GÉNÉTIQUE FONCTIONNELLE & MÉDICALE
Ecole nationale vétérinaire d'Alfort

EnvA
École nationale vétérinaire d'Alfort

Dealing with an Increasing Number of Canine DNA Tests

Marie Abitbol, DVM, PhD
Associate Professor in Medical Genetics
Alfort School of Veterinary Medicine (EnvA)
Maisons-Alfort, France

Challenges in DNA testing

- Breed and breeding line
- Genetic heterogeneity
- Genotype-phenotype correlation

Breed and breeding-line of origin

- DNA tests are mostly breed specific.
- Strong founder effect in dogs.
- For the same disease, several mutations in several genes may exist
- Examples: progressive retinal atrophies

Irish Setter: rcd1-PRA
PDE6B gene
Irish Setter: rcd4-PRA
C2orf71 gene

Photo: Luis Miguel Bugallo Sánchez

English Springer Spaniel:
Cord1-PRA
RPGRIP1 gene

Photo: Heinz Höfling

English Cocker Spaniel: prcd-PRA
PRCD gene

Photo: Ashbey Photography

Breed and breeding-line of origin

Example: Dilated cardiomyopathy (DCM) in Doberman Pinschers

- A mutation in the *PDK4* gene (Meurs *et al.* Hum Genet, 2012)
- This mutation was identified using US dogs
- But this mutation was not found in European dogs!
- Another locus is involved in European dogs! (Mausberg *et al.* PLoS One, 2011)
- The DNA test (*PDK4* mutation) is not relevant for European dogs

Hum Genet. 2012 Aug;131(8):1319-25. doi: 10.1007/s00439-012-1158-2. Epub 2012 Mar 25.

A splice site mutation in a gene encoding for PDK4, a mitochondrial protein, is associated with the development of dilated cardiomyopathy in the Doberman pinscher.

Meurs KM, Lahmers S, Keene BW, White SN, Oyama MA, Mauceli E, Lindblad-Toh K.

PDK4 is located on CFA14

PLoS One. 2011;6(5):e20042. doi: 10.1371/journal.pone.0020042. Epub 2011 May 20.

A locus on chromosome 5 is associated with dilated cardiomyopathy in Doberman Pinschers.

Mausberg TB, Wess G, Simak J, Keller L, Drögemüller M, Drögemüller C, Webster MT, Stephenson H, Dukes-McEwan J, Leeb T.

Genetic heterogeneity

The same symptoms may be caused by different mutations located in different genes: this situation is called genetic heterogeneity.

Example: Leonberger polyneuropathy

- Locus n°1: LPN1 mutation, 20% of polyneuropathy cases, certainly autosomal recessive
- Locus n°1: LPN2 mutation, 20-25% of polyneuropathy cases, autosomal dominant

(University of Minnesota, the University of Bern and the University of California San Diego)

- Two mutations can be tested: 2 DNA tests
- But about 50% of polyneuropathy cases are caused by mutation(s) in gene(s) that remain unknown.

Genotype-phenotype correlation

Example of genotype-phenotype correlation

Hereditary cataract in Boston Terriers and Australian Shepherds

Boston Terrier

Table 1. Distribution of genotypes with respect to *HSF4* mutation among dogs with different clinical status

		Clinical status			
		Unaffected >8 years	EHC affected <1 year	LHC affected >3 years	Unknown[a]
Genotype	+/+[b]	4	1	11	7
	+/−[c]	4	1	5	9
	−/−	0	20	0	0

[a] Includes apparently unaffected siblings and other relatives of affected dogs.

[b] "+" indicates wild-type *HSF4* allele.

[c] "−" indicates *HSF4* mutation (CFA5 g.85286582_85286583insC).

Mellersh *et al.*, J Hered. 2007

Genotype-phenotype correlation

Example of genotype-phenotype correlation

Hereditary cataract in Boston Terriers and Australian Shepherds

Australian Shepherd

Mellersh *et al.*, Vet Ohptalmology 2009

Table 1. Distribution of *HSF4* genotypes and alleles in Australian Shepherds with and without cataracts

	N (dogs)	Genotypes			Alleles		Mean age at examination	SD	Range
		−/−	+/−	+/+	+	−			
NAD	293	1 (0.34%)	46 (15.70%)	246 (83.96%)	538 (91.81%)	48 (8.19%)	4.06	2.98	0.04–15.62
Cataract	99	14 (14.14%)	54 (54.55%)	31 (31.31%)	116 (58.59%)	82 (41.41%)	4.82	2.82	0.13–12.30
Total	392								
Binocular	70	14 (20%)	44 (62.86%)	12 (17.14%)	68 (48.57%)	72 (51.43%)	5.09	2.73	0.24–12.30
Uniocular	24	0 (0%)	6 (25%)	18 (75%)	42 (87.5%)	6 (12.5%)	5.54	3.14	0.13–10.94
Unknown	5	0	4	1	6	4			
Total	99								
Total cataract vs. NAD		$\chi^2 = 109.62$	$P < 0.001$		$\chi^2 = 118.10$	$P < 0.001$			
Binocular cataract vs. NAD		$\chi^2 = 139.01$	$P < 0.001$		$\chi^2 = 153.12$	$P < 0.001$			
Uniocular cataract vs. NAD		$\chi^2 = 1.47$	$P = 0.48$		$\chi^2 = 1.06$	$P = 0.45$			

NAD, no abnormalities detected; +, normal or wild-type *HSF4* allele; −, *HSF4* deletion allele; +/+, homozygous normal or wild-type genotype; +/−, heterozygous wild-type/deletion genotype; −/−, homozygous deletion genotype.

Genotype-phenotype correlation

- A weak genotype-phenotype correlation may be explained by:
  - genetic heterogeneity
  - modifying genes
  - environmental factors
  - a combination of these factors

Strong genotype-phenotype correlation: DNA diagnosis and screening test

Weak genotype-phenotype correlation: DNA susceptibility test

Scientific accuracy of a canine DNA test
Information about
the genotype-phenotype correlation

Canine inherited diseases: databases and scientific information

Dr Marie Abitbol
2nd International Dog Health Workshop
Dortmund, 2015

OMIA database

OMIA database: *Online Mendelian Inheritance in Animals*

WELCOME TO OMIA

Online Mendelian Inheritance in Animals (**OMIA**) is a database of genes, inherited disorders and traits in 174 animal species (other than human and mouse, which have their own resources) authored by Professor Frank Nicholas of the University of Sydney, Australia, with help from many people over the years. The database contains textual information and references, as well as links to relevant PubMed and Gene records at the NCBI.

