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Stern Lab

Cardiac Genetics Part II

Influence on Cardiac Disease Phenotype and Pharmacologic Therapies

Conti Symposium 2025

Joshua Stern, DVM, PhD, DACVIM (Cardiology)
Associate Dean for Research & Graduate Studies

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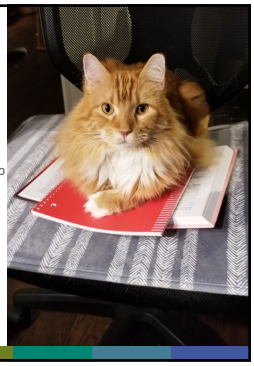
Disclosures

- NC State Operates a Cardiac Genetic Testing Service
- Scientific Advisory Board Member – TriviumVet
- Consultant & Collaborator Myokardia Inc. / Bristol Myers Squibb
- Relevant Sponsored Research Projects
  - Cytokinetics, Inc.
  - TriviumVet
  - Edgewise Therapeutics



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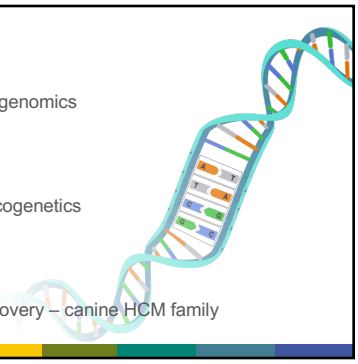
Outline

- Basis of pharmacogenetics/genomics
- Utility in human cardiology
- Companion animal pharmacogenetics
- Case Discussions
- Phenotype to genotype discovery – canine HCM family



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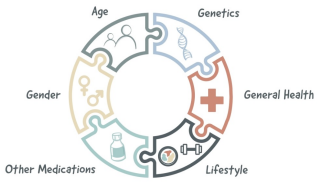
Individualized Medicine and Pharmacogenetics



https://precisionmedicine.uthealth.org/what-is-pharmacogenetics/

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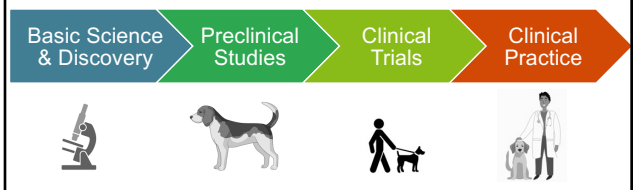
The Pathway



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Pharmacogenomics - The Human Experience

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CYP2C19 – Normal vs. Intermediate vs. Poor Metabolizers

CPIC UPDATE

Clinical Pharmacogenetics Implementation Consortium Guideline for *CYP2C19* Genotype and Clopidogrel Therapy: 2022 Update

Craig R. Lee[1], Jasmine A. Luzum[2], Katrin Sangkuhl[3], Roseann S. Gammal[4,5], Marc S. Sabatine[6], Charles Michael Stein[7], David F. Kisor[8], Nita A. Limdi[9], Yee Ming Lee[10], Stuart A. Scott[11,12], Jean-Sébastien Hulot[13], Dan M. Roden[14], Andrea Gaedigk[15], Kelly E. Caudle[3], Teri E. Klein[3], Julie A. Johnson[16] and Alan R. Shuldiner[17,*]

CYP2C19 catalyzes the bioactivation of the antiplatelet prodrug clopidogrel, and *CYP2C19* genotype impacts clopidogrel active metabolite formation. CYP2C19 intermediate and poor metabolizers who receive clopidogrel experience reduced platelet inhibition and increased risk for major adverse cardiovascular and cerebrovascular events. This guideline is an update to the 2013 Clinical Pharmacogenetics Implementation Consortium (CPIC) guideline for the use of clopidogrel based on *CYP2C19* genotype and includes expanded indications for *CYP2C19* genotype-guided antiplatelet therapy, increased strength of recommendation for CYP2C19 intermediate metabolizers, updated *CYP2C19* genotype to phenotype translation, and evidence from an expanded literature review (updates at www.cpicpgx.org).

