trimethylsilylation process. Fatty acid methyl ester retention indices (FAMEs) were utilized as retention index markers. To test the metabolite coverage of the library five mouse samples (brain, kidney, liver, plasma, and serum) were acquired by GC-QTOF and processed with the Agilent MassHunter software and compared to results from the publicly available MS-Dial software.

Accurate mass spectra and retention indices of 600 unique metabolites, totaling 800 individual entries are covered in this library. 25% of those spectra also have chemical ionization spectra recorded. Accurate mass data were also useful to verify trajectories predicted by theoretical fragmentation pathways using quantum chemistry molecular dynamics for electron ionization (QCEIMS). In routine metaboomics, over 100 compounds can be routinely annotated with high confidence. The library can be utilized for metabolic profiling experiments across a wide range of metabolic matrices.

Funding Sources (if applicable):

149

Qualitative characterization of NIST RM 8231- frozen human plasma suite for metabolomics

Tracey Schock[1], Clay Davis[1]

[1]National Institute of Standards and Technology, Chemical Science Division, Hollings Marine Laboratory, USA
Tracey.schock@nist.gov

Abstract

The field of metabolomics has prompted a new paradigm in metrological material development. Standard reference materials (SRM) are definitive physical sources of measurement traceability. Developing a metabolomics SRM, such as NIST SRM 1950 - Metabolites in Human Plasma, entails certification of multiple chemical properties across the complexity of the sample. Certification is an intensive process, more so for an untargeted material, and customer cost and accessibility increase with each value assignment. While certified quantitative values are ultimately necessary in validating clinical and candidate phenotypic biomarkers, the metabolomics community primarily requires quality control (QC) materials that establish method validations and analytical precision. Such materials evaluate and control systematic variance due to analyst, instrument performance, and intra/inter-laboratory protocols and do not require the stringent certification of an SRM in terms of SI traceable quantitative analyte determination. Consequently, a new generation of reference materials (RM) that are affordable and efficiently developed with qualitatively characterized metabolic profiles, are fundamental to untargeted metabolomics success.

The National Institute of Standards and Technology (NIST) is developing Reference Materials for Metabolomics Quality Control. A suite of human plasma RMs, comprising different metabolic health states (type 2 diabetes, hypertriglyceridemia, and normal young, African American) was designed to improve measurement harmonization, deep biomolecular profiling, differential analysis and data analytics for untargeted assessments. Here, we will present an important new metrology strategy to provide confidence levels for nominal metabolite identifications for each plasma within the RM suite. This process has the benefit of accelerating the RM development and certification procedures in delivering a fit-for-purpose material for the community at an affordable price.

Funding Sources (if applicable):

150

Oxidized complex lipids in bio-specimen by LC-DDA-MS/MS and iterative MS/MS

Tong Shen[1], Charles Evans[2], Jennifer E. Kyle[3], Thomas O. Metz[3], David A. Gaul[4], Facundo M. Fernández[4], Nathanial Chase Stevens[1], Uri Keshet[1], Raquel Cumeras[1,5], Oliver Fiehn[1]

[1]University of California-Davis, USA
[2]University of Michigan, USA
[3]Biological Sciences Division, Pacific Northwest National Laboratory, WA, USA
[4]School of Chemistry and Biochemistry, Georgia Institute of Technology, USA
[5]Institut d'Investigació Sanitària Pere Virgili, URV, CIBERDEM-ISCIII, CERCA, ES
tsshen@ucdavis.edu

Abstract

Oxidized lipids formed by enzymatic reactions or reactive oxygen species increase the complexity of the lipidome. Oxidized complex lipids bear significant biological functions, classic as oxidized cardiolipins in apoptosis, oxidized PE in ferroptosis, as well as oxLDL in association with inflammatory diseases including atherosclerosis, cardiovascular disease, pulmonary, renal, and liver diseases. Oxidized lipids are challenging to structurally characterize due to the various forms and numbers of oxidative modifications. To classify the extent of endogenous lipid oxidation versus potential oxidation during sample preparation, four laboratories from the NIH Common Fund Metabolomics Program Consortium used untargeted LC-MS/MS lipidomics in different plasma and tissues and critically compared the results across experiments. We studied low abundance oxidized complex lipids by LC-DDA-MS/MS and iterative MS/MS. More than 100 oxidized complex lipids. RPLC separated oxidized forms from unmodified lipids by characteristic retention time differences (smaller XlogP values led to 1-1.5 min earlier RTs). Oxidized lipids were generally of lower abundance but clearly detected by iterative MS/MS. Isomers of oxidized lipids were readily distinguished by specific diagnostic fragmentation patterns into classes of hydroperoxides, hydroxides, epoxides, and others. We annotated oxidized lipids including oxFA, oxTG, oxPC, oxPE, ox ether PC, ox ether PE, oxPI, and oxCer. In porcine brain, we found distinctly higher levels of oxGlcCer. OxPE were relatively more abundant in plasma than organs. Across the different specimens, the ratio of OxTG and unmodified TG was statistically different, indicating physiological regulation of the oxidation status rather than chemical autoxidation. Detected oxygenated species ranged from 1O to 3O with our current MS/MS libraries. Considering retention time modeling and specific fragmentation patterns of lipid species, we will highlight efforts into further expanding the coverage of oxidized lipids by MS/MS libraries in comparison to computational tools. Experimental tandem mass spectra will be uploaded to the open repository Massbank.us.