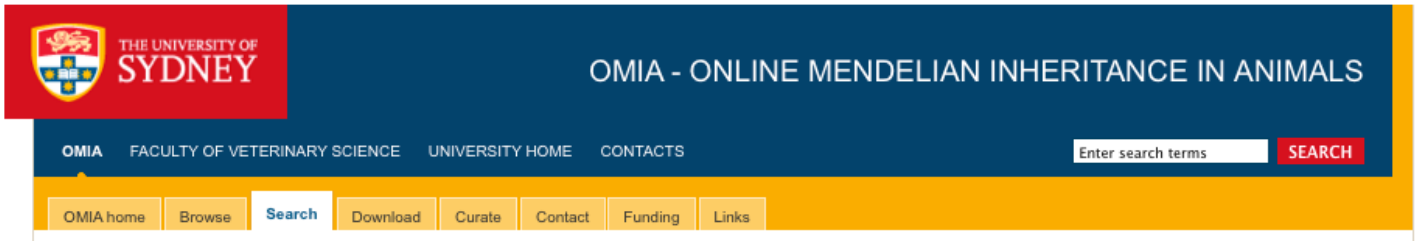
This database is manually curated by a team of specialists. If you see an error or wish to submit an entry, please contact us.

Summary

	dog	cattle	cat	pig	sheep	horse	chicken	goat	rabbit	Japanese quail	golden hamster	Other	TOTAL
Total traits/disorders	649	463	318	233	233	219	210	75	64	45	40	534	3083
Mendelian trait/disorder	256	199	83	54	97	45	127	14	33	33	28	170	1139
Mendelian trait/disorder; key mutation known	186	100	49	26	44	32	40	9	8	10	3	77	584
Potential models for human disease	353	166	183	90	98	117	43	31	38	13	15	278	1425

http://omia.angis.org.au/

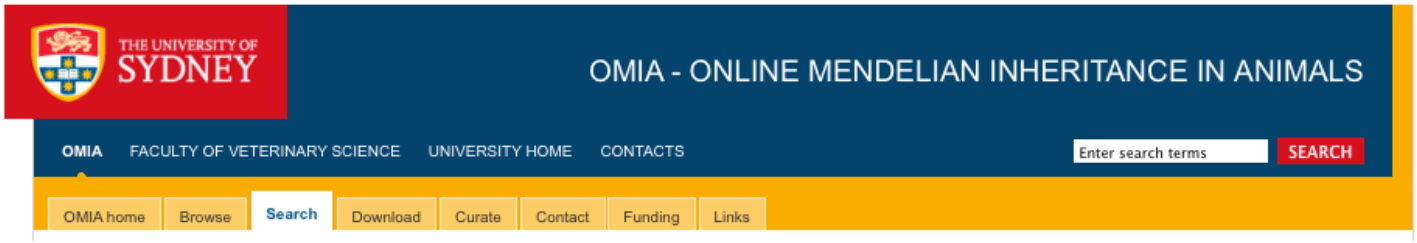
OMIA



- **OMIA 001309-9615 Mucopolysaccharidosis IIIA** in *Canis lupus familiaris* (dog) Gene: SGSH
- **OMIA 001342-9615 Mucopolysaccharidosis IIIB** in *Canis lupus familiaris* (dog) Gene not yet published
- **OMIA 000666-9615 Mucopolysaccharidosis VI** in *Canis lupus familiaris* (dog) Gene: ARSB
- **OMIA 000667-9615 Mucopolysaccharidosis VII** in *Canis lupus familiaris* (dog) Gene: GUSB
- **OMIA 001402-9615 Multidrug resistance 1** in *Canis lupus familiaris* (dog) Gene: ABCB1
- **OMIA 001444-9615 Multifocal retinopathy 1** in *Canis lupus familiaris* (dog) Gene: BEST1
- **OMIA 001553-9615 Multifocal retinopathy 2** in *Canis lupus familiaris* (dog) Gene: BEST1
- **OMIA 001554-9615 Multifocal retinopathy 3** in *Canis lupus familiaris* (dog) Gene: BEST1
- **OMIA 001081-9615 Muscular dystrophy, Duchenne type** in *Canis lupus familiaris* (dog) Gene: DMD
- **OMIA 000683-9615 Muscular hypertrophy (double muscling)** in *Canis lupus familiaris* (dog) Gene: MSTN
- **OMIA 001509-9615 Musladin-Lueke syndrome** in *Canis lupus familiaris* (dog) Gene: ADAMTSL2
- **OMIA 000685-9615 Myasthenic syndrome, congenital** in *Canis lupus familiaris* (dog) Gene: CHAT
- **OMIA 001928-9615 Myasthenic syndrome, congenital, Labrador Retriever** in *Canis lupus familiaris* (dog) Gene: COLQ
- **OMIA 000690-9615 Myoclonus epilepsy of Lafora** in *Canis lupus familiaris* (dog) Gene: NHLRC1
- **OMIA 001374-9615 Myopathy, centronuclear** in *Canis lupus familiaris* (dog) Gene: PTPLA

Dr Marie Abitbol
2nd International Dog
Dortmund, 2015

OMIA



OMIA 001374-9615 : Myopathy, centronuclear in *Canis lupus familiaris*

See the equivalent entry at NCBI

Possible human homologue (MIM number): 610467

Mendelian trait/disorder: yes

Mode of inheritance: Autosomal Recessive

Considered a defect: yes

Key mutation known: yes

Year key mutation first reported: 2005

Cross-species summary: Centronuclear Myopathy (CNM)

Species-specific name: Type II fiber deficiency; Autosomal recessive muscular dystrophy; Hereditary myopathy of Labrador retrievers (HMLR)

History: The first clinical description of this disorder was by Kramer et al. (1976).

Inheritance: Tiret et al. (2003) showed that this disorder is autosomal recessive.

