Sudden death and cardiomyopathy associated with *LMNA* in the Nova Scotia Duck Tolling Retriever

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Introduction

Dilated cardiomyopathy (DCM) is the most common cardiomyopathy in humans and is characterized by dilation of the left ventricle and decreased systolic function of the heart. Individuals with DCM develop heart failure, arrythmias and are at risk for premature and sudden death. DCM has a prevalence of one in 400 individuals, with 20-48% of those cases being familial DCM (FDCM)[1]. Human FDCM often has a dominant mode of inheritance, but a recessive mode of inheritance has been documented as well[2]. FDCM has also been reported in certain dog breeds and has been associated with genes that encode structural proteins of the cardiac myocyte[3]. FDCM has not been reported in the Nova Scotia Duck Tolling Retriever (NSDTR).

Echocardiograms of Toller Family

	WT (kg)	LVIDd	LVIDs	LVIDDn (normal <1.7)	Fractional Shortening (normal >25%)	LA:Ao (normal <1.6)
Affected	11.3kg	3.9cm	3.4cm	1.9	13.5%	2.5
Affected	15.4kg	4.5cm	3.6cm	2.0	20%	2.4
Sibling	17.6kg	3.6cm	1.7cm	1.6	53%	1.3
Parent	19.5kg	2.8cm	1.9cm	1.2	35%	1.0
Parent	16.3kg	3.3cm	1.6cm	1.4	54%	1.1

LVIDd: Left ventricle internal diameter diastolic
LVIDDn: left ventricle size normalized for weight
LVIDs: Left ventricle internal diameter systolic
LA:Ao: Left atrial to aortic root ratio

Approach

Genome Wide Association Study and Runs of Homozygosity

- 2 cases and 35 related controls
- Criteria of allele frequency equal to one

Whole Genome Sequencing

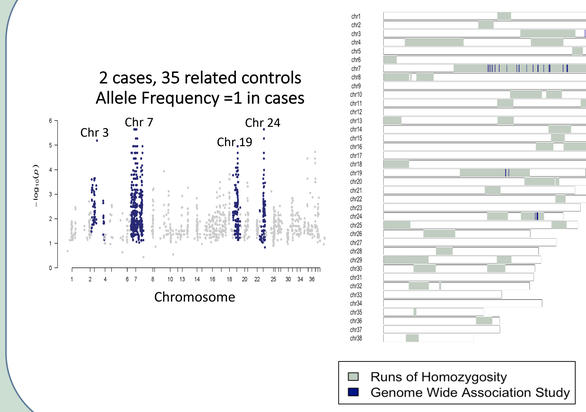
- 129 dogs
- Aligned to reference genome Mishka
- Recessive pedigree model used in WebGQT

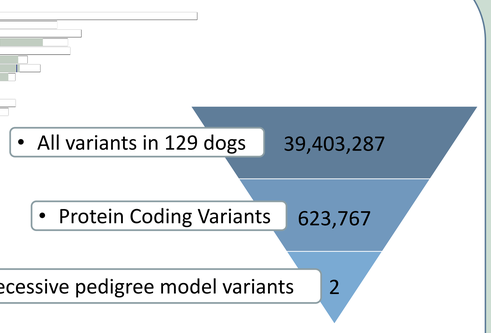
Genotyping

- Performed with Sanger sequencing on dogs from the pedigree as indicated
- 300 additional unrelated Tollers genotyped

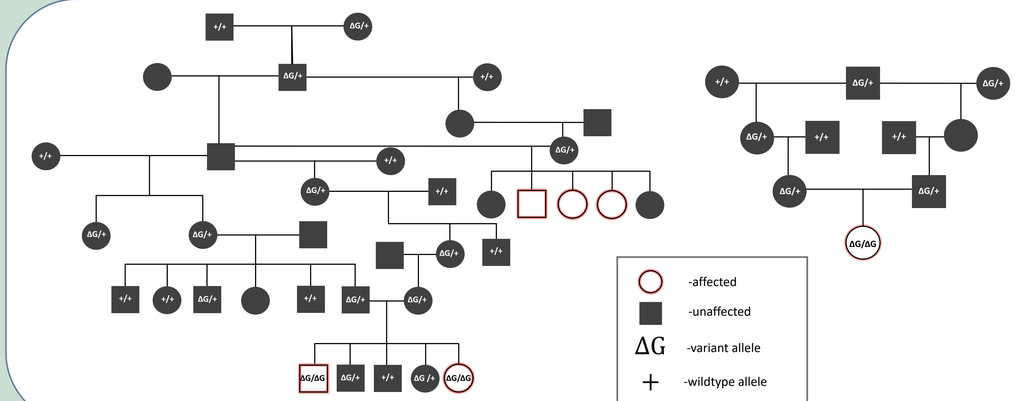
Functional Prediction

Genome Wide Association Study and Whole Genome Sequencing





Pedigrees



Conclusion

- We hypothesize that a guanine deletion in the LMNA gene causes a frameshift mutation which impairs the processing of prelamin to mature Lamin A but does not impair processing of mature Lamin C. Loss of Lamin A leads to DCM and sudden death in the NSDTR.
- Genotyping of 300 NSDTR revealed a carrier frequency of 8.7%.
- Future genetic testing of the LMNA deletion can be used to reduce the incidence of DCM in NSDTR.