Funding Sources (if applicable):

151

Enhanced metabolite and lipid imaging by Desorption Electrospray Ionization (DESI) MS using novel high-performance sprayer and heated transfer line

Bindesh Shrestha[1], Anthony Midey[1], Hernando Olivos[1], Greg Roman[1]

[1]Waters Corporation, Milford, MA, USA

bindesh_shrestha@waters.com

Abstract

Desorption electrospray ionization (DESI) mass spectrometry (MS) excels at imaging drugs, metabolites, and lipids. Here, we show enhanced high-resolution imaging of metabolites and lipids using a novel high-performance sprayer and heated transfer line. DESI imaging MS is performed by directly extracting and ionizing analytes pixel-by-pixel from sample surface. Metabolite and lipids ions, such as lactate, glutamate, Phosphatidylcholines, phosphatidylethanolamine, were imaged off tissue sections without any sample preparation. The quality of the DESI image is directly related to the ability of the stable and highly focused electrospray to extract and ionize molecules from the tissue. A novel high-performance DESI sprayer was designed based on precision machined ion-key emitters (Waters). DESI solvent was guided through the sprayer by a sheathing nebulization with a concentric and stable spray dimension of ~ 20 µm. The stability and the size of the DESI spray were related to solvent composition, solvent flow, voltage, and nebulization gas flow. In addition to high resolution and faster image acquisitions, the high-performance sprayer also improved the ease-of-use due to the cartridge-style sprayer needing minimal user assembly. DESI ion source was coupled with a quadrupole time-of-flight mass spectrometer (e.g., SYNAPT XS, Cyclic IMS, MRT). DESI solvent was pumped using a binary LC pump (ACQUITY UPLC M-Class). After generating ions, an efficient ion transfer was achieved by heating the transfer line from 100 to 500 °C. The heated transfer line improved sensitivity up to an order of magnitude, enabling visualization of lower abundant molecules. In conclusion, the novel high-performance sprayer with the heated transfer line was capable of high-resolution DESI imaging of metabolites and lipids in rat brain tissue and chicken liver sections routinely at 50 microns.

Funding Sources (if applicable):

152

A novel pan-class I Glucose transporter inhibitor targets metabolism of A549 cancer cells *in vitro* and A549 xenograft tumors *in vivo*

Pratik Shriwas[1,2,3], Xiaozhuo Chen[2,3,4]

[1]Department of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, Ohio State University, USA
[2]Edison Biotechnology Institute, Ohio University, USA
[3]Biological Sciences department, Ohio University, USA
[4]Heritage college of Osteopathic medicine, Ohio University, USA
shriwas.2@osu.edu

Abstract

Metabolic reprogramming is considered to be a hallmark of cancer. Cancer cells opportunistically try to increase uptake of extracellular nutrients and this phenomenon is an emerging hallmark of tumor metabolism. Glucose is a key nutrient taken in by cancer cells for satisfying their high demands for energy (ATP production) as well biosynthetic needs. Cancer cells drastically increase glucose uptake, a phenomenon called the Warburg effect, by overexpressing membrane bound glucose transporter (GLUT) proteins. Cancer cells overexpress specific glucose transporters (GLUTs), particularly GLUT1, on their membranes to transport glucose via facilitated diffusion. It is now well known that targeting glucose uptake by inhibiting glucose transporters is an attractive anticancer strategy. In this study, we have characterized DRB18, a pan-class GLUT1 inhibitor targeting glucose uptake via GLUT1-4 as an anticancer agent. We have used LC-MS/MS based metabolomics technique to determine the effect of DRB18 on glucose-based metabolism in A549 cancer cells *in vitro* and A549 tumor xenografts generated in nude mice *in vivo*. We identified metabolites in several different pathways such as glycolysis, TCA cycle, nucleotide metabolism, reactive oxygen species (ROS), fatty acid synthesis among others which were altered due to DRB18 treatment. We found that DRB18 affected these metabolite abundances similarly as well as differentially *in vitro* and *in vivo*. DRB18 reduced glycolytic metabolites *in vitro* and *in vivo*. We found that in vitro DRB18 reduced metabolites in glutaminolysis and TCA cycle *in vitro* but in vivo, some these metabolites increased suggesting that *in vivo* tumor cells can reply on glutamine to compensate for DRB18 mediated inhibition of glycolysis. Similarly, DRB18 increased metabolites in ROS pathway suggesting that DRB18 induced oxidative stress to kill cancer cells. All these data indicate that metabolomics of cancer cells is different between *in vitro* and *in vivo* conditions. It depends on availability of nutrients in extracellular tumor microenvironment.

Funding Sources (if applicable):

The study was supported by a R15 NIH grant CA242177-01 to Dr. Xiaozhuo Chen (Ohio University). The authors would also like to acknowledge OSU campus chemical instrument center (NIH Award Number Grant P30 CA016058) for performing LC-MS/MS analysis.

153

ADAP-KDB Spectral Knowledgebase: an online resource for searching and prioritizing untargeted metabolomics data

Aleksandr Smirnov[1], Yunfei Liao[1], Eoin Fahy[2], Shankar Subramaniam[2],
Yuanyuan Li[3], Blake Rushing[3], Susan McRitchie, Susan Sumner[3], and Xiuxia Du[1]

[1]Department of bioinformatics and genomics, University of North Carolina at Charlotte, USA
[2]Department of Cellular and Molecular Medicine, University of California at San Diego, USA
[3]Department of Nutrition, University of North Carolina at Chapel Hill, Chapel Hill, USA
asmirno1@uncc.edu

Abstract
The number of metabolomics studies in NIH's Metabolomics Data Repository (NMDR) acquiring untargeted data from the liquid chromatography (LC-) and gas chromatography coupled to mass spectrometry (GC-MS) analytical platforms has been steadily growing. Accompanying this growth is the enormous number of known and unknown compounds contained in that data, which empower various cross-species, cross-diseases, and cross-sample source analyses. Toward this end, we have developed ADAP-KDB, a mass spectral knowledgebase that contains consensus GC-MS and LC-MS/MS spectra extracted from untargeted metabolomics data in NMDR. ADAP-KDB enables efficient searching and prioritization of information about both known and unknown compounds across studies and makes those compounds easily findable.