CYP2C19 – Deranged Metabolism = Altered Drug Effects

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Table 1 Assignment of predicted CYP2C19 phenotype based on genotype

Predicted phenotype	Genotype	Examples of *CYP2C19* diplotypes[a]
CYP2C19 ultrarapid metabolizer	An individual carrying two increased function alleles	*17/*17
CYP2C19 rapid metabolizer	An individual carrying one normal function allele and one increased function allele	*1/*17
CYP2C19 normal metabolizer	An individual carrying two normal function alleles	*1/*1
CYP2C19 likely intermediate metabolizer[b]	An individual carrying one normal function allele and one decreased function allele or one increased function allele and one decreased function allele or two decreased function alleles	*1/*9, *9/*17, *9/*9
CYP2C19 intermediate metabolizer	An individual carrying one normal function allele and one no function allele or one increased function allele and one no function allele	*1/*2, *1/*3, *2/*17, *3/*17
CYP2C19 likely poor metabolizer[b]	An individual carrying one decreased function allele and one no function allele	*2/*9, *3/*9
CYP2C19 poor metabolizer	An individual carrying two no function alleles	*2/*2, *3/*3, *2/*3
Indeterminate metabolizer	An individual carrying one or two uncertain function alleles	*1/*12, *2/*12, *12/*14

[a]Please refer to the *CYP2C19* Diplotype-Phenotype Table online for a complete list. For allele functions and population-specific allele and phenotype frequencies, please refer to the *CYP2C19* Allele Functionality Table and the *CYP2C19* Allele Frequency Table online.[8,9]
[b]There are limited data to characterize the function of decreased function alleles.

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CYP2C19 – Recs for CV Disease – Normal - Rapid Metabolizer

Table 2 Antiplatelet therapy recommendations based on CYP2C19 phenotype when considering clopidogrel for cardiovascular indications

CYP2C19 phenotype[a]	Implications for phenotypic measures	Therapeutic recommendation	Classification of recommendation[b] - ACS and/or PCI[c]	Classification of recommendation[b] - non-ACS, non-PCI cardiovascular indications[d]
CYP2C19 ultrarapid metabolizer	Increased clopidogrel active metabolite formation; lower on-treatment platelet reactivity; no association with higher bleeding risk	If considering clopidogrel, use at standard dose (75 mg/day)	Strong	No recommendation
CYP2C19 rapid metabolizer	Normal or increased clopidogrel active metabolite formation; normal or lower on-treatment platelet reactivity; no association with higher bleeding risk	If considering clopidogrel, use at standard dose (75 mg/day)	Strong	No recommendation
CYP2C19 normal metabolizer	Normal clopidogrel active metabolite formation; normal on-treatment platelet reactivity	If considering clopidogrel, use at standard dose (75 mg/day)	Strong	Strong

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CYP2C19 – Recs for CV Disease – Intermediate Metabolizer

CYP2C19 likely intermediate metabolizer	Reduced clopidogrel active metabolite formation; increased on-treatment platelet reactivity; increased risk for adverse cardiac and cerebrovascular	Avoid standard dose clopidogrel (75 mg) if possible. Use prasugrel or ticagrelor at standard dose if no contraindication	Strong[e]	No recommendation[e]
CYP2C19 intermediate metabolizer	Reduced clopidogrel active metabolite formation; increased on-treatment platelet reactivity; increased risk for adverse cardiac and cerebrovascular events	Avoid standard dose (75 mg) clopidogrel if possible. Use prasugrel or ticagrelor at standard dose if no contraindication	Strong	No recommendation

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CYP2C19 – Recs for CV Disease – Poor Metabolizer

CYP2C19 likely poor metabolizer	Significantly reduced clopidogrel active metabolite formation; increased on-treatment platelet reactivity; increased risk for adverse cardiac and cerebrovascular events	Avoid clopidogrel if possible. Use prasugrel or ticagrelor at standard dose if no contraindication	Strong[e]	Moderate[f]
CYP2C19 poor metabolizer	Significantly reduced clopidogrel active metabolite formation; increased on-treatment platelet reactivity; increased risk for adverse cardiac and cerebrovascular events	Avoid clopidogrel if possible. Use prasugrel or ticagrelor at standard dose if no contraindication	Strong	Moderate

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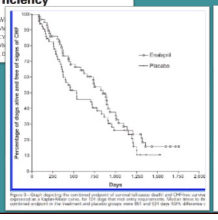
Can Genetics Explain Discrepant RCT Results?

