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Original Article

Evaluation of genes associated with human myxomatous mitral valve disease in dogs with familial myxomatous mitral valve degeneration

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ABSTRACT

Myxomatous mitral valve disease (MMVD) is the most common heart disease in the dog. It is believed to be heritable in Cavalier King Charles spaniels (CKCS) and Dachshunds. Myxomatous mitral valve disease is a familial disease in human beings as well and genetic mutations have been associated with its development. We hypothesized that a genetic mutation associated with the development of the human form of MMVD was associated with the development of canine MMVD. DNA was isolated from blood samples from 10 CKCS and 10 Dachshunds diagnosed with MMVD, and whole genome sequences from each animal were obtained. Variant calling from whole genome sequencing data was performed using a standardized bioinformatics pipeline for all samples. After filtering, the canine genes orthologous to the human genes known to be associated with MMVD were identified and variants were assessed for likely pathogenic implications.

No variant was found in any of the genes evaluated that was present in least eight of 10 affected CKCS or Dachshunds. Although mitral valve disease in the CKCS and Dachshund is a familial disease, we did not identify genetic cause in the genes responsible for the human disease in the dogs studied here.

Introduction

Myxomatous mitral valve degeneration (MMVD) is the most common heart disease in the dog (Gordon et al., 2017). Affected dogs can live with subclinical disease for years. However, many eventually develop clinical signs consistent with congestive heart failure and are at risk of sudden cardiac death. No medical cure for the disease exists. Instead, medical treatment is directed primarily at hemodynamic management of the clinical signs that result from severe mitral regurgitation with subsequent left atrial enlargement or congestive heart failure and in some cases, pulmonary hypertension (Gordon et al., 2017). While surgical repair or replacement of the valve is possible, the cost and expertise required to successfully implement these treatment strategies severely limit their applicability to the general pet population. The lack of knowledge about the factors that contribute to the development of this common and important canine disease has prevented the development of effective long-term clinical management plans.

Myxomatous degeneration of the mitral valve is believed to be heritable in at least some breeds, including the Cavalier King Charles spaniel (CKCS) and the Dachshund (Swenson et al., 1996; Olsen et al., 1999; Lewis et al., 2011). However, the mode of inheritance has not been determined and genetic mutation(s) responsible for the disease have not yet been identified. In the Whippet with mitral valve disease, the severity of the disease has been linked to a region on chromosome 15 (Stern et al., 2015).

Myxomatous mitral valve disease is a familial disease in human beings that exists either as part of a larger multisystem syndrome, or as a singular disease, and genetic mutations have been associated with its development (Padang et al., 2012; LaHaye et al., 2014). Several of the genes associated with the development of MMVD are associated with the tumor growth factor-β (Tgf-β) superfamily. Mitral valve abnormalities have been associated with mutations in the Fibrillin 1 (*FBN1*) gene and Fibrillin 1 contributes to the regulation of transforming growth factor beta (Tgf-β) by targeting and concentrating Tgf-β at specific locations. Fibrillin 1 deficiency in mice has been associated with enhanced Tgf-β

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signaling and development of a myxomatous and prolapsing mitral valve (Ng et al., 2004). Loeys-Dietz syndrome, characterized by cardiac changes including mitral valve prolapse is associated with mutations in Tgf-β receptors 1 and 2 (Loeys et al., 2005). A homozygous mutation was also identified in the *LTBP3* gene, a gene that produces a protein that increases the bioavailability of Tgf-β in a family with mitral valve disease as well as short stature and oligodontia (Dugan et al., 2015). These findings have led to the development of a hypothesis that MMVD may be associated with a dysregulation of Tgf-β signaling (Hulin et al., 2013).