Users of ADAP-KDB can (*i*) browse all consensus spectra and link them to the NMDR studies where those spectra come from, (*ii*) match their unannotated mass spectra to the consensus spectra in ADAP-KDB, (*iii*) find the studies where those spectra have been observed with a high statistical significance, and (*iv*) filter studies based on species, sample source, and disease information. Moreover, users can perform a batch search to match an entire list of mass spectra to the consensus spectra in ADAP-KDB and find all studies with similar spectral composition.

In addition to searching against existing consensus GC-MS and LC-MS/MS spectra, ADAP-KDB can be used by labs to search against their own compound libraries. Users can create their own private accounts and upload their library files with spectral, structural, mass, and retention time information. Then, they can search their query spectra against public spectra and compounds in ADAP-KDB and/or their private libraries. ADAP-KDB provides capabilities to search based on spectral similarities, mass differences, and retention time. Moreover, it uses predefined ontology levels to select the best matching results based on evidence and convey the confidence level of every match.

Funding Sources (if applicable):

This research and development were made possible through NSF award 1262416, NIH U01CA235507, NIH U2CDK119886, and NIH U2CES030857.

154

The effects of a prebiotic-supplemented diet on the bacteriome and metabolome

Kyle D. Spencer[1,2,3,4], Robert Jaggers[4], Ewy A. Mathé[2], Michael T. Bailey[4,5,6,7]

[1]Graduate Partnership Program, National Center for Advancing Translational Sciences, NIH, USA
[2]Division of Preclinical Innovation, National Center for Advancing Translational Sciences, NIH, USA
[3]Department of Biomedical Informatics, The Ohio State University, USA
[4]Center for Microbial Pathogenesis, Abigail Wexner Research Institute at Nationwide Children's Hospital, OH, USA
[5]Oral and GI Research Affinity Group, Abigail Wexner Research Institute at Nationwide Children's Hospital, OH, USA
[6]Department of Pediatrics, The Ohio State University College of Medicine, USA
[7]Institute for Behavioral Medicine Research, The Ohio State University College of Medicine, USA
spencer.698@buckeyemail.osu.edu

Abstract

Dietary prebiotics have been shown to impact animal behavior, and we found that giving mice the prebiotic galactooligosaccharides (GOS) reduces anxiety-like behavior. However, these mechanisms are not well understood. Because prebiotics, such as GOS, are readily metabolized by the gut microbiota, we hypothesized that GOS causes alterations in the bacteriome and metabolome which affect anxiety-like behavior.

C57BL/6 male mice (6-8 weeks old) were fed a defined mouse diet or a defined diet with 10% GOS (w:w) (Control: n=5, GOS: n=6) for 3 weeks. Anxiety-like behavior was assessed with open-field testing. Colonic and serum samples were collected afterwards. Metabolome (Metabolon platform) and bacteriome sampling (16S) were performed on colonic samples and metabolomic analyses were performed on the serum samples from the same mice. The serum data was normalized by sample volume. The colonic and serum datasets were median rescaled and missing values were imputed with the minimum. The bacteriome data was processed using DADA2 and QIIME2 was used for amplicon processing, QC of the reads, and taxonomic assignment.

GOS-fed mice had an increase in inner zone time (IZT) in the open-field, which is consistent with a reduction in anxiety-like behavior. *Peptococcus* and *Streptococcus* were decreased in the GOS diet whereas *Lachnospiraceae* was increased (FDR-corrected p-value<0.2 and log2 fold-change>0.75). In the colon, 6 lipids were decreased by GOS, and 30 metabolites were differentially abundant in the serum. One serum metabolite, indole-3-acetate was increased in GOS mice and correlated with IZT. Indole-3-acetate is a microbial tryptophan derivative which can enter the bloodstream, cross the blood-brain barrier, and activate the astrocyte AhR receptor-ligand to affect behavior.

This study demonstrates that GOS-prebiotic alters murine microbe and metabolite abundances that are related to changes in anxiety-like behavior. Follow-up studies are in progress to determine whether bacterial-derived tryptophan metabolites are responsible for effects on anxiety-like behavior.

Funding Sources (if applicable):

155

Analyzing the Crabtree effect through metabolomics

Harini Sridharan[1], April Miguez[1], Mark Styczynski[1]

[1]Department of Chemical and Biomolecular Engineering, Georgia Institute of Technology, USA
hsridharan6@gatech.edu

Abstract

Saccharomyces cerevisiae is a eukaryotic model organism that is straightforward to engineer yet complex enough to model higher organisms with biotechnological and biomedical relevance. The metabolism of *S.cerevisiae* exhibits multiple interesting metabolic phenotypes under different environmental conditions, including the Crabtree effect. In the Crabtree effect, even in the presence of oxygen, cells still undergo mostly glycolytic fermentation rather than respiration that would provide more energy for the cells. This phenotype has major applications in a variety of biotechnology applications. Extensive study of the Crabtree effect has helped uncover the contribution of certain genes, but the larger scale contributions of other genes and the impacts on metabolism remain poorly understood. Metabolomics is a powerful tool that could provide insight into the underlying metabolic mechanisms of the Crabtree effect, which may in turn enable improved metabolic engineering approaches in yeast or give insights into related topics in human metabolism. We moved towards this goal by selectively deleting individual genes of importance in the central carbon metabolism, including both enzymes and transcription factors. Metabolic profiles of these engineered knockout strains were compared to a wild type strain, S288C, in exponential and stationary phase growth. The knockout strains exhibited a wide array of temporal metabolic profiles depending upon the deleted gene, for example, some (e.g. PDC1 knockout) had a distinct metabolic profile in its log phase as compared to the wild-type, while others (e.g. PDA1 knockout) had similar log phase metabolite profiles but distinct plateau phase profiles. Using both multivariate and univariate analyses, we were able to compare and contrast the different strains' profiles, identify individual metabolites with statistically significant behaviors, and generate new hypotheses about respirofermentative metabolism.