Results of the veterinary enalapril trial to prove reduction in onset of heart failure in dogs chronically treated with enalapril alone for compensated, naturally occurring mitral valve insufficiency

Clarke E. Atkins, DVM, DACVIM; Bruce W. Keene, DVM, MS, DACVIM; W
Mary Ann Crawford, DVM, DACVIM; Teresa C. DeFrancesco, DV
Phillip R. Fox, DVM, DACVIM; Linda B. Lehmkuhl, DVM, DAC
Kathryn M. Meurs, DVM, PhD, DACVIM; Jean-Paul Petrie, DV
Steven L. Rosenthal, DVM, DACVIM; Jennifer A. Sadley, DVM,

VETPROOF
24 different breeds; n=124



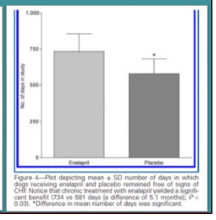


Figure 4—Plot depicting mean ± SD number of days in which dogs receiving enalapril and placebo remained free of signs of CHF. Notice that chronic treatment with enalapril yielded a significant benefit (734 vs 581 days [a difference of 5.1 months]; $P < 0.03$). *Difference in mean number of days was significant.

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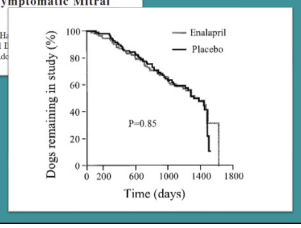
Can Genetics Explain Discrepant RCT Results?

J Vet Intern Med 2002;16:80–88

Efficacy of Enalapril for Prevention of Congestive Heart Failure in Dogs with Myxomatous Valve Disease and Asymptomatic Mitral Regurgitation

Clarence Kvart, Jens Häggström, Henrik Duelund Pedersen, Kerstin Hi
Anna-Kaisa Järvinen, Anna Tidholm, Karina Bsenko, Erik Ahlgren, Mikael E
Ellen Bjerkås, Susanne Gundler, Peter Lord, Gudrun Wegeland, Eva Ad

SVEP
100% CKCS; n=229 dogs



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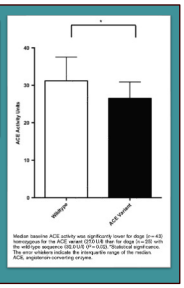
Can Genetics Explain Discrepant RCT Results?

Angiotensin-converting enzyme activity in Cavalier King Charles Spaniels with an ACE gene polymorphism and myxomatous mitral valve disease

Kathryn M. Meurs[a], Lisbeth H. Olsen[d], Maria J. Reimann[d], Bruce W. Keene[e], Clarke E. Atkins[a], Darcy Adin[a], Brent Aona[a], Julia Condit[a], Teresa DeFrancesco[a], Yamir Reina-Doreste[a], Joshua A. Stern[a,b], Sandra Tou[a], Jessica Ward[a,c] and Kathleen Woodruff[a]

N=73 CKCS – 66% Mutant!

43 of 73 (59%) = Homozygous Mutant
5 of 73 (7%). = Heterozygous Mutant
25 of 73 (34%) = Wildtype



Pharmacogenetics and Genomics 2018, Vol 28 No 2

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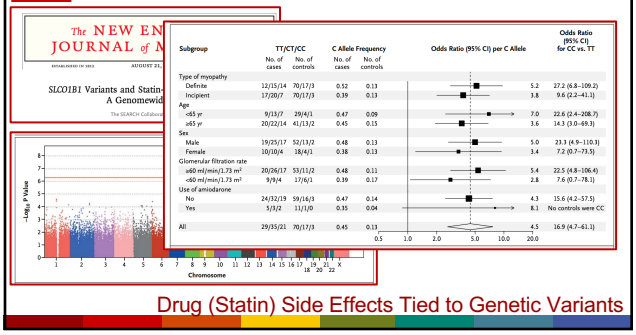

Drug (Statin) Side Effects Tied to Genetic Variants

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Can Genetics Explain AEs in Companion Animals?

J Vet Intern Med 2008;22:235–241

Effect of Spironolactone on Diastolic Function and Left Ventricular Mass in Maine Coon Cats with Familial Hypertrophic Cardiomyopathy

K.A. MacDonald, M.D. Kittleson, and P.H. Kass

4 of 13 cats from a closed breeding colony experienced severe facial dermatitis

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Can Genetics Explain AEs in Companion Animals?

