IOSR Journal of Agriculture and Veterinary Science (IOSR-JAVS)
e-ISSN: 2319-2380, p-ISSN: 2319-2372. Volume 9, Issue 12 Ver. II (December. 2016), PP 113-118
www.iosrjournals.org

Role of COL1A2 Gene Polymorphisms in Myxomatous Mitral Valve Disease in Poodle Dogs Genetic Study of Mitral Valve Disease

Torres-García, O.[1@], Rey-Buitrago, M.[1,2], Acosta-Virgüez, E.[2], Bernal-Rosas, Y.[1], Infante-González, J.[3], Gómez-Duarte, L.[4]

[1]*Faculty of Veterinary Medicine, Antonio Nariño University, Carrera 3 Este # 47 A - 15 Bogotá, Colombia.*
[2]*Faculty of Biology, National University of Colombia, Carrera 45 # 26-85 Bogotá D.C., Colombia.* [3]*Faculty of Veterinary Medicine, UNIAGRARIA Fundation of Colombia, Calle 170 No 54A -10 Bogotá, Colombia.*
[4]*Deparment of Cordiology, Veterinarian Medical Center, Carrera 18B # 145-62 Bogotá D.C., Colombia.*

Abstract: *Introduction - Myxomatous mitral valve disease is a common heart disease in dogs, characterized by chronic progressive, degenerative lesions of the mitral valve. The disease has many similarities with the human condition mitral valve prolapse; this is a polygenic disease in which more than one genetic locus is likely to contribute to disease susceptibility and clinical expression. Objective - Assess the implication of intronic variants rs9006567 and rs22372411 of COL1A2 gene in canine susceptibility to myxomatous mitral valve disease. Materials and methods - Case-control association study. Fifty canine patients with MMVD and 80 matched canine controls were evaluated. DNA from patients and controls was obtained from peripheral blood. Samples were genotyped for two intronic variants COL1A2 gene polymorphisms (rs9006567 A/G and rs22372411 C/T) using an allelic discrimination assay. Results - No significant differences were observed in genotype distribution among patients with MMVD and controls for the rs9006567 COL1A2 gene variant. However, the allele T of the rs22372411 variant was over-represented in MMVD patients compared with healthy controls (P = 0.009; OR = 1.98; 95% CI = 1.18 - 3.34). Conclusions and Clinical Relevance - Our results show for the first time an association of the rs22372411 COL1A2 gene variant with susceptibility to canine myxomatous mitral valve disease.*

Keywords: *COL1A2, Disease susceptibility, Dogs, Gene polymorphism, Genetic studies, Myxomatous mitral valve disease.*

I. Introduction

Myxomatous mitral valve disease (MMVD) is the most common cardiac disease in small breed dogs, such as Poodles, Cavalier King Charles Spaniels, Dachshunds, and Chihuahuas. It is characterized by progressive lesions affecting the mitral valve primarily [1]. They may be observed in approximately 30% of the small breed dogs over ten years old[2]. A similar disease has also seen in human beings [3] that is known as MVP. Additionally, the mitral valve changes that are seen in humans are alike to those mentioned at the MMVD in dogs. In the human population afflicted with MVP, it has been found that the mean patient age is 60 years old [4]. The physiopathology of the disease is similar in both species [5].

Previous studies have identified MMVD and MVP as a genetic disease with an autosomal dominant mode of inheritance [6, 7]. However, there seems to be a high degree of genetic heterogeneity of the disease in the human population, and the inheritance of MVP is now believed to be polygenic [8-10]. Similarly the predisposition of some breeds of dogs to an early onset of MMVD, it has been suggested that the disease has a strong genetic background [11, 12]. Some gene polymorphisms have been associated with either disease susceptibility [13, 14] or disease progression [14, 15], both in human patients as in animals, with this valvular disease. Many of these genes are also susceptibility factors for heritable connective tissue diseases, suggesting that they are shared connective tissue disease susceptibility genes. This fact may support the paradigm of common dysregulated pathways across multiple heritable diseases, such as Marfan and Ehlers–Danlos syndromes [7]. These illnesses with its abnormalities of the aorta and mitral valve that are associated with abnormalities of skin, joints and osteogenesis imperfecta have associated skeletal and vascular abnormalities that are due to mutations in collagen genes [16]. However, there are some studies reporting negative associations between collagen polymorphisms and outcomes of MMVD [17, 18].