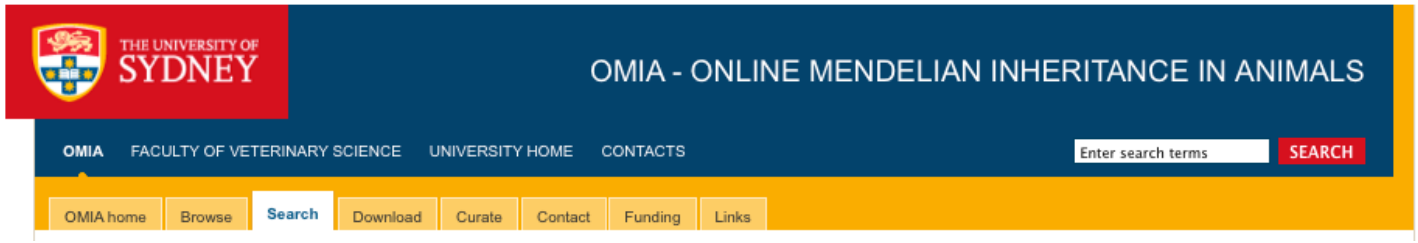
Mapping: Conducting a genome scan with 66 microsatellites on a four-generation pedigree that "comprised 40 dogs among which 20 were affected (12 females and 8 males)" Tiret et al. (2003) mapped this disorder to the centromeric region of chromosome CFA2. Subsequent fine mapping reduced this to an "18.1-cM interval between markers FH2087U and AHT132". Subsequent FISH-mapping "established orthology between the centromeric region of CFA2 and the GDI2-cREM human segment (HSA10p15/HSA10p12.1-p11.1)".

Molecular basis: Pelé et al. (2005) determined the molecular basis of this disorder by adopting a comparative positional cloning approach. Having mapped the canine disorder as described in the Mapping section above, they then studied the 208 human genes that are located in the orthologous region of chromosome HSA10p. Based on tissue expression and sequence motif, the most likely of these 208 genes was PTPLA (protein tyrosine phosphatase-like, member A). Sequencing of the canine PTPLA gene revealed the causative mutation as an insertion of a "tRNA-derived short interspersed repeat element (SINE)" in exon 2 ("PTPLA*g9459-9460ins236") which "has a striking effect on the maturation of PTPLA mRNA, whereby it can be spliced out, partially exonized or involved in multiple exon-skipping. As a result, the amount of wild-type transcripts falls to 1% in affected muscles."

Prevalence: Maurer et al. (2012) conducted a comprehensive world-wide survey by genotyping 7,426 Labradors from 18 countries for the PTPLA mutant reported by Pelé et al. (2005). All 80 affected dogs from 8 countries were homozygous for the same mutant allele, and none of the 1.172 heterozygous dogs from 13 countries was affected. The highest % of carriers were "found in the UK (19%), the USA (13%) and Canada (11,5%)". The UK estimate is similar to the UK estimate of 22%, reported by Owczarek-Lipska et al. (2011). Maurer et al. (2012) concluded that the mutant allele "resulted from a single and recent mutational event that may have rapidly disseminated through the extensive use of popular sires".

Breed: Labrador Retriever.

OMIA



Associated gene:

Symbol	Description	Species	Chr acc	Chr name	Start	Stop	OMIA gene details page	Other Links
PTPLA	protein tyrosine phosphatase-like (proline instead of catalytic arginine), member A	*Canis lupus familiaris*	no genomic information	-	-	-	PTPLA	Homologene, Ensembl, NCBI gene

References

Note: the references are listed in reverse chronological order (from the most recent year to the earliest year), and alphabetically by first author within a year.

2013 Broeckx, B.J., Coopman, F., Verhoeven, G.E., Van Haeringen, W., van de Goor, L., Bosmans, T., Gielen, I., Saunders, J.H., Soetaert, S.S., Van Bree, H., Van Neste, C., Van Nieuwerburgh, F., Van Ryssen, B., Verelst, E., Van Steendam, K., Deforce, D. :
The prevalence of nine genetic disorders in a dog population from belgium, the Netherlands and Germany. *PLoS One* 8:e74811, 2013. Pubmed reference: 24069350. DOI: 10.1371/journal.pone.0074811.

2012 Maurer, M., Mary, J., Guillaud, L., Fender, M., Pelé, M., Bilzer, T., Olby, N., Penderis, J., Shelton, G.D., Panthier, J.J., Thibaud, J.L., Barthélémy, I., Aubin-Houzelstein, G., Blot, S., Hitte, C., Tiret, L. :
Centronuclear myopathy in Labrador retrievers: a recent founder mutation in the PTPLA gene has rapidly disseminated worldwide. *PLoS One* 7:e46408, 2012. Pubmed reference: 23071563. DOI: 10.1371/journal.pone.0046408.

2011 Gentilini, F., Zambon, E., Gandini, G., Rosati, M., Spadari, A., Romagnoli, N., Turba, M.E., Gernone, F. :
Frequency of the allelic variant of the PTPLA gene responsible for centronuclear myopathy in Labrador Retriever dogs as assessed in Italy. *J Vet Diagn Invest* 23:124-6, 2011. Pubmed reference: 21217042.

Owczarek-Lipska, M., Thomas, A., André, C., Hölzer, S., Leeb, T. :
[Frequency of gene defects in selected European retriever populations]. *Schweiz Arch Tierheilkd* 153:418-20, 2011. Pubmed reference: 21866517. DOI: 10.1024/0036-7281/a000236.

2005 Pelé, M., Tiret, L., Kessler, J.L., Blot, S., Panthier, J.J. :
SINE exonic insertion in the PTPLA gene leads to multiple splicing defects and segregates with the autosomal recessive centronuclear myopathy in dogs. *Hum Mol Genet* 14:1417-27, 2005. Pubmed reference: 15829503. DOI: 10.1093/hmg/ddi151.