Funding Sources (if applicable):

National Institutes of Health (NIH) GM119701

156

Untargeted identification and analysis of oxidized complex lipids in mouse organs

Nathanial C. Stevens[1], Tong Shen[1], and Oliver Fiehn[1]

[1]Genome Center, University of California Davis, USA
ncstevens@ucdavis.edu

Abstract

Oxidized complex lipids are emerging as important bioactive mediators in vital cellular signaling roles such as apoptosis, ferroptosis, and innate and adaptive immune responses. Despite the increasing importance of this lipid class in cell biology, accurate and robust untargeted characterization of oxidized complex lipids remains a significant challenge. Lack of commercially available reference standards and inherent structural diversity have prompted the development of *in silico* MS/MS libraries to characterize compounds based on accurate mass and spectral matching. However, these approaches lack orthogonal information to improve the reliability of annotations and are limited in scope, failing to observe other chemically plausible sub classes of oxidized complex lipids. Our study details an alternative means for identification of oxidized complex lipids using untargeted lipidomics. We constructed a spectral library consisting of experimentally acquired MS/MS data from biological samples. Annotations were manually validated based on known lipid fragmentation rules and characteristic mass shifts in fragment ions corresponding to the addition of oxygen. We also used retention time as a criterion used for compound annotation, which improves upon previous methods only using accurate mass and spectral matching. This library was implemented in untargeted lipidomics analysis of liver, kidney, brain, and heart samples from mouse models. Importantly, we observed distinct differences in the relative abundance of multiple oxidized complex lipid subclasses between organs, including oxLPC, oxPC, oxPE, oxTG, and oxFA. These findings demonstrate that oxidized complex lipids are detectable using conventional untargeted lipidomics approaches and exhibit phenotypical differences depending on the type of organ, indicating that the origin of a major portion of the lipid oxidation is by biological regulation rather than by chemical mishaps in the laboratory. Our study provides a framework for subsequent untargeted analyses that could yield valuable insights regarding biologically important oxidized complex lipid species and identify targets for future mechanistic studies.

Funding Sources (if applicable):

Nathanial C. Stevens is supported by NIEHS Grant T32 ES007059. Compound identification was funded by NIH U2C ES030158.

157

Evaluation of natural products in Mamaki Tea using untargeted metabolomics

Alanna Sun[1,2], Arpana Vaniya[1], Oliver Fiehn[1]

[1]University of California Davis, West Coast Metabolomics Center, USA
[2]Waiakea High School, HI, USA

Mamaki tea, a native Hawaiian plant species, is best known for its medicinal properties. It is endemic to and geographically restricted to the Hawaiian Islands. Mamaki tea contains many types of antioxidants, including chlorogenic acid and catechin. Along with that, antioxidants were reported to play an important role in cancer chemoprevention and development. In previous years of research, Mamaki tea extract samples showed promising biological properties in anticancer and cancer chemopreventive assays. Normal-phase and reverse-phase flash column chromatography was used to purify extracts to identify the chemical basis and discover potential new natural products. We here have used untargeted metabolomics to analyze natural products in Mamaki tea leaves. Prior to this study, very little has been researched about this tea, especially using untargeted metabolomics.

Samples were extracted and analyzed using LC-MS/MS to identify secondary metabolites. Two different types of tea were used, fresh tea leaves from the farmer's market and store-bought tea leaves. Fresh tea leaves were lyophilized prior to extraction. The leaves were extracted using two different methods for comparison, first with 100% ethanol and 80:20 methanol/water. Samples were injected onto an Kinetex PFP liquid chromatography column and analyzed using LC-MS/MS on a Thermo Fisher Q Exactive HF mass spectrometer coupled to a Vanquish UHPLC. Iterative MS/MS was also acquired to expand the metabolite identification coverage. 13 rounds of iterative MS/MS were performed on the tea samples. The data was then analyzed using MS-DIAL 4.70 with m/z-RT library and MS/MS reference library from MassBank of North America (MoNA) combined with NIST 20 MS/MS. MS-FLO was used for post data curation. A total of 90 metabolites have been annotated so far which include benzenoids, organic heterocyclic compounds, and carboxylic acids.

Funding Sources (if applicable):

158

Metabolic reprogramming in mutant KRAS colorectal cancer cells

Dorna Varshavi[1,2], Dorsa Varshavi[1,2], Nicola McCarthy[3,4], Kirill Veselkov[5], Jeremy Everett[1]

[1]School of Science, University of Greenwich, UK
[2]Department of biological science, University of Alberta, CA
[3] Horizon Discovery, UK
[4]Milner Therapeutic Institute, University of Cambridge, UK
[5]Department of Surgery & Cancer, Imperial College London, UK
varshavi@ualberta.ca

Abstract

Colorectal cancer (CRC) is one of the leading causes of cancer-related death worldwide. KRAS mutations occur in approximately one-third of CRC tumours and have been associated with the poor prognosis and resistance to therapeutics. About 80% of KRAS mutations are heterozygous. However, it has been demonstrated that the wild-type KRAS gene is frequently lost during tumour progression in many types of cancer and hence may serve as a tumour-suppressor gene in the presence of mutant KRAS. Until now, little is known about the function of WT-KRAS in the context of cancer.

Here, we applied an explorative NMR-based metabonomics approach to obtain more comprehensive understanding of metabolic dysregulation driven by KRAS mutations and more importantly the role of WT-KRAS in cancer metabolism and oncogenic KRAS signalling through comparing the metabolic profile of parental KRAS $^{G13D/+}$ HCT116 cell line with its isogenic derivative cell lines KRAS $^{+/-}$ and KRAS $^{G13D/-}$.