Physiopathological changes associated with MMVD include excessive deposition of proteoglycan and glycosaminoglycan, fragmentation of elastin, overexpression of proteolytic enzymes such as MMP-1, MMP-2, and MMP-13; and disruption of collagen [19-21]. It has been reported that MMVD is characterized by the accumulation of collagen in the valve [22] as a result of an imbalance between production-deposition of collagen and collagenolytic enzymes [23-25]. In this sense, some studies have shown a decrease in collagen

while others argue that the collagen does not decrease. However, in each case the collagen fibrils have an altered pattern of organization [26].

Type I collagen is the major component of extracellular matrix, composed of two α1 (I) and one α2 (I) chains, which are the products of COL1A1 and COL1A2 genes [27]. In the mitral valve, the type I and III collagens are predominant, however, it also is found type V collagen in smaller amounts [28]. MMVD is characterized by alterations involving the central and marginal region of the valve that comprising coincidentally to the collagen type I in both cases [24].

In human, genetic segregation analysis of familial MVP, performed to evaluate the relationship between COL1A defects and MVP have excluded the involvement of COL1A gene polymorphisms in MVP [17]. Nevertheless, evidence shows that the disease being the result of mutations in the genes encoding the major fibrillar collagens [18]. Further, in a rare autosomal recessive cardiac valvular form of Ehlers-Danlos Syndrome, mutations in the COL1A2 gene has been published [29].

In dogs, it has not been established whether there is a relationship between the different polymorphisms previously reported in various types of collagen with the presentation of MMVD. However, recently it was conducted a genome-wide association study that identified two loci associated with the development of MMVD in Cavalier King Charles Spaniels Dogs [13]. The region on CFA14 contains the COL1A2 gene that is a good candidate gene for the development of MMVD because they play a role in the composition of connective tissue as mentioned above. In this way, the hypothesized for this work, is that mutations in COL1A2 (located on CFA 14 at 22.8 Mb) [13] might cause in dogs the MMVD.

Because there are no data available in the literature to Poodle dogs regarding the role of COL1A2 gene polymorphisms in the pathogenesis of MMVD, the current study was designed to determine whether two SNPs of COL1A2 reported in the dbSNP of GenBank are associated with MMVD. For this, were selected the intronic variants of COL1A2 gene: rs9006567 and rs22372411, which may affect the splicing efficiency of the full-length of COL1A2, as it has been observed in other studies [30] and specifically with COL1A2 [29, 31]; or affect regulatory elements since it has been reported that there may be regulatory elements in intronic regions of genes [32, 33].

II. Materials And Methods

Patients

Fifty Poodle breed canine patients diagnosed with MMVD between 2013 and 2015 were included in our study. A canine control population (n = 80), matched by age, sex, and breed with the canine patients with MMVD was also investigated. Patients and controls were included in this study after its owners gave their written informed consent. Ethical committee approval was obtained for this study. According to clinical status all patients were classified between stage B and D [34]. The diagnosis of MMVD was made at the Veterinary Cardiology Unit of the Veterinary Medical Center by means of cardiac auscultation and echocardiography, the presence of MMVD was identified, and its severity was assessed by quantifying the MMVD jet as a percentage of the left atrial area, as described by Pedersen et al. [35]. Control dogs were defined as dogs older than 8 years with no or mild MMVD. All dogs were unrelated at the parental level.

The studied canine population is a homogeneous mixture and there is not the concentration of breed groups (F_{sT} 0.009) [36].