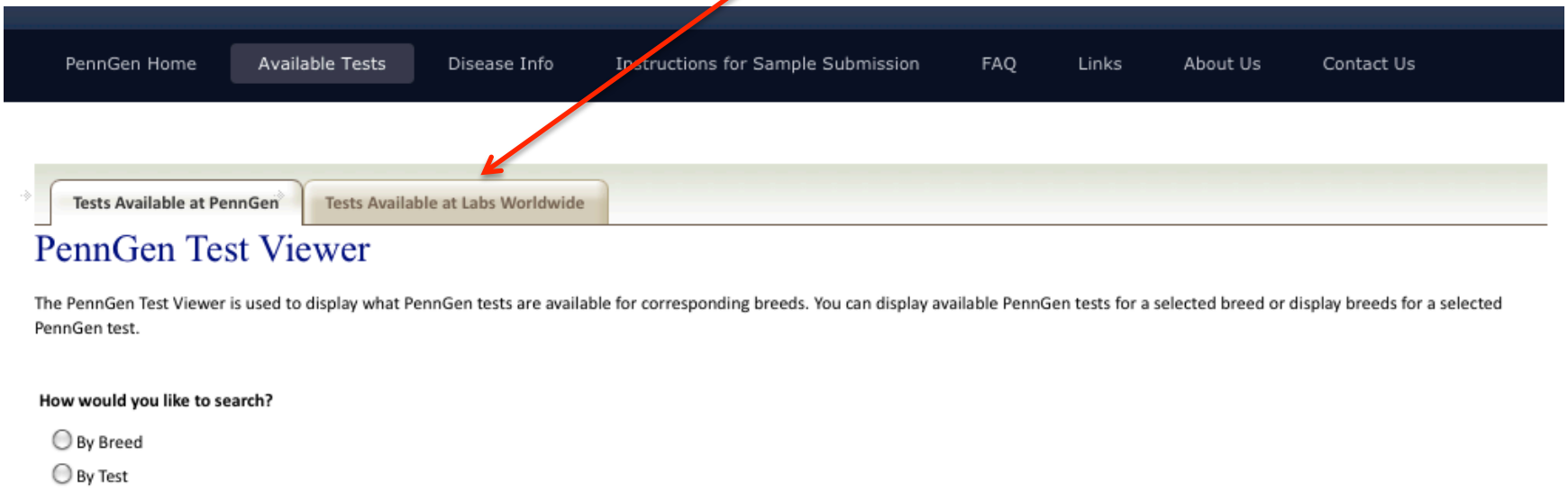
2003 Tiret, L., Blot, S., Kessler, J.L., Gaillot, H., Breen, M., Panthier, J.J. :
The cnm locus, a canine homologue of human autosomal forms of centronuclear myopathy, maps to chromosome 2 *Human Genetics* 113:297-306, 2003. Pubmed reference: 12884002. DOI: 10.1007/s00439-003-0984-7.

2002 Bley, T., Gaillard, C., Bilzer, T., Braund, K.G., Faissler, D., Steffen, F., Cizinauskas, S., Neumann, J., Vogtli, T., Equey, R., Jaggy, A. :
Genetic aspects of labrador retriever myopathy *Research in Veterinary Science* 73:231-236, 2002. Pubmed reference: 12443679.

Blot, S., Tiret, L., Devillaire, A.C., Fardeau, M., Dreyfus, P.A. :
Phenotypic description of a canine centronuclear myopathy. *Journal of Neurological Science* 199:S9, 2002.