In this study, a clear discrimination was observed not only between KRAS mutants (KRAS $^{G13D/+}$, KRAS $^{G13D/-}$) and wild-type (KRAS $^{+/-}$) but also between KRAS $^{G13D/+}$ and KRAS $^{G13D/-}$.

The findings of this study provide potential prognostic markers and new targets for the development of effective therapies against oncogenic KRAS.

Funding Sources (if applicable):

159

Discovery of an anti-obesogenic agent from the microbiome of a genetically modified mouse

Dorsa Varshavi[1,2], Sunil Veeravalli[3], Flora H. Scott[3], Dorna Varshavi[1,2], Ian R. Phillips[3,4], Jeremy R. Everett[1], Elizabeth A. Shephard[3]

[1]School of Science, University of Greenwich, UK
[2]Department of biological science, University of Alberta, CA
[3]Department of Structural and Molecular Biology, University College London, UK
[4]School of Biological and Chemical Sciences, Queen Mary University of London, UK
dvarshav@ualberta.ca

Abstract

Metabolomics is a powerful tool for studying the impact of genetic changes and the interplay of genetic and microbiome factors. We recently used an NMR-based metabonomic approach to investigate the impact of flavin monooxygenase 5 knockout (FMO5 KO) in C57BL/6 mice. These mice exhibit a remarkable phenotype of greater glucose tolerance and insulin sensitivity, low body fat and slow ageing, of potential relevance to both obesity and T2DM. NMR-based metabolite analyses of the urine of Fmo5-/- and wild-type mice identified a compound of microbial origin present in the urine of Fmo5 KO but not of WT mice. Subsequent treatment of WT mice with this compound mimicked some characteristics of the Fmo5 KO mouse phenotype e.g. statistically significant reductions in plasma cholesterol, plasma triglycerides and epididymal fat. This study demonstrates the importance of genome - microbiome interactions in determining clinical phenotypes.

Funding Sources (if applicable):

160

Predicting electron ionization mass spectra by excited state molecular dynamics

Shunyang Wang[1,2], Tobias Kind[1], Dean J. Tantillo[2], Oliver Fiehn[1]

[1]West Coast Metabolomics Center, University of California-Davis, USA
[2]Department of Chemistry, University of California, USA

Abstract

Compound identification by mass spectrometry needs reference mass spectra. While there are over 111 million compounds in PubChem, less than 300,000 curated electron ionization (EI) mass spectra are available from NIST or MassBank mass spectral databases. This gap must be closed by predicting spectra. QCEIMS is a quantum chemistry method to generate in silico EI mass spectra (MS) by combining molecular dynamics (MD) with statistical methods. Yet, on average, QCEIMS spectra overestimated the abundance of molecular ions and missed many fragment ions. Excited state molecular dynamics is a possible way to solve the problems.

We made several modifications to the QCEIMS v4.0 code to conduct excited state molecular dynamics. Default settings were applied for ground state calculations at the GFN2-xTB level. The MNDO99 program was used for excited state MD calculations at the semiempirical OM2/MRCI level. Excited state (D_1, D_2…) spectra were used as a correction to the ground state (D_0) spectrum according to the relative ratio obtained from the BEB model.

We considered the two lowest electronic states (D_0, D_1) as reference states, ignoring possible non-adiabatic crossing. Excited state calculations were performed to correct results from ground-state calculations, enabling straightforward extensions to calculations of other higher excited states in the future. We predicted QCEIMS spectra of ten intermediates from the histidine pathway. These excited state calculations successfully corrected ground-state QCEIMS models by adding on average 35% more fragment ions and improving the dot-score similarity score by 7% in comparison to GC-MS experimental data. We also investigated the effect of active space choice in MD. We found that a larger active space made the simulations more accurate, requiring to find a balance between active space and computational time. Overall, this work provides an affordable correction method based on excite state MD for better prediction of electron ionization-MS spectra.

Funding Sources (if applicable):

Funding for the “West Coast Metabolomics Center for Compound Identification” was provided by the National Institutes of Health under the award number NIH U2C ES030158.

161

Altered omega fatty acid derived lipid mediators in relapsing-remitting multiple sclerosis patients

Jeffrey Waters[1*], Insha Zahoor[1*], Indrani Datta[2], Theresa L. Pedersen[3], Mirela Cerghet[1], Laila Poisson[2], Silva Markovic-Plese[4], Ramandeep Rattan[3], John W. Newman[5,6,7], Ameer Y. Taha[3,8], Shailendra Giri[1]

[1]Department of Neurology,
[2]Department of Public Health Sciences,
[3]Division of Gynaecology Oncology, Department of Women's Health Services, Henry Ford Health System, USA
[4]Department of Neurology, Thomas Jefferson University, USA
[5]Department of Food and Technology, University of California-Davis, USA
[6]United States Department of Agriculture, Agricultural Research Service, Western Human Nutrition Research Centre, USA.
[7]West Coast Metabolomics Centre, Genome Centre, University of California-Davis, USA
[8]Department of Nutrition, University of California-Davis, USA
JWaters3@hfhs.org