Journal of Veterinary Cardiology (2018) 20, 1–12

ELSEVIER

Journal of Veterinary Cardiology

www.elsevier.com/locate/jvc

The SEISICAT study: a pilot study assessing efficacy and safety of spironolactone in cats with congestive heart failure secondary to cardiomyopathy☆

No cat experienced facial excoriations over 15 months of study (0 of 9)

Rachel James, VetMB [a], Emilie Guillot, DVM [a], Catherine Garelli-Paar, Pharm D [b], Jacqueline Huxley, BVSc [a], Vanessa Grassi, MSc [b], Malcolm Cobb, PhD [a,*]

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Pharmacogenomics - The Companion Animal Experience

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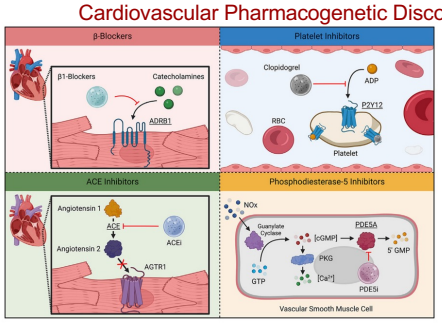
Cardiovascular Pharmacogenetic Discoveries



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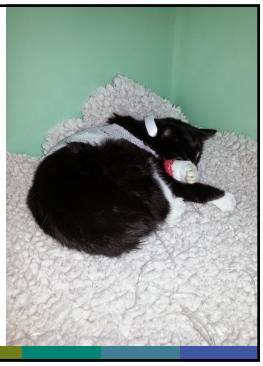
CASE - FRANKIE

- 4yr MC DSH
- rDVM ausculted gallop and HCM B2 was diagnosed
- Clopidogrel 18.75mg PO q24 prescribed

- 1 month later
- Acute decompensation – R thoracic limb paralysis & respiratory distress



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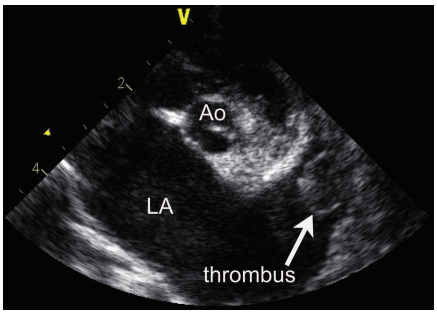

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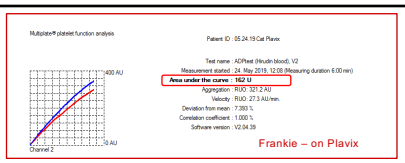
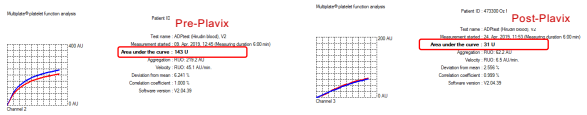
FRANKIE



- Normal cat response



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Could understanding Frankie's genetics at HCM diagnosis have altered therapy?

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CLOPIDOGREL RESISTANCE?

- Did patient receive recommended dose?
  - Compliance is a big issue
  - Taste aversion - always give in gel capsules
  - Compounded
- Clopidogrel resistance associated with on-treatment CV events in humans

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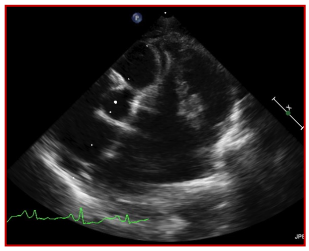
Clopidogrel Resistance Discovery

- 18-30% of cat population across studies has less robust or no measurable response to clopidogrel on advanced platelet testing
- Aspirin resistance 20-100%



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SNPs in *P2RY1*, *P2RY12*, and *CYPC19* for Clopidogrel Resistance in Cats

Nonsynonymous single nucleotide polymorphisms in candidate genes *P2RY1*, *P2RY12* and *CYP2C19* for clopidogrel efficacy in cats

Yu Ueda, Ronald Hak Long Li, Fern Tablin, Eric S. Ontiveros, Joshua A. Stern

Table 1 *P2RY1*, *P2RY12*, and *CYP2C19* polymorphisms.