SNP genotyping

Genomic DNA was extracted from peripheral blood mononuclear cells, using standard methods. The genotyping of the COL1A2 (rs9006567 and rs22372411) polymorphisms were performed using a TaqMan SNP genotyping assay (Applied Biosystems, Foster City, CA, USA). Allele-specific probes were labelled with the fluorescent dyes VIC and FAM. PCR, was carried out in a total reaction volume of 5 µl, containing 50 ng genomic DNA as template, 2.5 µl of TaqMan genotyping master mix, 0.25 µl of 20× assay mix, and ddH2O up to 5 µl of the final volume. The amplification protocol used, was the following: initial denaturation at 203°F for 10 min followed by 40 cycles of denaturation at 198°F for 15 s, and annealing/extension at 140°F for 1 min. Post-PCR, the genotype of each sample was attributed automatically by measuring the allelic-specific fluorescence on a Eco™ Real-Time PCR system (Illumina, San Diego, CA, USA) and Illumina Eco study software package (Illumina, San Diego, CA, USA).

Statistical analysis

We used the chi-squared test for assessment of Hardy-Weinberg equilibrium. Odds ratios and 95% CI were calculated according to Woolf's method using PLINK v1.9 (http://pngu.mgh.harvard.edu/~purcell/plink/index.shtml) a public software [37]. Haploview software (Broad Institute, Cambridge, MA, USA), was used to obtain linkage disequilibrium pairwise values. In addition, we

performed a haplotype analysis of 2 SNPs according to the method of Gabriel et al. [38]. P values < 0.05 were considered statistically significant.

III. Results

Patients

Fifty canine patients of the poodle breed with MMVD were enrolled. Most were male (64%) (n = 50; median age at disease diagnosis 12.34 ± 2.34 yr; range 8-17 yr). According to clinical status all patients were classified between stage B and D of illness.

SNP genotyping

A genotyping success rate 100 % was achieved in canine patients with MMVD and controls. No evidence of departure from Hardy-Weinberg equilibrium was observed in controls. The case: control ratio obtained was 1:1.6. The estimated power of this study for an estimated OR between 1.5 and 2.0 was 72%–95%, for a type I error rate of 0.05. Genotype distributions in canine MMVD patients and controls are shown in Table 1. No significant association between rs9006567 variant of the COL1A2 gene and MMVD was observed. However, when the rs22372411 COL1A2 polymorphism was assessed, we found that the frequency of allele T was significantly increased in MMVD patients compared to controls (P = 0.009; OR: 1.98; 95% CI 1.18 - 3.34). It was due to an increased frequency of heterozygous (C/T) for the rs22372411 variant genotype in the group of patients with MMVD (52.0%) compared to controls (35.0%) (P = 0.057; OR: 2.00; 95% CI 0.97 - 4.16). In addition to the analysis based on a single variant, we performed haplotypes estimations. Nevertheless, these COL1A2 polymorphisms did not form haplotype blocks according to the method of Gabriel et al. [38].

IV. Discussion

Evidence indicates that susceptibility to myxomatous mitral valve disease or other heritable disorders of connective tissue may be related to genetic variability at collagen loci[14, 39-42]. In the same way, mouse models of valve disease with a spontaneous mutation in the Collagen COL1A2 gene, exhibit distal leaflet thickening and increased proteoglycan composition characteristic of myxomatous valve disease[43]. The above shows the role of collagen type I in the pathogenesis of MMVD.

Type I collagen, encoded by the COL1A1 and COL1A2 genes, exists as a heterotrimeric triple-helical protein, that is synthesized as a soluble, procollagen form, composed of globular C - and N-propeptides domains at both ends [44]. Procollagen is composed of two related, yet genetically distinct, procollagen chains, each of about 1000 amino acids. The heterotrimer is formed by the incorporation of two proα1(I) collagen chains and one proα2(I) collagen chain [α1(I)2α2(I)] [45, 46]. After the procollagen molecule has entered the extracellular space is cleaved by C- and N-proteases, and the collagen monomer is required for the initiation of fibril formation, which represents the aggregate form of the protein found in tissues [47].