Familial MMVD in human beings is also observed as an isolated disease, associated with variants in the urokinase-plasminogen activator (*PLAU*) gene, Dachsous Cadherin-Related 1 (*DCHS1*) gene and several of the collagen genes (Chou et al., 2004a,b; Lardeux et al., 2011; Padang et al., 2012). *PLAU* encodes for a protein that has been suggested to be a trigger to activate the matrix metalloproteinase pathway (Chou et al., 2004a,b). *DCHS1* encodes for a protein thought to be associated with atrioventricular canal development and cellular stability of the mitral valve (Durst et al., 2015). Finally, abnormalities of collagen have been identified in other syndromic forms of MMVD including Ehler-Danos, Stickler and Osteogenesis imperfecta (Padang et al., 2012).

Similarities in the disease have been noted between human and canine MMVD (Pedersen and Haggstrom, 2000; Aupperle and Disatian, 2012). In both species, familial MMVD starts in adulthood and increases in prevalence with increasing age. The clinical course is generally one of fairly slow progression and mitral valve prolapse (MVP) is a common finding such that the disease is commonly referred to as MVP in human beings. Pathologic findings include an accumulation of glycosaminoglycans, and fragmentation of collagen bundles and elastic fibers within the valve leaflets (Pedersen and Haggstrom, 2000; Aupperle and Disatian, 2012).

Given the similarities between the human and canine forms of the disease, we hypothesized that a genetic mutation associated with the development of the human form of MMVD might be associated with the development of the canine form of the disease. The objective of this study was to evaluate genes known to be associated with human form of MMVD in two dog breeds in which the disease is known to be heritable, the CKCS and the Dachshund.

Materials and methods

This study was conducted in accordance with the guidelines of the North Carolina State University Institutional Animal Care and Use Committee (Approval No. IACUC 13-103-0; Approval date 4 November 2012).

DNA sequencing

DNA was isolated from blood samples from 10 Cavalier King Charles spaniels and 10 Dachshunds diagnosed with MMVD by the presence of a systolic left apical heart murmur and echocardiographic findings consistent with mitral valve degeneration (valve thickening, prolapse, mitral regurgitation), as identified by board-certified veterinary cardiologists (Borgarelli and Haggstrom, 2010). Approximately 3 μg of DNA from each animal was submitted for library preparation and whole genome sequencing.[1] All sequencing experiments were designed as 150 bp paired-end reads and samples were run in one lane of an Illumina HiSeq 4000 high-throughput sequencing system.

Variant calling and filtering

Variant calling from whole genome sequencing data was performed using a standardized bioinformatics pipeline for all samples as described previously (Friedenberg and Meurs, 2016). Briefly, sequence reads were trimmed using Trimmomatic 0.32 to a minimum phred-scaled base quality score of 30 at the start and end of each read with a minimum read length of 70 bp (Bolger et al., 2014). Sequences were then aligned to the canFam3 reference sequence using BWA 0.7.13 (Li and Durbin, 2009; Lindblad-Toh et al., 2005). Aligned reads were prepared for analysis using Picard Tools 2.5[2] and GATK 3.7 following best practices for base quality score recalibration and indel realignment as specified by the Broad Institute, Cambridge, MA (McKenna et al., 2010; DePristo et al., 2011; Van Der Auwera et al., 2014). Variant calls were made using GATK's HaplotyeCaller walker, and variant quality score recalibration (VQSR) was performed using sites from dbSNP 146 and the Illumina 174K CanineHD BeadChip as training resources. A VQSR tranche sensitivity cutoff was applied to SNPs at 99.9% and 99% to indels for use in downstream analyses; genotype calls with a phred-scaled quality score < 20 were flagged but not removed from the variant callset.

Table 1
Gene names and chromosomal coordinates.

