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

1Faculty of Veterinary Medicine, Antonio Nariño University, Carrera 3 Este # 47 A - 15 Bogotá, Colombia.
2Faculty of Biology, National University of Colombia, Carrera 45 # 26-85 Bogotá D.C., Colombia.
3Faculty of Veterinary Medicine, UNIAGRARIA Foundation of Colombia, Calle 170 No 54A -10 Bogotá, Colombia.
4Department of Cardiology, 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 been 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 in 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 (Fst 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 allele-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]. Haplovie 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 proc(1) collagen chains and one proc(2) collagen chain [u1(1)2a2(1)] [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 pro(1) 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 which 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 regions 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

Role of COL1A2 gene polymorphisms in myxomatous mitral valve disease in Poodle Dogs..

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

<table>
<thead>
<tr>
<th>rsno</th>
<th>MMVD</th>
<th>CONTROLS</th>
</tr>
</thead>
<tbody>
<tr>
<td>9006567</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AA</td>
<td>11 (22.0)</td>
<td>16 (20.0)</td>
</tr>
<tr>
<td>AG</td>
<td>19 (38.0)</td>
<td>40 (50.0)</td>
</tr>
<tr>
<td>GG</td>
<td>20 (40.0)</td>
<td>24 (30.0)</td>
</tr>
<tr>
<td>AA</td>
<td>41 (81.0)</td>
<td>72 (45.0)</td>
</tr>
<tr>
<td>G</td>
<td>59 (59.0)</td>
<td>88 (55.0)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>rsno</th>
<th>MMVD</th>
<th>CONTROLS</th>
</tr>
</thead>
<tbody>
<tr>
<td>2237241</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>41 (40.0)</td>
<td>72 (45.0)</td>
</tr>
<tr>
<td>G</td>
<td>59 (59.0)</td>
<td>88 (55.0)</td>
</tr>
</tbody>
</table>

DOI: 10.9790/2380-091202113118 www.iosrjournals.org 117 | Page
Role of COL1A2 gene polymorphisms in myxomatous mitral valve disease in Poodle Dogs.

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

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