DNA tests available worldwide: WSAVA-PennGen

Worldwide



www.wsava.org/guidelines/hereditary-diseases

http://research.vet.upenn.edu

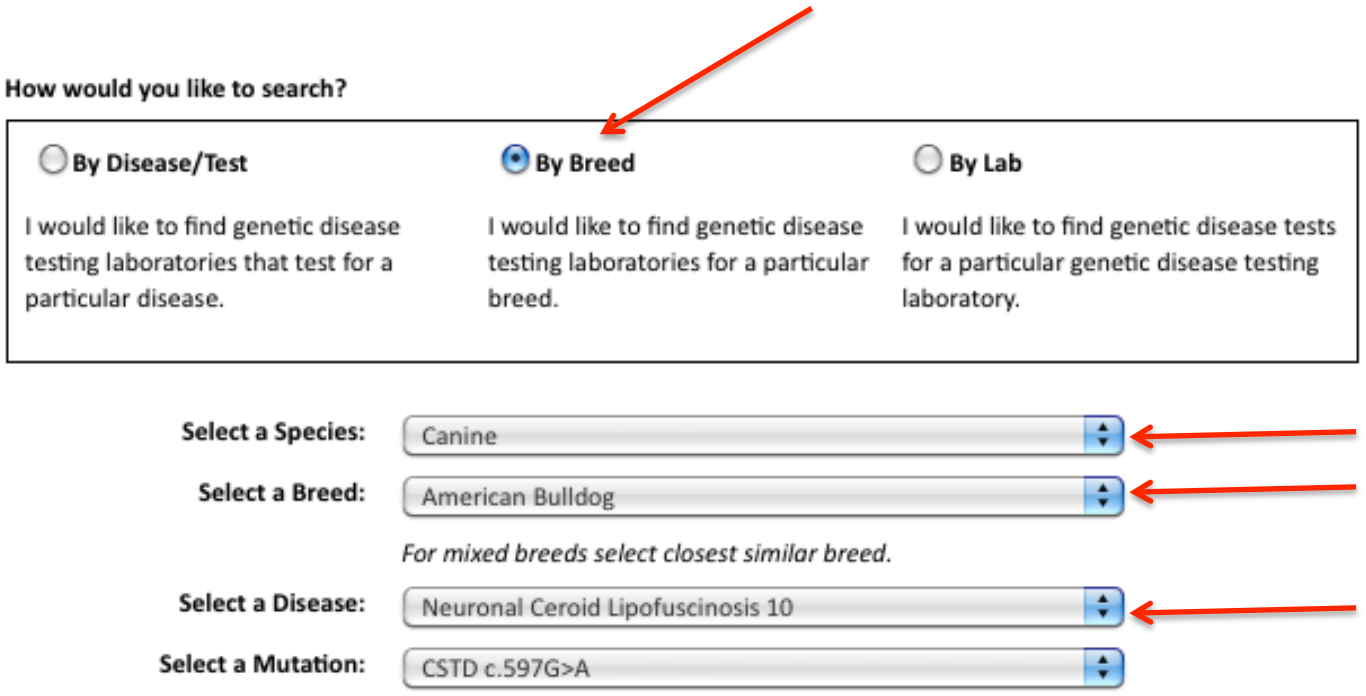
WSAVA Global Veterinary Community

Dr Marie Abitbol
2nd International Dog Health Workshop
Dortmund, 2015

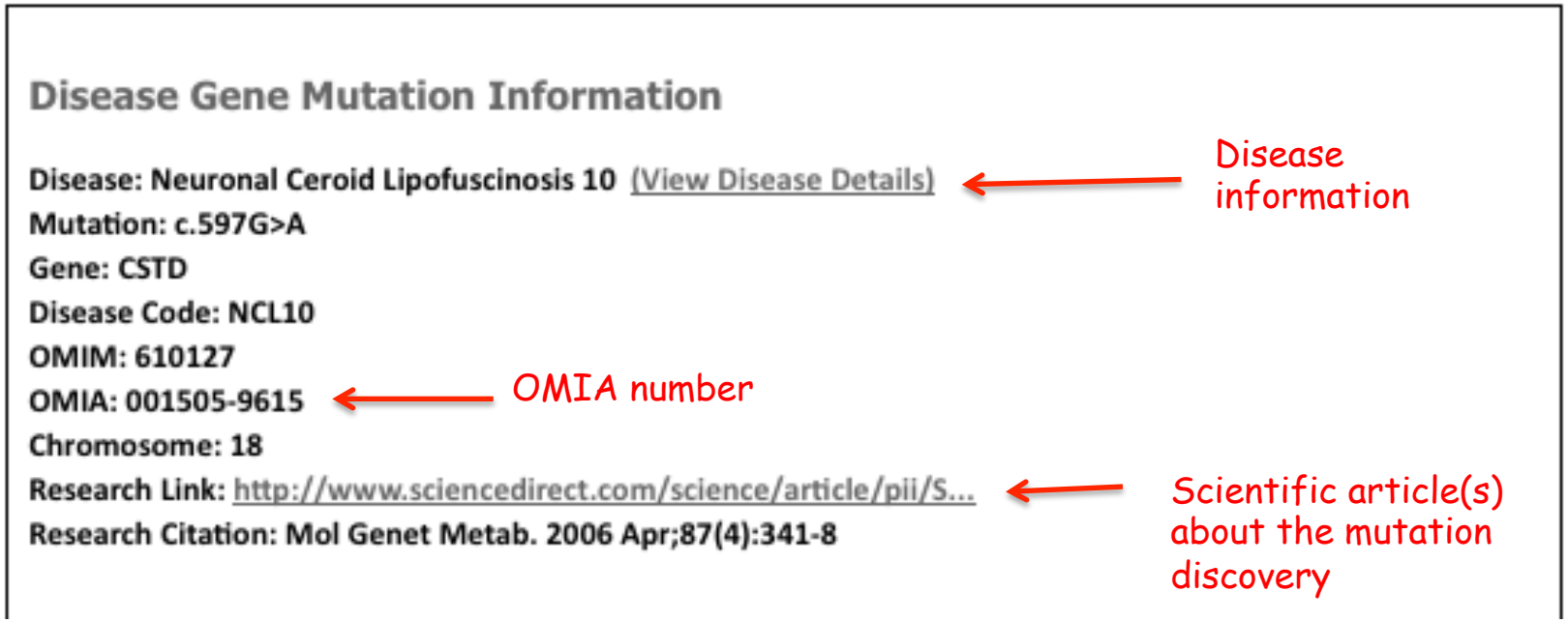
DNA tests available worldwide: WSAVA-PennGen

Canine and Feline Hereditary Disease (DNA) Testing Laboratories

This page is used to search for Genetic Testing Laboratories and their corresponding tests for hereditary diseases in **dogs** and **cats**. Practically all DNA tests for hereditary diseases are breed specific.



DNA tests available worldwide: WSAVA-PennGen



DNA tests available worldwide: WSAVA-PennGen

Laboratories

Animal Molecular Genetics Lab - U of Missouri 321 Connaway Hall Columbia, MO 65211-5120 UNITED STATES	http://www.caninegeneticdiseases.net/ HansenL@missouri.edu
Genetic Technologies Ltd. 60-66 Hanover Street Fitzroy Vic 3065 AUSTRALIA	http://www.animalnetwork.com.au/dnatesting/ askus@animalnetwork.com.au
Genindexe 6, rue de sports La Rochelle 17000 FRANCE	http://www.genindexe.com/uk/index.php contact@genindexe.com
Laboklin Steubenstraße 4 Post box 1810 Bad Kissingen D-97688 GERMANY	http://www.laboklin.de/ info@laboklin.de
Paw Print Genetics 850 E Spokane Falls Blvd, Suite 200 Spokane, WA 99202 UNITED STATES	https://www.pawprintgenetics.com AskUs@pawprintgenetics.com
Van Haeringen Agro Business Park 100, PO ox 408 6700 AK Wageningen NETHERLANDS	http://www.vhlgenetics.com info@vhlgenetics.com
VetGen 3728 Plaza Drive, Suite 1 Ann Arbor, MI 48108 UNITED STATES	http://www.vetgen.com/ vetgen@vetgen.com
Vetnostic Laboratories 2439 Kuser Road Hamilton Township, NJ 08690 UNITED STATES	https://www.vetnostic.com/index.php?route=common/home rmason@mdlab.com

Information to extract from databases

OMIA

- Gene and mutation
- Breed(s)
- Prevalence
- Scientific articles

WSAVA-PennGen

- Gene and mutation
- Breed(s)
- Scientific article about the mutation
- Testing laboratories

