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The association of MMP1 1G>2G polymorphism with aortic valve calcification

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Summary

Introduction: The most common cause of aortic stenosis is active calcium accumulation in the valve cusps, which implies serious clinical consequences. Various extracellular matrix metalloproteases (MMPs) have been implicated in the development of this disease. Therefore, the possible association between a functional MMP1 polymorphism and the amount of calcium deposited on the aortic valve is studied.

Patients and methods: 45 patients undergoing valve replacement were included in the study. The calcium content in valve cusps removed during surgery was determined by computed micro-tomography. DNA was extracted from peripheral blood samples for genotyping the -1607 1G>2G polymorphism of MMP1 by PCR and subsequent digestion.

Results: Significant differences were observed in the calcium content in aortic valves in individuals with different -1607 1G>2G genotypes (p=0.042). Thus, 2G allele carriers (homozygous or heterozygous) present higher calcium levels measured as BMD (p=0.004) as well as BV/TV (p=0.002). The association with BV/TV was independent of sex, age, degree of renal function and anatomy of the valve (p=0.02). BMD tendency (p=0.07) was also observed.

Conclusion: The association between 1G>2G MMP1 polymorphism and calcium content of the aortic valve suggests that the 1G allele would have a protective effect against calcium deposits. These results support the importance of further study to confirm whether this polymorphism could be used as a possible predictor of aortic stenosis development.

Key words: *aortic valve disease, matrix metalloproteinase polymorphisms, microCT, calcium content.*

Introduction

Aortic stenosis degeneration is the most common valve disease in the industrialized countries[1]. Initially considered a passive process, it is now described as an active calcium buildup in the valve cusps, accompanied by changes in the morphology and function of valvular cells, characterized by notable osteoblast differentiation that increases valve stiffness. This leads to a reduction in the orifice opening of the valve and an increase in blood pressure gradient, with serious clinical consequences[2].

There is abundant evidence that implicates extracellular matrix metalloproteinases (MMPs) in this process[3]. MMPs are a large family of zinc-dependent enzymes that exert their function in both pathologic and physiological conditions[4]. Traditionally they have been grouped according to their ability to degrade various components of the extracellular matrix, but also exert functions in other locations. In fact, recently researchers have suggested that they also act on non-matrix proteins and highlight their role in inflammatory processes[5,6]. It has also been found that there is increased expression of MMP-1, -2 and -3 in calcified aortic valves than in normal aortic valves, and the exclusive presence of MMP-9 in diseased valves[7].

The association of MMPs with valve disease has also been studied from the genetic point of view. Thus, the MMP1 1G>2G polymorphism gene at -1607 is reportedly associated with the presence of bicuspid aortic valve anamoly[8]. This same polymorphism has also been associated with levels of bone mineral density in postmenopausal women[9]. Based on the data presented and in order to identify a possible early marker of disease calcific aortic valve, the association of 1G>2G polymorphism with parameters indicative of mineralization in aortic valves from valve replacement was studied.

Patients and methods

Population group

Aortic valves from 45 patients diagnosed with aortic valve disease (stenosis 91%, failure 9%) undergoing aortic valve replacement between April 2012 and May 2014 in the Department of Cardiac Surgery of the Central University Hospital of Asturias were studied. Table 1 shows some features of greater clinical interest are shown, including cardiovascular risk factors.

A current smoker was considered active if that person had smoke during the previous year. An ex-smoker was an individual who gave up smoking more than a year prior and non-smoking to a person who has never taken up the habit. Dyslipidemia was defined according to compliance with any of the following criteria: history of diagnosed hyperlipidemia and/or treated with medication, diet and/or exercise, figures of total cholesterol above 200 mg/dl, LDL cholesterol greater than or equal to 130 mg/ dl, HDL cholesterol less than 40 mg/dl or lipid-lowering therapy. Hypertension was defined as meeting one of the following criteria: history of diagnosed or treated with medication, diet and/or exercise hypertension; systolic blood pressure less than 140 mmHg or diastolic less than 90 mmHg, at least two determinations; or antihypertensive treatment not administered as therapy to anything other than hypertension disorder. The existence of diabetes mellitus was based on the presence of any of the following: accredited medical history of diabetes mellitus, blood glucose greater than or equal to 200 mg/dl fasting in any situation and symptoms of diabetes mellitus, the least two determinations of blood glucose greater than or equal to fasting 126 mg/dl (fasted understood as a period without intake for at least 8 hours) or use of oral hypoglycemic current treatments and/or insulin. The estimation of glomerular filtration was carried out using the MDRD-4 variable equation. The classification of the valve anatomy was made based on intraoperative findings, in addition to the ECG description prior to surgery.