Genotyping 300 Dogs

	Number of samples	Genotype Frequency	Allele Frequency
WT +/+	274	91.3%	95.7%
Heterozygous ΔG/+	26	8.7%	-
Homozygous ΔG/ΔG	0	0	4.3%

References

1. Burkett, E. L., & Hershberger, R. E. (2005). Clinical and genetic issues in familial dilated cardiomyopathy. Journal of the American College of Cardiology, 45(7), 969–981.
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3. Maurer-Stroh, S., Washietl, S., & Eisenhaber, F. (2003). Protein prenyltransferases. Genome biology, 4(4), 212.
4. Simpson, S., Edwards, J., Ferguson-Mignan, T. F., Cobb, M., Mongan, N. P., & Rutland, C. S. (2015). Genetics of Human and Canine Dilated Cardiomyopathy. International journal of genomics, 2015, 204823.
5. Zhang, F. L., & Casey, P. J. (1996). Protein prenylation: molecular mechanisms and functional consequences. Annual review of biochemistry, 65, 241–269.

LMNA Variant

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LMNA_Canine_Variant/1-699  534 STGEEVAMRKLVRSVTVVEDDEDEDGDDLLHHHHGSHCSSS 574
LMNA_Canine/1-665          534 STGEEVAMRKLVRSVTVVEDDEDEDGDDLLHHHHGSHCSSS 574
LMNA_Human/1-664           533 STGEEVAMRKLVRSVTVVEDDEDEDGDDLLHHHHGSHCSSS 573
LMNA_Canine_Variant/1-699  575 CTPPSTTCARAPCCAGLAGSLQTRLPPAAREPRWADPSPLA 615
LMNA_Canine/1-665          575 CDPAEYNLRSRTVLCGTCGQPADKASASSSGAQVGGSISSG 615
LMNA_Human/1-664           574 CDPAEYNLRSRTVLCGTCGQPADKASASGSGAQVGGPISSG 614
LMNA_Canine_Variant/1-699  616 LPPPVSQSPAATAVWGAVGVAASGTAWSPAPTSWAAPAPEP 656
LMNA_Canine/1-665          616 SSASSVTVTRSYRSVGGSGGGSFGDSLVTRSYLLGSSSPRT 656
LMNA_Human/1-664           615 SSASSVTVTRSYRSVGGSGGGSFGDNLVTRSYLLGNSSPRT 655
LMNA_Canine_Variant/1-699  657 RAPRTAASCDLGPARPGEGGGCLPPFCLTPTLPTPAQHLMG 697
LMNA_Canine/1-665          657 QSPQNCSIM---------------------------------- 665
LMNA_Human/1-664           656 QSPQNCSIM---------------------------------- 664
LMNA_Canine_Variant/1-699  698 GA
LMNA_Canine/1-665          --
LMNA_Human/1-664           --
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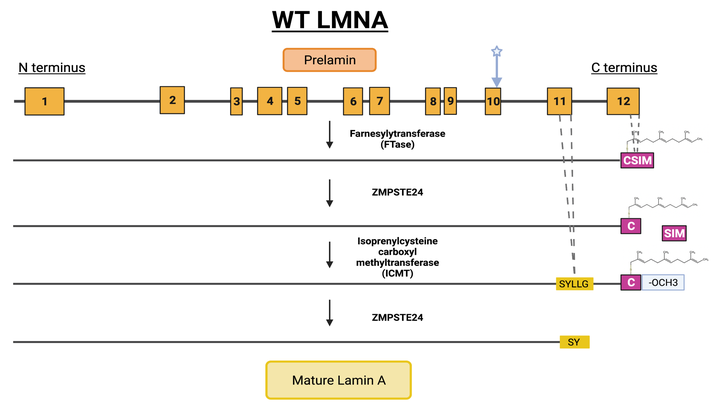
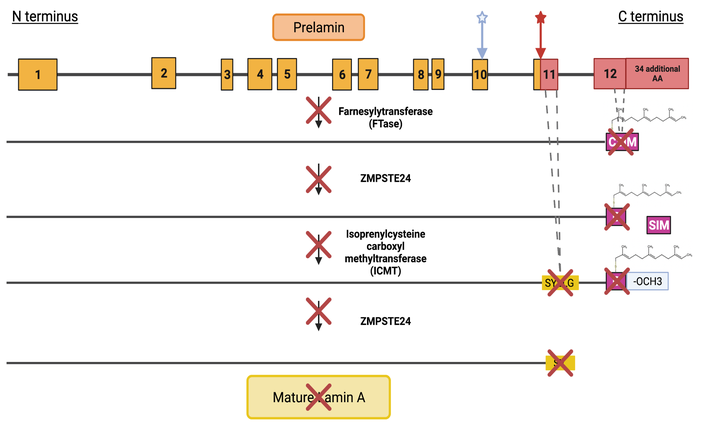
WGS identified a guanine deletion on CFA 7 corresponding to the *LMNA* gene which encodes scaffolding proteins of the nuclear membrane. The deletions causes a frameshift mutation and residue 576 (red box) is changed from an Aspartic acid to a Threonine. An additional 34 amino acids are added to the sequence (red underline). The *Caax* motif, CSIM, (blue box) is required for processing of prelamin to mature lamin A[4,5]. The last 13 amino acids of prelamin are cleaved between a tyrosine and leucine (blue dotted box) residue to complete the processing of mature lamin A. Lamin C is cleaved between residues 566 and 567 (red dashed line) for processing.



LMNA produces a prelamin that is alternatively spliced to mature Lamin A or Lamin C. The blue star and arrow indicate where prelamin is cut to produce Lamin C. Specific residues in prelamin guide processing of prelamin to mature Lamin A.



The frameshift mutation (red star) alters the amino acids in exon 11 and exon 12 of *LMNA*.

Acknowledgments

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