Genes	Mutation	Location	Polymorphisms	Distribution *n* (%)
P2RY1	p.Ala236Gly	D2.110685285	C/C (wildtype)	14/34 (41.2)
			C/G	15/34 (44.1)
			G/G	5/34 (14.7)
P2RY12	p.Val34Ile	D2.112204070	G/G (wildtype)	12/34 (35.3)
			G/A	16/34 (47.1)
			A/A	6/34 (17.6)
CYP2C19	p.Pro479Leu	D3.56761958	C/C (wildtype)	13/34 (38.2)
			C/T	16/34 (47.1)
			T/T	5/34 (14.7)

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SNPs in *P2RY1*, *P2RY12*, and *CYPC19* for Clopidogrel Resistance in Cats

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			C/T	16/34 (47.1)
			T/T	5/34 (14.7)

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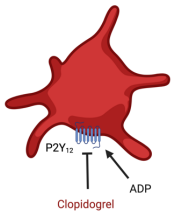
Clopidogrel Resistance – Clinical Trial

scientific reports

OPEN **A genetic polymorphism in *P2RY*$_1$ impacts response to clopidogrel in cats with hypertrophic cardiomyopathy**

Yu Ueda[1], Ronald H. L. Li[2], Nghi Nguyen[2], Eric S. Ontiveros[2], Samantha L. Kovacs[2], Maureen S. Oldach[2], Karen M. Vernau[2], Michael H. Court[2]* & Joshua A. Stern[2]*



- n = 49 HCM cats
- A236G variant in the *P2RY1* gene
- Reduced response to clopidogrel
- High prevalence in HCM-affected cats (51% heterozygous, 16.3% homozygous)

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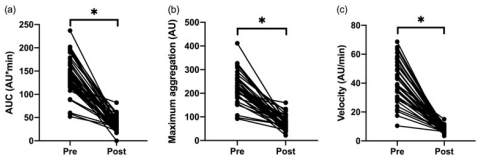
P2Y1-Mediated Clopidogrel Resistance

A genetic polymorphism in *P2RY*$_1$ impacts response to clopidogrel in cats with hypertrophic cardiomyopathy

Yu Ueda, Ronald H. L. Li, Nghi Nguyen, Eric S. Ontiveros, Samantha L. Kovacs, Maureen S. Oldach, Karen M. Vernau, Michael H. Court & Joshua A. Stern



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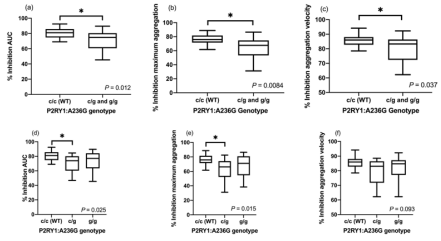
Genotype-Dependent Percent Platelet Inhibition

A genetic polymorphism in *P2RY*$_1$ impacts response to clopidogrel in cats with hypertrophic cardiomyopathy

Yu Ueda, Ronald H. L. Li, Nghi Nguyen, Eric S. Ontiveros, Samantha L. Kovacs, Maureen S. Oldach, Karen M. Vernau, Michael H. Court & Joshua A. Stern



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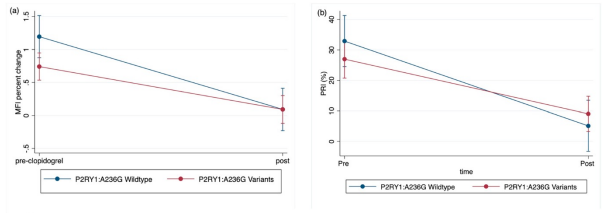
P-Selectin Mean Fluorescence

A genetic polymorphism in *P2RY$_1$* impacts response to clopidogrel in cats with hypertrophic cardiomyopathy

Yu Ueda, Ronald H. L. Li, Nghi Nguyen, Eric S. Ontiveros, Samantha L. Kovacs, Maureen S. Oldach, Karen M. Vernau, Michael H. Court & Joshua A. Stern



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Could understanding Frankie's genetics at HCM diagnosis have altered therapy?