Abstract

Unresolved and uncontrolled inflammation is considered a hallmark of pathogenesis in chronic inflammatory diseases like multiple sclerosis (MS), suggesting a defective resolution process. Inflammatory resolution is an active process partially mediated by endogenous metabolites of dietary polyunsaturated fatty acids (PUFA), collectively termed specialized pro-resolving lipid mediators (SPMs). Altered SPM levels have been reported in several inflammatory diseases and may explain impaired inflammatory resolution. Performing LC-MS/MS-based targeted lipid mediator profiling we observed distinct changes in fatty acid metabolites in serum from 29 relapsing-remitting MS (RRMS) patients relative to 29 matched healthy controls (HC). An unpaired Welch's t-test revealed 12 altered lipid mediators ($p < 0.05$). Of these, 11,12-EpETrE, 5-HEPE, 15 deoxy PGJ2, LTB5 and PGE3 were increased while, 12-HETE, 12-HEPE, 14 HDoHE, 5,6-diHETrE, 8,9-DiHETrE, and docosahexaenoyl ethanolamide were reduced in patients with RRMS. In MS patients, linoleic acid-derived 12,13-DiHOME, 9,10-DiHOME, and 9,12,13-TriHOME were positively correlated with disease severity (EDSS), while the pro-resolving docosahexaenoic acid-derived 10,11-EpDPE was negatively correlated with disease duration. Precursor unesterified fatty acids did not differ between groups, except for alpha-linolenic acid (ALA) relative abundance, which was higher in RRMS. Using an AdaBoost ensemble machine learning model, discrimination between healthy control and RRMS was achieved with an average area under the receiver operator characteristic curve (AUC) of 0.84 based on 5 metabolites. Together, these findings are consistent with previous studies, confirming the metabolic dysfunction of lipid mediators in MS, suggesting an imbalance between inflammation and resolution.

Funding Sources (if applicable):

This work was supported by the National Multiple Sclerosis Society (US) (RG-1807-31964), the National Institutes of Health (NS112727, AI44004), and Henry Ford Hospital Internal Grant (A10270) to SG. The UCSF Biorepository is supported by grant SI-2001-35701 from the National Multiple Sclerosis Society. Additional support was provided by USDA CRIS Project 2032-51530-025-00D to JWN. The USDA is an equal opportunity employer and provider. The funders had no role in study design, data collection, and interpretation or the decision to submit the work for publication.

162

A simple, iterative algorithm for RT alignment and integration of multi-batch untargeted LCMS data

Janis Wigginton[1], William Duren[2], Maureen Kachman[1], Tanu Soni[1], Charles Burant[3]

[1] Biomedical Research Core Facilities – Metabolomics, University of Michigan Medical School, United States
[2] Department of Computational Medicine and Bioinformatics, University of Michigan Medical School, United States
[3] Department of Internal Medicine, *University of Michigan Medical School, United States*
wiggie@umich.edu

Abstract

Integration of multi-batch LCMS data sets remains a primary challenge for the field of Metabolomics. While many software tools have been developed for this purpose, nearly all implement algorithms that impose assumptions that significantly simplify the data and that may not be valid in the presence of sample or other QC issues. To bridge this gap, we've developed an entirely data-driven algorithm that first constructs an alignment across multiple batches and then makes use of the structure of that alignment to self-correct. Iterative application of this approach makes it possible to construct a "best alignment" snapshot of the data that can be used both as a QC and a data integration tool for multi-batch data. We demonstrate how our algorithm might be applied both in the presence and the absence of data errors. For typical data sets, our iterative approach generates roughly 40-50% more feature matches than single pass alignments and does so by following the observed structure of the data rather than imposing a predetermined curve or smoothing function.

Funding Sources (if applicable):

MoTrPAC (NIH Funding)

163

Molecular network-oriented parameter optimizations enhanced the annotation of small molecules in metabolomics

Rui Xu[1], Jisun Lee[1], Li Chen[1], Jiangjiang Zhu[1,2]

[1]Human Nutrition Program, The Ohio State University, USA
[2]James Comprehensive Cancer Center, The Ohio State University, USA
xu.3819@osu.edu

Abstract

Metabolomics, especially large-scale untargeted metabolomics, generate massive amounts of data on a regular basis, which often needs to be filtered, screened, analyzed, and annotated via a variety of approaches. Data-dependent acquisition (DDA) mode including inclusion and exclusion rules for tandem mass spectrometry (MS) is routinely used to perform such analyses. While parameters of data acquisition are important in these processes, there is a lack of systematic studies of these parameters that can be used in data collection to generate metabolic features for molecular network (MN) analysis on the Global Natural Product Social Molecular Networking platform (GNPS). To explore the key parameters that impacting the formation and quality of MNs, several data acquisition parameters for metabolomic studies were proposed in this study. The influences of MS^1 resolution, normalized collision energy (NCE), intensity threshold, and exclusion time to GNPS analyses were demonstrated. Among 4 parameters tested in this study, resolution and intensity threshold were optimized for enhanced MS^2 signal intensity, while the optimization of NCE could increase the spectra diversity and enable the annotations of many unique compounds based on their fragmentation patterns. Resolution, NCE and intensity threshold have greater influences on MS^2 spectra quality, while exclusion time have less influence. Moreover, an optimization workflow dedicated to Thermo Scientific QE Hybrid Orbitrap instruments is described, and a comparison of phytochemical contents from two forms of black raspberry extracts were performed based on the GNPS MN results. By optimizing the parameters of the two types of BRB extracts separately, we noted that the parameter optimization can be sample type-specific and MS polarity dependent. Overall, we expect this study to provide additional thoughts on developing natural product analysis workflow using GNPS network, and shed some lights to future analyses that utilizing similar instrumental setups.

Funding Sources (if applicable):

The authors thank the support from National Institute of General Medical Sciences of the National Institutes of Health Award R35GM133510.