Biochemical studies demonstrated that normal human heart valves contained 74% type I, 24% type III and 2% type V collagen [28]. However, in human MVP, the amount of collagen I is decreased and collagen III increased [28], similar findings were observed in dogs with MMVD [24]. In keeping with this study, Chou et al. demonstrate association between COL3A1 collagen gene exon 31 polymorphism and risk of mitral valve prolapse[14].

In the present study, we examined for the first time the contribution of rs9006567 (c.595-295A>G) and rs22372411 (c.1351-46C>T) intronic polymorphisms of the COL1A2 gene in the susceptibility to canine MMVD. In this study, the frequency of the T allele, was found to be higher in patients with myxomatous mitral valve disease than in healthy individuals, indicating that this allele may be a risk factor for genetic susceptibility to canine MMVD. Our results support a potential role of the COL1A2 c.1351-46C>T (rs22372411) gene polymorphism in the predisposition to canine myxomatous mitral valve disease. Previous studies, have shown that mutations in the COL1A2 gene that change the primary structure of the proα1 (I) chain so as to make the N-propeptide resistant to cleavage by procollagen N-proteinase [41, 48, 49]. The intronic location of the rs22372411 (c.1351-46C>T) polymorphism might have functional consequences on the transcription of the COL1A2 gene, this finding coincides with others reports where many collagen-related diseases are caused by splicing variants [39, 40, 50].

Advances in computational analyzes have found flanking intronic regions important in regulating both constitutive as alternative splicing. In addition to sequences adjacent to the boundaries between exons and introns, other sequences located in introns and exons can modulate the recognition of splice sites, either by facilitating or preventing the binding of factors in trans. Changes in these splicing regulatory sequences known as "enhancers" or "silencers" pathologies can lead to considerably increasing the weight of these alterations in splicing process as causes of genetic diseases [51, 52].

In addition, specific changes in the DNA adjacent to or during transcription, errors may activate a cryptic splice site of the hnRNA that usually does not overlap. This results in a mature messenger RNA with a

section missing an exon. Thus, a point mutation, which usually affects only a single amino acid, may manifest as a deletion in the final protein. Furthermore activation of cryptic sites results in altered mRNAs with the reading frame that lead to non-functional truncated protein [53, 54]. In Fig. 1 shows as rs22372411 polymorphism (C/T) is located in a region of cryptic splicing in intron 23 of the gene for collagen (COL1A2) and reported as regulatory region in alternative splicing donor. This change could explain possible changes in alternative splicing who is involved in the susceptibility to canine MMVD [53, 54].

Recently, a genome-wide association study performed in Cavalier King Charles Spaniels dogs, identified a 1.58 Mb region on CFA13 and a 1.68 Mb region on CFA14 associated with the development of MMVD [13]. Interestingly, the COL1A2 gene is located in the region on CFA14. All these data are in accordance with our results and support a potential role of these region in the susceptibility to MMVD.

V. Conclusions

In conclusion, our study constitutes the first attempt to establish the potential influence of intronic polymorphism of the COL1A2 gene in the susceptibility to myxomatous mitral valve disease. The pathogenesis of type I collagen abnormality in MMVD might be partially explained by this finding. However, the association between polymorphisms and disease progression is still unclear. Given the crucial role of collagen in mitral valve leaflets, further studies on other functional polymorphisms of COL1A2 gene are required to clarify the role of COL1A2 locus in the pathogenesis of MMVD. Besides, we demonstrate that a dog breed is sufficiently homogeneous with regard to the genetic background for a polygenic disease to provide evidence for loci inherent MMVD in a somewhat modest number of cases and controls.

Acknowledgements

Supported by a grant from Antonio Nariño University. The authors thank the contribution of veterinarians of the Clinical Center of Faculty of Veterinary Medicine - UAN. The authors declare no conflict of interest.