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Could … s genet … ave altere…

NC State College of Veterinary Medicine

Veterinary Cardiac Genetics Laboratory

1060 William Moore Dr., RB 326
Raleigh, NC 27607

cvm-cardiacgenetics@ncsu.edu
(919) 515-3277

P2RY1 Mutation Genetic Testing Results

Clopidogrel is a drug frequently used to prevent blood clot formation in cats with heart disease. A portion of cats are resistant to this medication or have less than expected response to treatment. A mutation in the P2RY1 gene is associated with reduced response to this medication and cats harboring this mutation may benefit from altered drug dosing, selection of a different anti-thrombotic drug, or combined anti-thrombotic therapies under the consultation of a veterinarian.

Owner Last Name:

Animal Name: Frankie

Result: **Positive Homozygous**

ID: **494958**

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Ongoing Observational Clinical Research

7 cases with atrial thrombus, ATE or smoke despite consistent clopidogrel therapy

6 of 7- mutant

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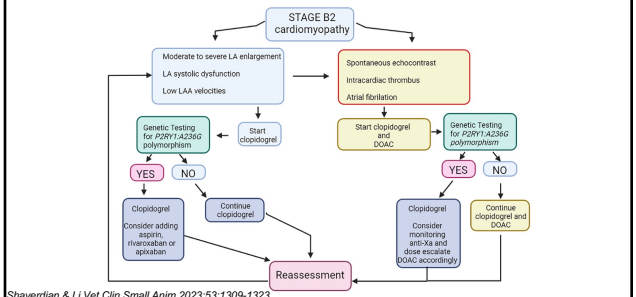
Clopidogrel Resistance – Clinical Decision-Making



Shaverdian & Li Vet Clin Small Anim 2023;53:1309-1323

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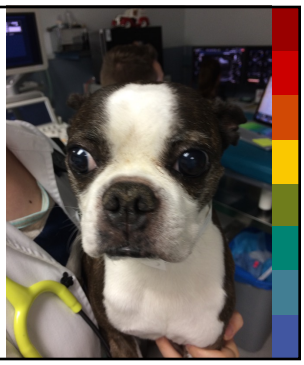
Pulmonary Hypertension & Variable Response to Sildenafil



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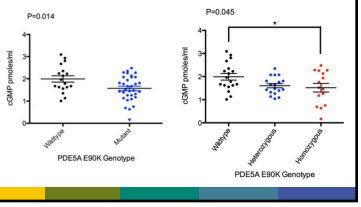
PDE5A:E90K SNP Affects cGMP in Healthy Dogs

Identification of PDE5A:E90K: A Polymorphism in the Canine Phosphodiesterase 5A Gene Affecting Basal cGMP Concentrations of Healthy Dogs

J. A. Stern, Y. Reina-Doreste, L. Chdid, and K. M. Meurs

Species	Amino Acid Sequence
Rat	T V S F L A D S E K H E Q M P L T P P R F D N
Platypus	T V S F L A D S E K H E Q M P L T P P R F D N
Opossum	T V S F L A D S E K H E Q M P L T P P R F D N
Chameleon	T V S F L A D S E K H E Q M P L T P P R F D N
Turkey	T V S F L A D S E K H E Q M P L T P P R F D N
Chicken	T V S F L A D S E K H E Q M P L T P P R F D N
Pufferfish	T V S F L A D S E K H E Q M P L T P P R F D N
Frog	T V S F L A D S E K H E Q M P L T P P R F D N
Human	T V S F L S D S E K H E Q M P L T P P R F D N
Cat	T V S F L S D S E K H E Q M P L T P P R F D N
Horse	T V S F L S D S E K H E Q M P L T P P R F D N
Elephant	T V S F L S D S E K H E Q M P L T P R R F D N
Dog	T V S F L A D S E K H E Q M P L T P P R F D N
Mutant Dog	T V S F L S D S E K H K Q M P L T P R R F D



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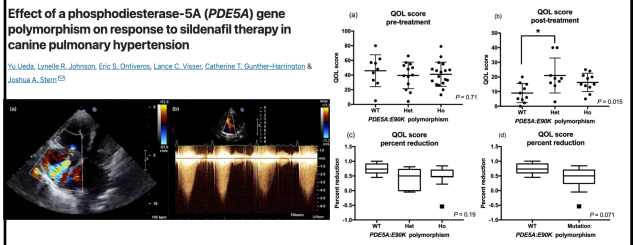
PDE5A:E90K In Dogs with Pulmonary Hypertension

Effect of a phosphodiesterase-5A (*PDE5A*) gene polymorphism on response to sildenafil therapy in canine pulmonary hypertension

Yu Ueda, Lynelle R. Johnson, Eric S. Ontiveros, Lance C. Visser, Catherine T. Gunther-Harrington & Joshua A. Stern



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Consider dose escalation or multimodal therapy in PDE5A mutants. Can we genotype fast enough to aid clinical decision-making

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What's yet to be discovered?