Gene name	Chromosome	Start	End
FBN1	30	14,638,844	14,864,146
LTBP3	18	51,653,800	51,668,012
PLAU	4	24,327,934	24,334,907
DCHS1	21	29,932,714	29,949,159
FLNA	X	122,061,570	122,0883,141
LTBP2	8	47,659,413	47,758,444
TGFBR1	11	56,192,030	56,223,557
TGFBR2	23	13,886,405	13,945,343
COL1A1	9	26,183,852	26,199,927
COL1A2	14	19,883,733	19,920,718
COL2A1	27	6,756,994	6,787,846
COL3A1	36	30,487,822	30,526,563
COL5A1	9	50,741,552	50,856,744
COL5A2	36	30,537,639	30,677,658
COL5A3	20	51,037,378	51,074,394
COL11A1	6	47,425,307	47,622,225
COL11A2	12	2,626,822	2,655,698

Variants present in affected CKCS and Dachshunds were selected and filtered against a database of variants derived from whole genome sequencing of 98 medium to large breed dogs. Selected variants had to be present in at least eight of 10 of the affected dogs from each breed to allow for possible areas of poor coverage. The whole genome sequencing database included 13 different dog breeds not known to have an increased prevalence of MMVD including: American staffordshire terrier, Boxer, Doberman pinscher, German shepherd, Goldendoodle, Golden retriever, Great dane, Labradoodle, Portuguese water dog, Rhodesian ridgeback, Rottweiler, Scottish deerhound and Standard poodle. Variants were then annotated using Variant Effect Predictor 89 (Mclaren et al. 2016). Two files of variants were developed. The first file (no pipeline) contained variants found in at least eight of 10 dogs of each breed and not in any of the medium- and large-breed dogs. The second file (rare pipeline) contained variants found in at least eight of 10 dogs of each breed, but allowed variants present at an allele frequency of up to 5% within the population of medium- and large-breed dogs. After filtering, the orthologous canine genes to the human genes known to be associated with mitral valve degeneration (either as a syndromic or single trait) were identified. The following genes were evaluated: Collagen Type I Alpha 1 (COL1A1), Collagen Type 1 Alpha 2 (COL1A2), Collagen Type II Alpha 1 (COL2A1), Collagen Type III Alpha 1 (COL3A1), Collagen Type V Alpha 1 (COL5A1), Collagen Type 5 Alpha 2 (COL5A2), Collagen Type 5 Alpha 3 (Col5A3), Collagen Type XI Alpha 1 (COL11A1), Collagen Type 11 Alpha (COL11A2), Dachsous Cadherin-Related 1 (*DCHS1*), Fibrillin 1 (*FBN1*), Filamin A (*FLNA*), Latent Transforming Growth Factor Beta Binding Protein 2 and 3 (*LTBP2*, *LTBP3*), Plasminogen Activator Urokinase (*PLAU*), Transforming Growth Factor Beta Receptor 1 and 2 (*TGFBR1*, *TGFBR2*) (Chou et al., 2004a,b; Padang et al., 2012; LaHaye et al., 2014; Dugan et al., 2015; Durst et al., 2015) (Table 1).

Variant evaluation

Variants were then evaluated for likely pathogenic implications using the Standards and Guidelines for the Interpretation of Sequence Variants (Richards et al., 2015). First, identified variants were evaluated to determine any that encoded for a premature stop codon, frameshift, altered splice site, altered start codon, and/or a single or multi-exon deletion. Next, any missense variants were evaluated using the in-silico programs Polyphen,[3] Sift,[4] and Provean[5] to assess the predicted impact of any amino acid change. Variants that were predicted to be deleterious in at least two of the three programs were selected for further evaluation. Variants identified within noncoding regions (upstream, downstream, intronic) were evaluated for evidence of conservation with additional in-silico programs (GERP, PhyloP and Phastcons) as described (Nalpathamkalam et al., 2014; Pollard et al., 2010). Intronic variants were also evaluated with three prediction programs to determine if the

[1] See: https://www.genewiz.com/en (Accessed 2 December 2017).

[2] See: http://broadinstitute.github.io/picard (Accessed 2 December 2017).
[3] See: http://genetics.bwh.harvard.edu/pph2/ (Accessed 2 December 2017).
[4] See: http://sift.jcvi.org/ (Accessed 2 December 2017).
[5] See: http://provean.jcvi.org/index.php (Accessed 2 December 2017).