Number of dogs
Origin of the dogs
Genotype-phenotype correlation

Genotype	Healthy	Affected
+/+		
+/-		
-/-		

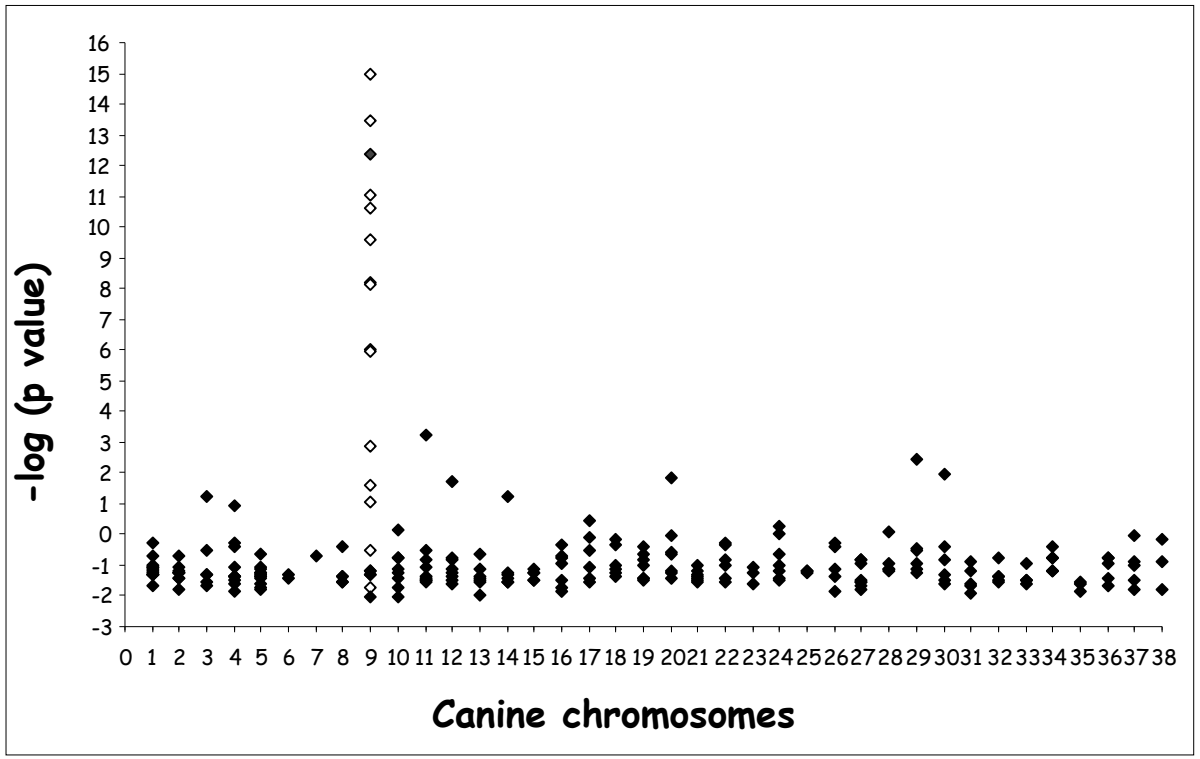
First example of scientific articles

Cerebellar ataxia in American Staffordshire Terriers

Linkage study in 48 US dogs

Association study in 77 French dogs

➢ **a unique region**



Abitbol *et al.*, PNAS 2010

First example of scientific articles

Cerebellar ataxia in American Staffordshire Terriers

Clinical status of dogs	Healthy			Affected
Genotype at the *ARSG*-SNP locus	*G/G*	*G/A*	*A/A*	*A/A*
French ASTs	53.5% (*n* = 38)	45.1% (*n* = 32)	1.4% (*n* = 1)	100% (*n* = 66)
US ASTs	43.6% (*n* = 48)	53.6% (*n* = 59)	2.7% (*n* = 3)	100% (*n* = 72)

Abitbol *et al.*, PNAS 2010

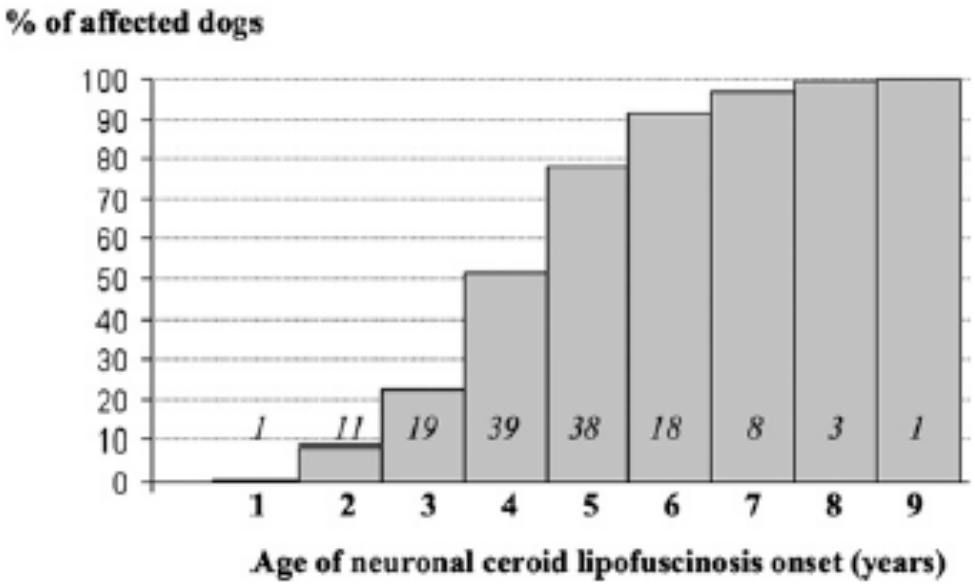
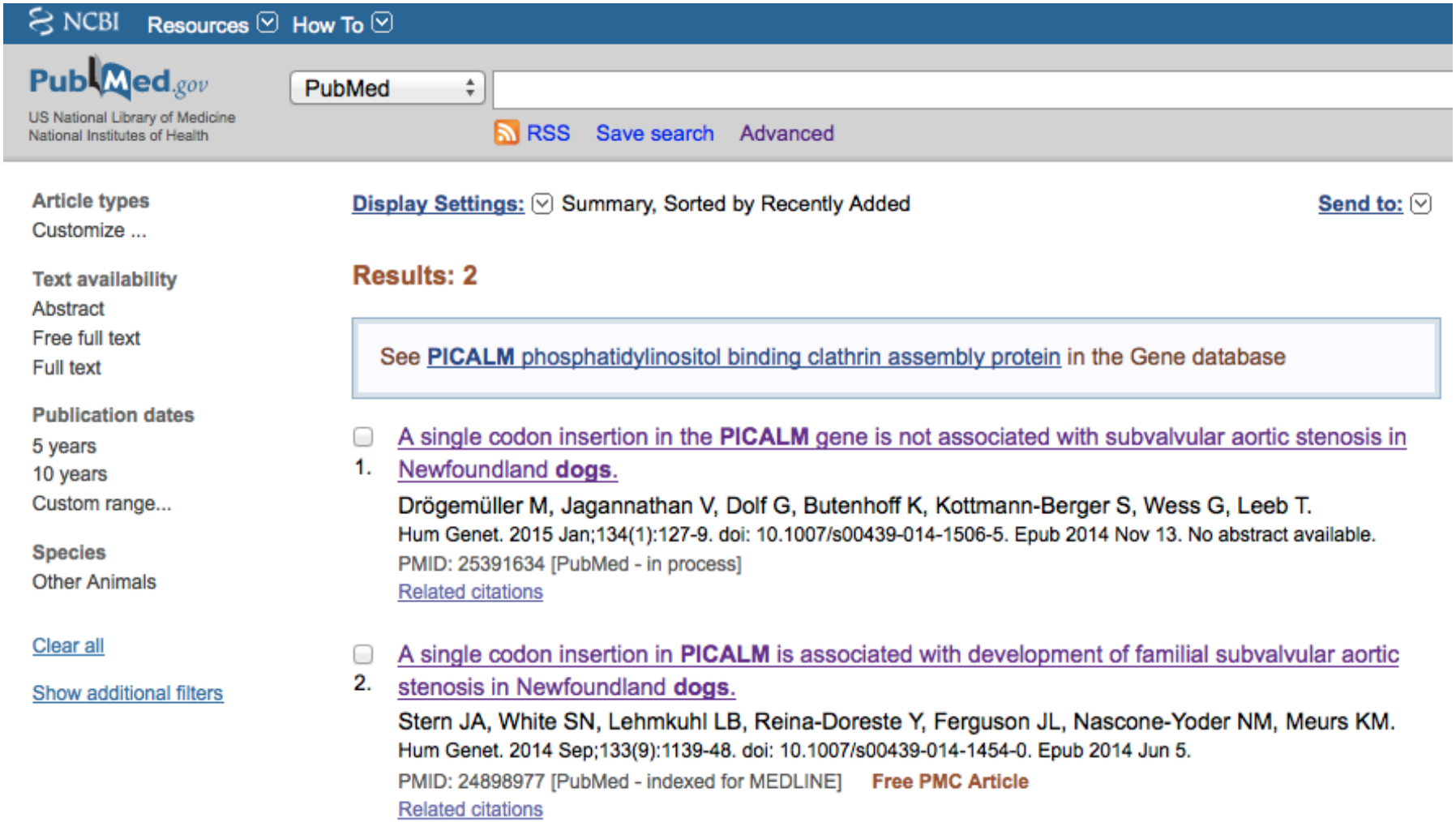