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MMVD Dog – PDE5i Super Responders?

- August 2016
  - B2; LA:Ao 2.1
  - No obvious c.t. rupture
  - Pimobendan initiated
- February 2017
  - LA:Ao 1.5
- September 2020
  - LA:Ao 1.7
  - Non-cardiac COD 16.5yrs

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Phenotype to Genotype Discovery

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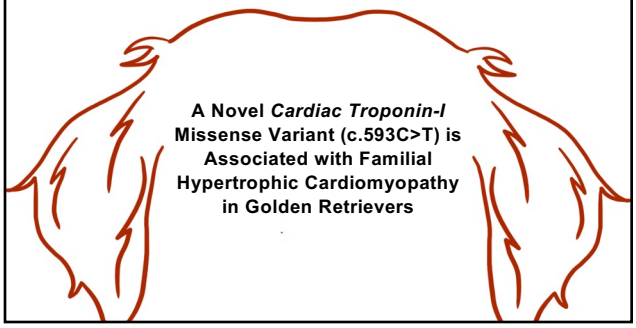

A Novel *Cardiac Troponin-I* Missense Variant (c.593C>T) is Associated with Familial Hypertrophic Cardiomyopathy in Golden Retrievers

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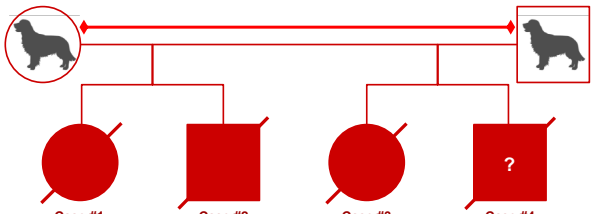
Golden Retriever HCM Cases



Case #1
September 2023
- 12mo, intact

Case #2
October 2023
- 14mo, spayed

Case #3
November 2023
- 10mo, intact

Case #4

Full Necropsy

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Hypothesis

HCM in this Golden Retriever family is caused by a genetic variant(s) harbored in sarcomere-related gene(s)

Aim

Identify the first-ever reported genetic etiology of canine HCM via a Whole-Genome Association Study (WGAS)

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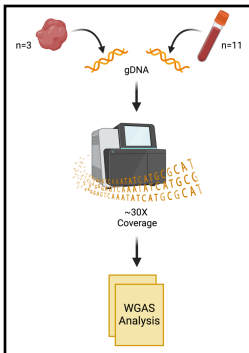
Methods



Autosomal Recessive ($P_{Allelic}$)

	HCM-Affected	Control
Variant	6	1
Wildtype	0	10

$P_{Allelic}$=0.0237

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Results: Necropsy

Presumed cause of death: Sudden cardiac death following arrhythmogenic event

Shared Features

Gross Pathologic
- Biventricular hypertrophy
- Evidence of L-CHF

Histopathologic
- Cardiomyocyte hypertrophy
- ↑ interstitial fibrosis
- Myofiber disarray
- Myocytolysis

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Results: WGAS

- Total Freebayes-called variants: **13,186,318**
- Total $P_{Allelic}$<0.024: **98,268**
- 'MODERATE' $P_{Allelic}$<0.024: **225**
- 'HIGH' $P_{Allelic}$<0.024: **33**
- Segregating 'MODERATE' $P_{Allelic}$<0.024 called: **8**
- Segregating 'HIGH' $P_{Allelic}$<0.024 called: **2**

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Results: Unique Variants

Validation cohort
- 2,771 unphenotyped dogs (>400 breeds)
  - Including 52 GRs
- Additional 42 GRs
  - Total of 94 GRs