References

[1]. Oyama MA, Levy RJ. Insights into serotonin signaling mechanisms associated with canine degenerative mitral valve disease. *J Vet Intern Med. 2010;24(1):27-36.*
[2]. Borgarelli M, Haggstrom J. Canine degenerative myxomatous mitral valve disease: natural history, clinical presentation and therapy. *Vet Clin North Am Small Anim Pract. 2010;40(4):651-663.*
[3]. Boudoulas H, Sparks EE, Wooley CF. Mitral valvular regurgitation : etiology, pathophysiologic mechanisms, clinical manifestations. *Herz. 2006;31(1):6-13.*
[4]. Stewart BF, Siscovick D, Lind BK, Gardin JM, Gottdiener JS, Smith VE, et al. Clinical factors associated with calcific aortic valve disease. Cardiovascular Health Study. *J Am Coll Cardiol. 1997;29(3):630-634.*
[5]. Pedersen HD, Haggstrom J. Mitral valve prolapse in the dog: a model of mitral valve prolapse in man. *Cardiovasc Res. 2000;47(2):234-243.*
[6]. Hayek E, Gring CN, Griffin BP. Mitral valve prolapse. *Lancet. 2005;365(9458):507-518.*
[7]. Grau JB, Pirelli L, Yu PJ, Galloway AC, Ostrer H. The genetics of mitral valve prolapse. *Clin Genet. 2007;72(4):288-295.*
[8]. Yosefy C, Ben Barak A. Floppy mitral valve/mitral valve prolapse and genetics. *J Heart Valve Dis. 2007;16(6):590-595.*
[9]. Orton EC, Lacerda CM, MacLea HB. Signaling pathways in mitral valve degeneration. *J Vet Cardiol. 2012;14(1):7-17.*
[10]. Lincoln J, Garg V. Etiology of valvular heart disease-genetic and developmental origins. *Circ J. 2014;78(8):1801-1807.*
[11]. Buchanan JW. Chronic valvular disease (endocardiosis) in dogs. *Adv Vet Sci Comp Med. 1977;21:75-106.*
[12]. Lewis T, Swift S, Woolliams JA, Blott S. Heritability of premature mitral valve disease in Cavalier King Charles spaniels. *Vet J. 2011;188(1):73-76.*
[13]. Madsen MB, Olsen LH, Haggstrom J, Hoglund K, Ljungvall I, Falk T, et al. Identification of 2 loci associated with development of myxomatous mitral valve disease in Cavalier King Charles Spaniels. *J Hered. 2011;102 Suppl 1:S62-67.*
[14]. Chou HT, Hung JS, Chen YT, Wu JY, Tsai FJ. Association between COL3A1 collagen gene exon 31 polymorphism and risk of floppy mitral valve/mitral valve prolapse. *Int J Cardiol. 2004;95(2-3):299-305.*
[15]. Nesta F, Leyne M, Yosefy C, Simpson C, Dai D, Marshall JE, et al. New locus for autosomal dominant mitral valve prolapse on chromosome 13: clinical insights from genetic studies. *Circulation. 2005;112(13):2022-2030.*
[16]. Milewicz DM. Molecular genetics of Marfan syndrome and Ehlers-Danlos type IV. *Curr Opin Cardiol. 1998;13(3):198-204.*
[17]. Henney AM, Tsipouras P, Schwartz RC, Child AH, Devereux RB, Leech GJ. Genetic evidence that mutations in the COL1A1, COL1A2, COL3A1, or COL5A2 collagen genes are not responsible for mitral valve prolapse. *Br Heart J. 1989;61(3):292-299.*
[18]. Wordsworth P, Ogilvie D, Akhras F, Jackson G, Sykes B. Genetic segregation analysis of familial mitral valve prolapse shows no linkage to fibrillar collagen genes. *Br Heart J. 1989;61(3):300-306.*
[19]. Disatian S, Ehrhart EJ, 3rd, Zimmerman S, Orton EC. Interstitial cells from dogs with naturally occurring myxomatous mitral valve disease undergo phenotype transformation. *J Heart Valve Dis. 2008;17(4):402-411.*
[20]. Barnette DN, Hulin A, Ahmed AS, Colige AC, Azhar M, Lincoln J. Tgfbeta-Smad and MAPK signaling mediate scleraxis and proteoglycan expression in heart valves. *J Mol Cell Cardiol. 2013;65:137-146.*
[21]. Gupta V, Barzilla JE, Mendez JS, Stephens EH, Lee EL, Collard CD, et al. Abundance and location of proteoglycans and hyaluronan within normal and myxomatous mitral valves. *Cardiovasc Pathol. 2009;18(4):191-197.*
[22]. Grande-Allen KJ, Borowski AG, Troughton RW, Houghtaling PL, Dipaola NR, Moravec CS, et al. Apparently normal mitral valves in patients with heart failure demonstrate biochemical and structural derangements: an extracellular matrix and echocardiographic study. *J Am Coll Cardiol. 2005;45(1):54-61.*
[23]. Ljungvall I, Rajamaki MM, Crosara S, Olsen LH, Kvart C, Borgarelli M, et al. Evaluation of plasma activity of matrix metalloproteinase-2 and -9 in dogs with myxomatous mitral valve disease. *Am J Vet Res. 2011;72(8):1022-1028.*
[24]. Aupperle H, Marz I, Thielebein J, Kiefer B, Kappe A, Schoon HA. Immunohistochemical characterization of the extracellular matrix in normal mitral valves and in chronic valve disease (endocardiosis) in dogs. *Res Vet Sci. 2009;87(2):277-283.*