Fig. S1. Distribution of age at disease onset in the 138 French and US affected ASTs.

Second example of scientific articles

Aortic stenosis in Newfoundland dogs



Second example of scientific articles

18 affected dogs
20 control dogs

Aortic stenosis in Newfoundland dogs

Stern *et al.*, Hum Genet 2014

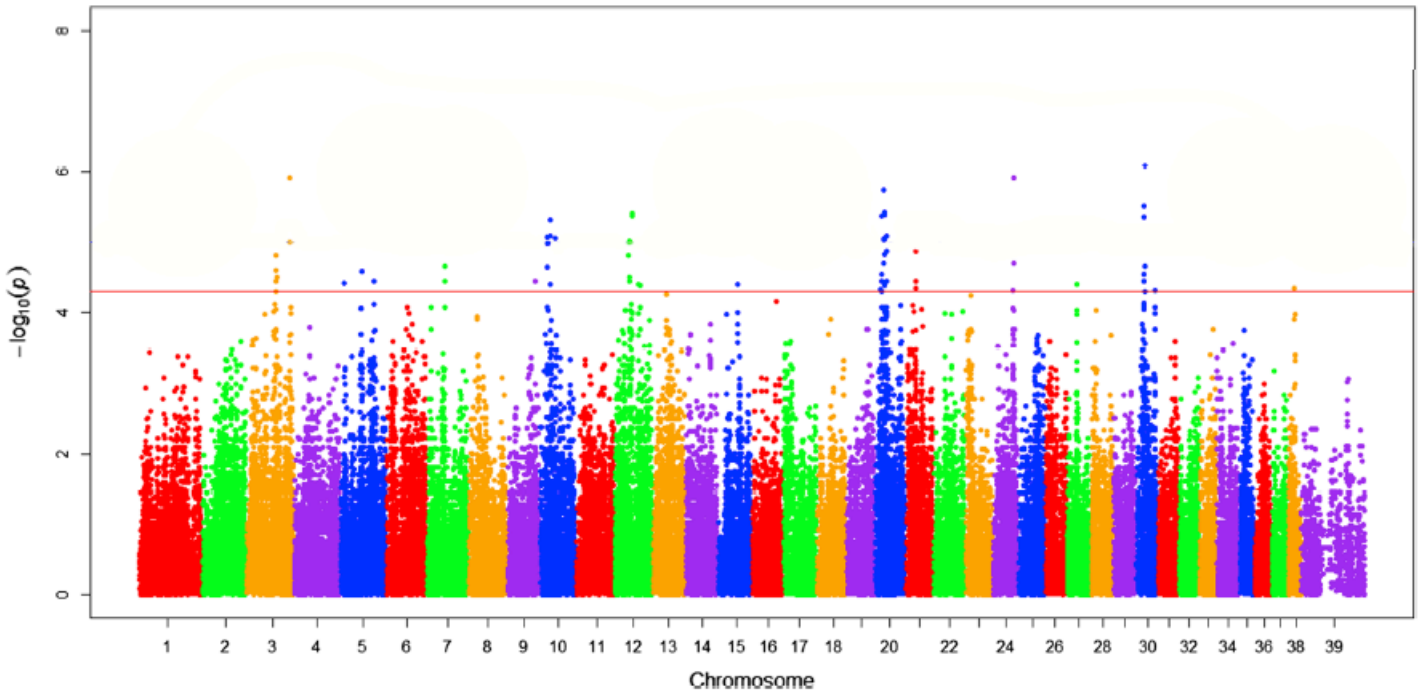


Fig. 2 Manhattan plot demonstrating association of SNP markers with SAS in Newfoundlands graphed as chromosomal location versus –Log10 of P_{raw} value. The *red line* represents the threshold of genome-wide suggestive loci