***Cardiac Troponin-I* (*TNNI3*)**
- 'MODERATE' missense variant
  - Chr1:103244333 (canFam4)
  - c.593C>T; p.Ala198Val
  - Perfect segregation

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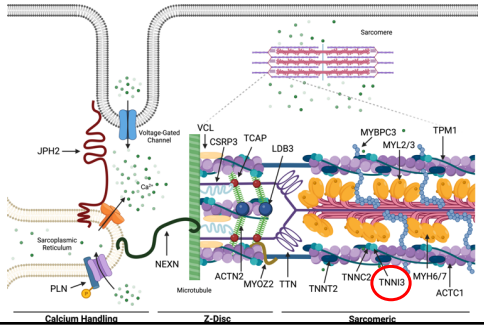
TNNI3: In Humans



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TNNI3: In Humans

RESEARCH ARTICLE

Founder Mutation in N Terminus of Cardiac Troponin I Causes Malignant Hypertrophic Cardiomyopathy

Nath Heart J (2011) 19:344–351
DOI 10.1007/s12471-011-0135-z

SPECIAL ARTICLE

Recurrent and founder mutations in the Netherlands: cardiac Troponin I (*TNNI3*) gene mutations as a cause of severe forms of hypertrophic and restrictive cardiomyopathy

A. van den Wijngaard · P. Volders · J. P. Van Tintelen · J. D. H. Jongbloed · M. P. van den Berg · R. H. Lekanne Deprez · M. M. A. M. Mannens · N. Hofmann · M. Siegerhorst · D. Dooijes · M. Michels · Y. Arens · R. Jongbloed · B. J. M. Smeets

Mutations in the cardiac troponin I gene associated with hypertrophic cardiomyopathy

Akinori Kimura[1], Haruhito Harada[1,2], Jeong-Euy Park[3], Hirofumi Nishi[2,4], Manatsu Satoh[1], Megumi Takahashi[1], Shitoshi Hiroi[1,5], Taishi Sasaoka[1], Nobuhisa Ohbuchi[1], Takeyuki Nakamura[1,5], Takeshi Koyanagi[2], Tae-Hoeng Hwang[3], Jin-A Choo[3], Kyu-Sung Chung[3], Akira Hasegawa[3], Ryozo Nagai[2], Osamu Okazaki[6], Hirobi Nakamura[7], Masunori Matsuzaki[7], Tsuguya Sakamoto[8], Hironori Toshima[8], Yoshinori Koga[8], Tsutomu Imaizumi[2] & Takehiko Sasazuki[10]

Functional Consequences of the Mutations in Human Cardiac Troponin I Gene Found in Familial Hypertrophic Cardiomyopathy

Fumi Takahashi-Yanaga[1]¶, Sachio Morimoto¶, Keita Harada[1]§, Reiko Minakami[2], Fumie Shiraishi[1], Mika Ohta[1,3], Qun-Wei Lu[1], Toshiyuki Sasaguri[1] and Iwao Ohtsuki[1]

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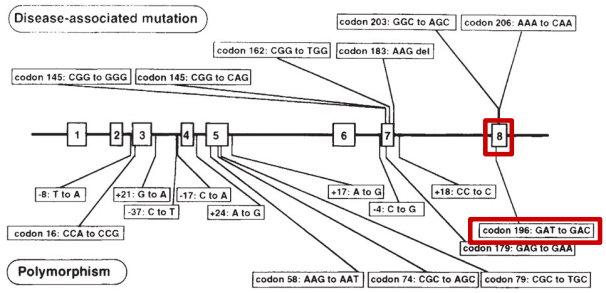
TNNI3: In Humans



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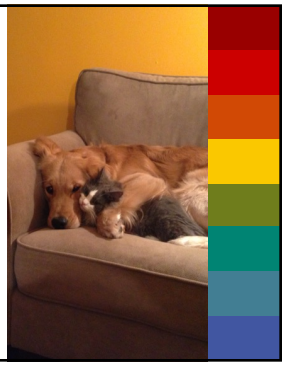
Stern Lab

Questions?



Joshua A. Stern, DVM, PhD, DACVIM (Cardiology)
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MORE POWERFUL THAN APPLAUSE

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ACVIM FORUM 2025

Questions?

Joshua A. Stern, DVM, PhD, DACVIM (Cardiology)
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