[25]. Aupperle H, Thielebein J, Kiefer B, Marz I, Dinges G, Schoon HA. An immunohistochemical study of the role of matrix metalloproteinases and their tissue inhibitors in chronic mitral valvular disease (valvular endocardiosis) in dogs. *Vet J. 2009;180(1):88-94.*
[26]. Richards JM, Farrar EJ, Kornreich BG, Mosmall yi UNS, Butcher JT. The mechanobiology of mitral valve function, degeneration, and repair. *J Vet Cardiol. 2012;14(1):47-58.*
[27]. Retief E, Parker MI, Retief AE. Regional chromosome mapping of human collagen genes alpha 2(I) and alpha 1(I) (COLIA2 and COLIA1). *Hum Genet. 1985;69(4):304-308.*
[28]. Cole WG, Chan D, Hickey AJ, Wilcken DE. Collagen composition of normal and myxomatous human mitral heart valves. *Biochem J. 1984;219(2):451-460.*
[29]. Schwarze U, Hata R, McKusick VA, Shinkai H, Hoyme HE, Pyeritz RE, et al. Rare autosomal recessive cardiac valvular form of Ehlers-Danlos syndrome results from mutations in the COL1A2 gene that activate the nonsense-mediated RNA decay pathway. *Am J Hum Genet. 2004;74(5):917-930.*
[30]. Greenwood TA, Kelsoe JR. Promoter and intronic variants affect the transcriptional regulation of the human dopamine transporter gene. *Genomics. 2003;82(5):511-520.*
[31]. Melis D, Cappuccio G, Ginocchio VM, Minopoli G, Valli M, Corradi M, et al. Cardiac valve disease: an unreported feature in Ehlers Danlos syndrome arthrocalasia type? *Ital J Pediatr. 2012;38:65.*
[32]. Voigtlander T, Ripperger A, Ganten D, Bader M. Transcriptional silencer in intron I of the rat renin gene. *Adv Exp Med Biol. 1995;377:285-292.*
[33]. Galvagni F, Oliviero S. Utrophin transcription is activated by an intronic enhancer. *J Biol Chem. 2000;275(5):3168-3172.*
[34]. Atkins C, Bonagura J, Ettinger S, Fox P, Gordon S, Haggstrom J, et al. Guidelines for the diagnosis and treatment of canine chronic valvular heart disease. *J Vet Intern Med. 2009;23(6):1142-1150.*
[35]. Pedersen HD, Haggstrom J, Falk T, Mow T, Olsen LH, Iversen L, et al. Auscultation in mild mitral regurgitation in dogs: observer variation, effects of physical maneuvers, and agreement with color Doppler echocardiography and phonocardiography. *J Vet Intern Med. 1999;13(1):56-64.*
[36]. Infante J, Bernal Y, Acosta E, Gómez L, Torres O. Estudio Preliminar de la Diversidad Genética de Perros con Fenotipo Poodle en Colombia Usando Microsatélites. *Arch Zootec. 2015;64(247):303 - 306.*
[37]. Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MA, Bender D, et al. PLINK: a tool set for whole-genome association and population-based linkage analyses. *Am J Hum Genet. 2007;81(3):559-575.*
[38]. Gabriel SB, Schaffner SF, Nguyen H, Moore JM, Roy J, Blumenstiel B, et al. The structure of haplotype blocks in the human genome. *Science. 2002;296(5576):2225-2229.*
[39]. Vasan NS, Kuivaniemi H, Vogel BE, Minor RR, Wootton JA, Tromp G, et al. A mutation in the pro alpha 2(I) gene (COL1A2) for type I procollagen in Ehlers-Danlos syndrome type VII: evidence suggesting that skipping of exon 6 in RNA splicing may be a common cause of the phenotype. *Am J Hum Genet. 1991;48(2):305-317.*