164

Profiling blood-based markers using combination of highly sensitive single molecule array technology (SIMOA) and untargeted metabolomics in multiple sclerosis

Insha Zahoor[1], Jeffrey Waters[1], Indrani Datta[2], Mirela Cerghet[1], Laila M Poisson[2], Ramandeep Rattan[3], Shailendra Giri[1]

[1]Department of Neurology, Henry Ford Health System, USA
[2]Department of Public Health Sciences, Henry Ford Health System, USA
[3]Women's Health Services, Henry Ford Health System, USA
izahoor1@hfhs.org

Abstract

Multiple Sclerosis is a chronic non-traumatic disease mainly affecting young adults. There is a dire need for less invasive and sensitive assay to detect biomarkers with prognostic and therapeutic value. The purpose of our study was to profile the blood-based markers including, cytokines, neurofilament light chain (NFL) and glial fibrillary acid protein (GFAP) in serum samples from patients with relapsing-remitting (RRMS) and progressive (PMS) disease course and correlate them with altered metabolites and metabolic pathways using a combination of highly sensitive single molecule array technology (SIMOA, Quanterix) and untargeted metabolomics (Metabolon, NC) approach. We obtained serum samples of 32 MS patients (12 RRMS and 20 PMS) and 20 age-gender matched healthy subjects (HS) from Accelerated Cure Project (ACP). We profiled cytokines and neural markers including IFNγ, IL6, IL17, 1L12p70, IL10, IL8, IL22, TNFα, neurofilament light chain (NFL) and glial fibrillary acid protein (GFAP) using SRX/SPX. The data obtained from Quanterix and Metabolon was subjected to statistical and bioinformatics analysis using Welch's t-test, principal component analysis (PCA), Metaboanalyst (KEGG), and weighted correlation network analysis (WGCNA). We observed significant increase in IL12p70 ($p<0.05$), NFL ($p<0.001$), and GFAP ($p<0.0001$) in PMS compared to HS, with IL12p70 and GFAP also significantly increased in PMS compared to RRMS. We found a unique biomarker panel of 16 variables, including 14 metabolites and 2 neuronal markers (NFL and GFAP), as highly predictive biomarker panel for MS. A bi-weight mid-correlation of cytokines and neural markers with EDSS, while controlling for age, found significant correlations in RRMS with IL8 (negative correlation) and NFL (positive correlation, $p < 0.05$) and in PMS with IL-10 (negative correlation, $p < 0.05$) with EDSS. In conclusion, our study is the first to show the utility of a combination approach involving SIMOA and metabolomics to reveal a unique biomarker profile that has the potential to diagnose MS and predict RRMS and PMS classifications, including disease severity score (EDSS) with a high degree of accuracy.

Funding Sources (if applicable):

165

A comprehensive targeted metabolomic assay for uremic toxin quantification

Lun Zhang[1,2], Jiamin Zheng[1,2], Rupasri Mandal[1,2], David S. Wishart[1,2,3]

[1]The Metabolomics Innovation Centre, University of Alberta, CA
[2]Department of Biological Sciences, University of Alberta, CA
[3]Department of Computing Science, University of Alberta, CA
Lun2@ualberta.ca

Abstract

Uremic toxins are ubiquitous compounds produced as byproducts of food consumption or microbial digestion. Most of these compounds are removed by the kidneys but the accumulation of uremic toxins in the blood can lead to the development of a condition known as uremia. At low abundance, most uremic toxins are relatively harmless, however at high levels, uremic toxins can contribute to diabetes, liver failure, heart disease, memory loss, kidney disease, jaundice and a variety of intractable of skin conditions. Indeed, it is believed that uremic toxins contribute to many chronic conditions among the elderly, even in those without overt kidney disease. Given the importance of uremic toxins in health and disease, we believe that an improved method for detecting and quantifying uremic toxins is needed. To date, the methods published for uremic toxin characterization are only able to quantify a handful of uremic toxins. Here we describe a new assay based on liquid chromatography-tandem mass spectrometry (LC-MS/MS) that allows us to identify and quantify 88 small-molecule uremic toxins in plasma and serum. This includes 38 organic acids, 31 amino acids, 12 biogenic amines, 4 nucleotides/nucleosides, and 3 saccharide derivatives. In developing the assay, we carefully checked its accuracy, sensitivity, and reproducibility. The accuracies of quality control solutions and the recovery rates of spiked NIST SRM 1950 human plasma samples at 3 different concentrations were in the range of 100 ± 10% and 100 ± 20%, respectively. As far as we are aware, this is the most comprehensive, quantitative assay that has been reported for the analysis of uremic toxins. We further demonstrate the utility of this uremic toxin assay by reporting the levels of many previously unmeasured and potentially important uremic toxins in the plasma samples of patients undergoing renal dialysis.

Funding Sources (if applicable):

166

Untargeted metabolomics sensitively differentiates gut bacterial species in single culture and co-culture systems

Shiqi Zhang[1], Jiangjiang Zhu[2]

[1]Human Nutrition Program, Department of Human Sciences, The Ohio State University, USA
[2]James Comprehensive Cancer Center, The Ohio State University, USA
Zhang.6517@buckeyemail.osu.edu

Abstract

Gut microbiome plays vital role in human health, and its characteristic has been widely identified through next-generation sequencing techniques. Although with great genomic insight of gut microbiome, their functional information is not clearly elaborated through metagenomics techniques. Besides, mass spectrometry (MS)-based metabolomics, due to its advantages of high sensitivity and broad range of metabolites detection, has been developed to be an appropriate tool to investigate gut microbial metabolome. It is suggested that fecal metabolome can be used as a functional readout of microbiome composition, therefore, we designed a proof-of-concept study to first characterize the metabolome of different gut microbes, and then investigate relationship between bacterial metabolomes and their compositions in co-culture systems. Resulting from the complexity of gut microbiome interactions in human large intestine, we selected eight representative bacteria species from *Bifidobacterium(2), Bacteroides(1), Lactobacillus(4)*, and *Akkermansia(1)* genera as our model microbes and cultured them in vivo. Liquid chromatograph coupled mass spectrometry based untargeted metabolomics was utilized to explore microbial metabolome of single cultures and co-culture systems. Through spectra comparison, our results showed that untargeted metabolomics could capture similarity and differences in metabolic profiles from eight representative gut bacteria. Also, untargeted metabolomics could sensitively differentiate gut bacterial species from principal component analysis. For example, citrulline and histamine level were significantly different among four *Lactobacillus* species. In addition, in the co-culture systems with different bacteria population ratio, gut bacterial metabolomes can be used to quantitatively reflect bacterial population in a mixed culture. For instance, 2-hydroxybutyric acid relative abundance changed proportionately with population ratio of *Lactobacillus reuteri* in the co-culture system. In summary, we proposed a workflow that could demonstrate the capability of untargeted metabolomics in differentiating gut bacterial species and detecting their characteristic metabolites proportionally to the microbial population in co-culture systems.