- **Several regions**

Second example of scientific articles

Aortic stenosis in Newfoundland dogs

Stern *et al.*, Hum Genet 2014

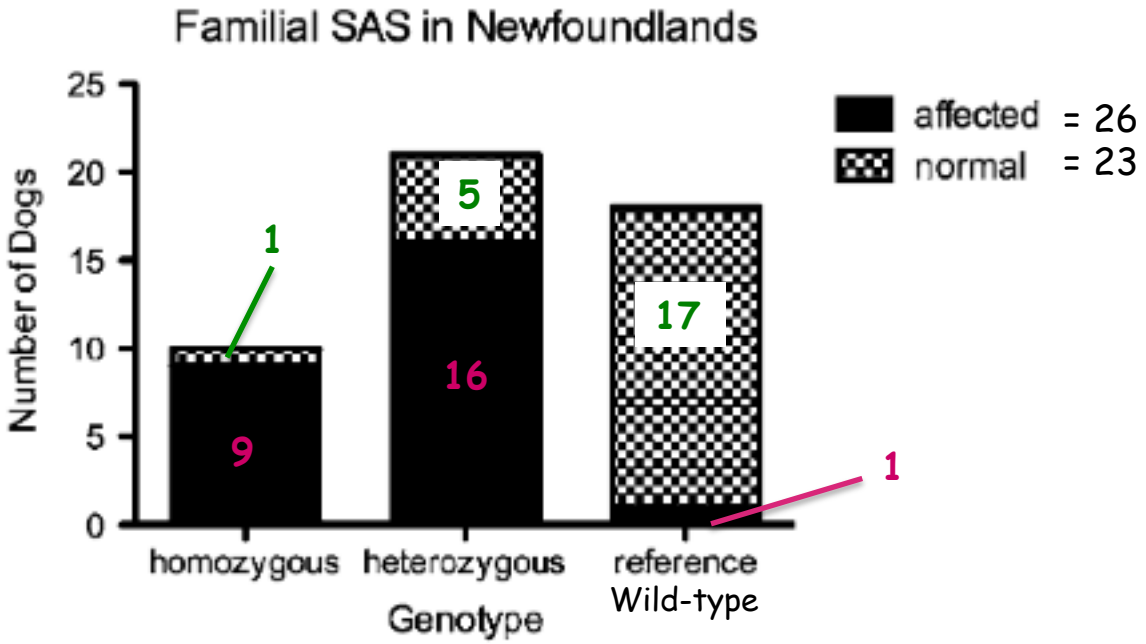


Fig. 4 A *bar graph* represents genotypes of Newfoundland dogs sequenced for the PICALM insertion associated with SAS. *Checkered regions* represent normal Newfoundland dogs while *blackened* regions demonstrate SAS-affected Newfoundland dogs

Second example of scientific articles

Aortic stenosis in Newfoundland dogs

Table 1 PICALM:p.K599_L600insL allele and genotype distribution in Newfoundland dogs

References	Phenotype class	*n*	Allele distribution		Genotype distribution		
			wt	ins	wt/wt	wt/ins	ins/ins
Stern et al. (2014)							
	SAS non-affected	23	39 (0.85)	7 (0.15)	17 (0.74)	5 (0.22)	1 (0.04)
	SAS affected	26	18 (0.35)	34 (0.65)	1 (0.04)	16 (0.62)	9 (0.35)
This study							
	Phenotype unknown	286	344 (0.60)	228 (0.40)	98 (0.34)	148 (0.52)	40 (0.14)
	SAS non-affected, LVOT V_{max} unknown	11	15 (0.68)	7 (0.32)	6 (0.55)	3 (0.27)	2 (0.18)
	SAS non-affected, LVOT V_{max} <1.9 m/s	88	106 (0.60)	70 (0.40)	30 (0.34)	46 (0.52)	12 (0.14)
	SAS equivocal, LVOT V_{max} = 1.9–2.4 m/s	8	11 (0.69)	5 (0.31)	4 (0.50)	3 (0.38)	1 (0.13)
	SAS affected, LVOT V_{max} >2.4 m/s	6	8 (0.67)	4 (0.33)	2 (0.33)	4 (0.67)	0 (0.00)

While Stern et al. reported a significant association of the insertion with SAS in their cohort, there is no statistically significant difference between the allele or genotype distribution in any of the five phenotype classes investigated in this study ($p_{allelic} = 0.91$; $p_{genotypic} = 0.80$; Fisher's exact test)

Drögemüller *et al.*, Hum Genet 2015

Choosing a Lab!

Criteria to choose a reliable genetic testing laboratory

Dr Marie Abitbol
2nd International Dog Health Workshop
Dortmund, 2015

Different types of Lab

- University Laboratory

  Has performed the research to discover the mutation?

- For-profit Laboratory
  - With a R and D department
  - Without a R and D department

- Staff of the Lab
  - With a graduated scientist (PhD)
  - With a commercial staff and a technical staff only
    - Interpretation of the results
    - Additional research to resolve a discrepancy in a result?

Conclusion

- Scientific accuracy of the new DNA test
  - Publication
  - Number of dogs
  - Sampling method
  - Genotype-phenotype correlation

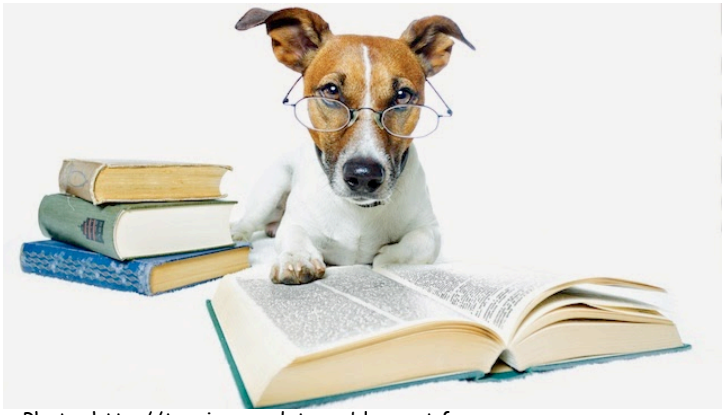


Photo: http://terriermandotcom.blogspot.fr

- Laboratory
  - Staff
  - Research department
  - Communications