[40]. Yoneyama T, Kasuya H, Onda H, Akagawa H, Hashiguchi K, Nakajima T, et al. Collagen type I alpha2 (COL1A2) is the susceptible gene for intracranial aneurysms. *Stroke. 2004;35(2):443-448.*
[41]. Campbell BG, Wootton JA, Macleod JN, Minor RR. Canine COL1A2 mutation resulting in C-terminal truncation of pro-alpha2(I) and severe osteogenesis imperfecta. *J Bone Miner Res. 2001;16(6):1147-1153.*
[42]. Ahram DF, Cook AC, Kecova H, Grozdanic SD, Kuehn MH. Identification of genetic loci associated with primary angle-closure glaucoma in the basset hound. *Mol Vis. 2014;20:497-510.*
[43]. Cheek JD, Wirrig EE, Alfieri CM, James JF, Yutzey KE. Differential activation of valvulogenic, chondrogenic, and osteogenic pathways in mouse models of myxomatous and calcific aortic valve disease. *J Mol Cell Cardiol. 2012;52(3):689-700.*
[44]. Prockop DJ, Kivirikko KI. Collagens: molecular biology, diseases, and potentials for therapy. *Annu Rev Biochem. 1995;64:403-434.*
[45]. Phillips CL, Morgan AL, Lever LW, Wenstrup RJ. Sequence analysis of a full-length cDNA for the murine pro alpha 2(I) collagen chain: comparison of the derived primary structure with human pro alpha 2(I) collagen. *Genomics. 1992;13(4):1345-1346.*
[46]. Byers PH. Folding defects in fibrillar collagens. *Philos Trans R Soc Lond B Biol Sci. 2001;356(1406):151-157.*
[47]. Kauppila S, Stenback F, Kacinski BM, Carcangiu ML, Risteli J, Risteli L. Characterization of type I collagen synthesis and maturation in uterine carcinosarcomas. *Cancer. 1999;86(7):1299-1306.*
[48]. Wirtz MK, Glanville RW, Steinmann B, Rao VH, Hollister DW. Ehlers-Danlos syndrome type VIIB. Deletion of 18 amino acids comprising the N-telopeptide region of a pro-alpha 2(I) chain. *J Biol Chem. 1987;262(34):16376-16385.*
[49]. Weil D, Mattei MG, Passage E, N'Guyen VC, Pribula-Conway D, Mann K, et al. Cloning and chromosomal localization of human genes encoding the three chains of type VI collagen. *Am J Hum Genet. 1988;42(3):435-445.*
[50]. Takahara K, Schwarze U, Imamura Y, Hoffman GG, Toriello H, Smith LT, et al. Order of intron removal influences multiple splice outcomes, including a two-exon skip, in a COL5A1 acceptor-site mutation that results in abnormal pro-alpha1(V) N-propeptides and Ehlers-Danlos syndrome type I. *Am J Hum Genet. 2002;71(3):451-465.*
[51]. Faustino NA, Cooper TA. Pre-mRNA splicing and human disease. *Genes Dev. 2003;17(4):419-437.*
[52]. Cooper TA, Wan L, Dreyfuss G. RNA and disease. *Cell. 2009;136(4):777-793.*
[53]. Yeo GW, Van Nostrand EL, Liang TY. Discovery and analysis of evolutionarily conserved intronic splicing regulatory elements. *PLoS Genet. 2007;3(5):e85.*
[54]. Voelker RB, Berglund JA. A comprehensive computational characterization of conserved mammalian intronic sequences reveals conserved motifs associated with constitutive and alternative splicing. *Genome Res. 2007;17(7):1023-1033.*