Funding Sources (if applicable):

National Institute of General Medical Sciences of the National Institutes of Health under Award Number R35GM133510

167

A comprehensive targeted metabolomics assay for crop plant sample analysis

Jiamin Zheng[1], Mathew Johnson[1], Rupasri Mandal[1], David S. Wishart[1,2]

[1]Departments of Biological Sciences and
[2]Computing Science, Faculty of Science, University of Alberta, CA
jiamin3@ualberta.ca

Abstract

Metabolomics plays an important role in various fields from health to agriculture. However, the comprehensive, quantitative metabolomics analysis of plants and plant metabolites has not been widely performed. Liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS)-based plant metabolomics offers the high sensitivity and breadth of coverage for both phenotyping and disease diagnosis of plants. Here, we report a high-coverage and quantitative LC-MS-based assay for plant metabolite analysis. The assay detects and quantifies 206 primary and secondary plant metabolites, including many key plant hormones. This assay permits the detection of 28 amino acids and derivatives, 27 organic acids, 20 biogenic amines and derivatives, 40 acylcarnitines, 90 phospholipids and hexose. All the analysis methods in this assay are based on LC-MS/MS using both positive and negative ion-mode multiple reaction monitoring (MRM). The recovery rates of spiked plant samples at three different concentration levels ranged from 80% to 120% with satisfactory precision values of less than 20%. This targeted plant metabolomic assay has been successfully applied to the analysis of large numbers of pine and spruce needles, canola root samples, grass samples, as well as cannabis samples. Moreover, the assay was specifically developed in a 96-well plate format, which enables automated, high-throughput sample analysis.

Funding Sources (if applicable):

Funding for this work has been provided by Genome Canada and the Canada Foundation for Innovation (CFI).

168

Lipid oxidation as critical contributor of the cholesterol dysregulation in and its Implications in neurological diseases

Lisa R. Zou[1], Medina-Meza, Ilce G[1], Carlo Barnaba[2]

[1]Department of Biosystems and Agricultural Engineering, Michigan State University, USA
[2]Institute of Quantitative Health Science and Engineering, Michigan State University, USA
ilce@msu.edu

Abstract

The human brain contains up to 25% of cholesterol in the body, where cholesterol makes up to 2% of brain. Cholesterol plays a significant role in the brain's health and function. Factors that can affect the cholesterol metabolism include oxidative stress, weakened antioxidant defenses and gene expression. The crucial role of lipids and cholesterol in brain tissue physiology and cell signaling is demonstrated by many neurological disorders, including bipolar disorders and schizophrenia, and neurodegenerative diseases such as Alzheimer's, Parkinson's, Niemann-Pick and Huntington diseases, that involve deregulated lipid metabolism. Brain contains a high amount of lipids, especially cholesterol, and is vulnerable to oxidative stress and lipid peroxidation, characterizing traumas of vast clinical importance.

The aim of this study was to evaluate cholesterol alterations in human brains at different clinical stages and its association with brain aging. Cholesterol was quantified by gas chromatography-mass spectrometry (GC-MS). Brain tissues were separated into four major sections: frontal cortex, occipital cortex, and cerebellum. In the control group, cholesterol was 22.79 ug per mg compared to the illness group with 11.73 ug per mg. Using an independent samples T test, the decreased levels of cholesterol in illness versus control brains was found to be statistically significant ($p < 0.001$). This shift in cholesterol homeostasis due to brain aging can be a biomarker that indicates the development of neurological diseases such as dementia and Alzheimer's. Further research in lipid homeostasis can benefit neurological diseases early detection. The next step would be to evaluate the oxidized lipids such as cholesterol oxidation products (COPs) in human brains.

Funding Sources (if applicable):

169

NIST Interlaboratory study of human stool reference materials: towards metabolomics analysis harmonization

Sandra Da Silva[1], Stephanie Servetas[1], Paulina Piotrowski[2], Christina Jones[2], Katrice Lippa[2], Nancy Lin[1], and Scott Jackson[1]

[1]Biosystems and Biomaterials Division, National Institute of Standards and Technology, USA
[2]Chemical Sciences Division, National Institute of Standards and Technology, USA
sdasilva@nist.gov

Abstract

Researchers have established substantial evidence regarding the impact of the gut microbiome on human health and the potential for its use as a diagnostic tool. Many studies depend on the analysis of stool samples; a complex matrix composed of microbes, protein, undigested plant matter, and fat content that is strongly influenced by diet. Given the complexity of the gut microbiome (microbes and metabolites) and its importance for human health, the gut microbiome community has recognized the need for quality control standards (e.g. reference material) to validate analytical workflows, improve reproducibility, and allow comparability among different studies. To address some of these needs, NIST has organized an interlaboratory study to gather information on the level of comparability among different metabolomic platforms to characterize vegan and omnivore candidate human stool reference materials preserved in lyophilized and aqueous formats. Participants analyzed the candidate RM by nuclear magnetic resonance spectroscopy (^{1}H NMR), gas chromatography-mass spectrometry (GC-MS), and liquid chromatography-mass spectrometry (LC-MS) and provided the 20 top metabolites observed by their chosen analytical platform as well as metabolites that distinguished the different diets and storage preservation methods. Preliminary results of this exercise will be described, including a review of the analytical approaches employed and opportunities for metabolomic measurement harmonization.

Funding sources (if applicable):

Institute for the Advancement of Food and Nutrition Sciences (IAFNS)