Table 1. COL1A2 gene polymorphisms in canine patients with myxomatous mitral valve disease and healthy controls.

COL1A2 rs9006567	MMVD n = 50 (%)	CONTROLS n = 80 (%)	P	OR (CI 95%)
AA	11 (22.0)	16 (20.0)	0.79	1.13 (0.46 – 2.69)
AG	19 (38.0)	40 (50.0)	0.18	0.62 (0.30-1.26)
GG	20 (40.0)	24 (30.0)	0.24	1.55 (0.73-3.28)
A	41 (41.0)	72 (45.0)	0.53	0.85 (0.51 – 1.41)
G	59 (59.0)	88 (55.0)	0.53	1.18 (0.71-1.96)
COL1A2 rs22372411	MMVD n = 50 (%)	CONTROLS n = 80 (%)	P	OR (CI 95%)

CC	14 (28.0)	42 (52.5)	0.006	0.35 (0.16 – 0.75)
CT	26 (52.0)	28 (35.0)	0.057	2.00 (0.97-4.16)
TT	10 (20.0)	10 (12.5)	0.251	1.74 (0.65-4.66)
C	54 (54.0)	112 (70.0)	0.009	0.50 (0.30 – 0.85)
T	46 (46.0)	48 (30.0)	0.009	1.98 (1.18-3.34)

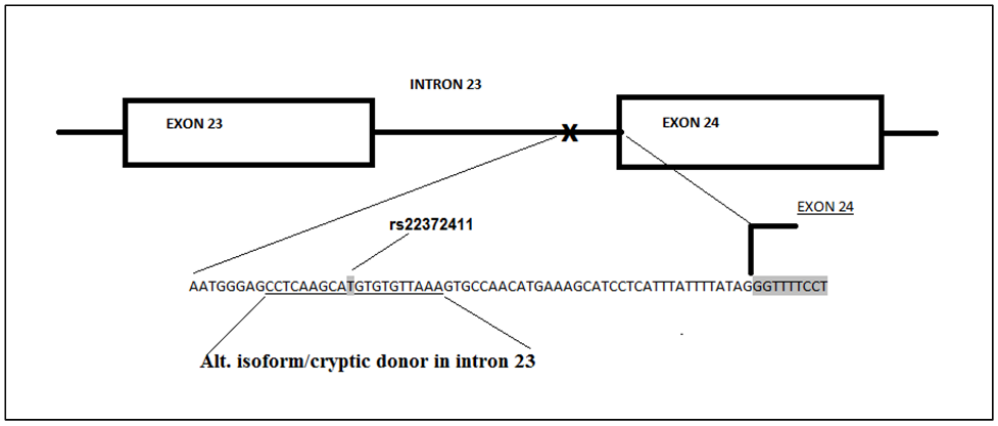

Figure 1. Location polymorphism rs22372411 (C/T) in the collagen gene COL1A2