Tissues removed during surgery were treated for 24 h with 4% formaldehyde and after several washings with water, preserved in 70% ethanol at 4° C in the Principality of Asturias Biobank. Peripheral blood sample tube with EDTA, which was processed in the biobank for extraction of genomic DNA was stored at -20° C until use. Patients signed an informed consent form for use of their biological samples and the study was approved by the Ethics Committee of the Principality of Asturias for Clinical Research.

Genotyping

The MMP1 1G>2G polymorphism at position -1607 (rs1799750) was genotyped by polymerase chain reaction and subsequent digestion with restriction enzyme (PCR-RFLP), following a previously reported procedure[10].

Quantification of calcium content in the aortic cusp

Valvular tissue samples preserved in ethanol were analyzed by computerized microtomography (microCT) in a SkyScan 1174 (Bruker, Kontich, Belgium) available at the University of Oviedo Vivarium research center. Images were obtained using 50 kV and 800 µA parameters. 1,300 images of each of the samples with a pitch of 0.3° rotation and an average frame 2 for a 180° scan were obtained. Scanning each lasted 10 to 20 minutes (depending on valve size) using an exposure time of 6,200 ms. flat field correction at the beginning of each scan.

The images obtained were reconstructed with the NRecon (Bruker) software (Figure 1). Correction values of attenuation coefficient, light ray sharpness, smoothness and ring artifacts were the same in all samples. 3D morphometric analysis was carried out using CTAn (Bruker) software. The volume of interest was manually delimited in each of the samples. The threshold used for all of 0.74 to 3.39 was g/cm^3 of bone mineral density (BMD).

BMD parameters and bone volume/total volume (BV/TV) were considered as measures of the amount of calcium deposited.

Statistical analysis

All statistical analyzes were carried out using SPSS version 15.0 software. It was first confirmed that genotype and allele frequencies of polymorphism were in Hardy-Weinberg Equilibrium Model by χ^2 test. ANOVA test was used to compare the mean values of the parameters studied in the different genotypes and then Bonferroni test to discriminate which genotype pairs showed statistical significance. Then, based on these results, the genotypes were grouped into two categories whose average values for BMD and BV/TV were compared by T. Finally test, an adjusted linear regression analysis was performed for variables of sex, age, glomerular filtration rate measured using the MDRD-4 and presence of bicuspid aortic valve anamoly. A p-value <0.05 was considered statistically significant.

Table 1. Clinical and anthropometric characteristics of the study population

Characteristics	Values
Age[a] (years)	69±11
Mens	63%
Smoking	17.4%
Dyslipidemia	43.5%
Hypertension	67.4%
Diabetes	21.7%
MDRD-4[b] (ml/min)	82±28
Bicuspid aortic valve	20%

[a] average ± standard deviation.
[b] glomerular filtration rate in ml/min/1.73 m^2 (mean ± standard deviation).

Results

Individuals are analyzed in Hardy-Weinberg equilibrium for the 1G>2G, polymorphism at position -1607 with a frequency of 0.49 for the minor allele (1G in our population), similar to that of other European populations (dbSNP).

The average values of BMD and BV/TV in population groups defined by different MMP1 polymorphism genotypes at position -1607 and differences found in both variables were calculated, although they were statistically significant only in the case of BV/TV (Table 2).

Post hoc analysis found that homozygous individuals presented significant differences for the 2G allele compared with homozygous for 1G (p = 0.042), with calcium content values similar to those of homozygous for 2G allele heterozygous individuals. Thus, applying a model of recessive for the allele effect 1G, it was found that allele carriers 2G had significantly higher values of calcium content in the aortic valve (3 times in BMD and 2 times the BV/TV) than noncarriers (BMD values of 62.52±10.99 mg/cm^3 in 2G allele carriers ± 8.54 versus 20.08 mg/cm^3 in the 1G allele homozygotes, and values BV/TV 5.44±0.62% in 2G allele carriers versus 2.52±0.59% in homozygotes for the 1G allele) (Figure 2). These significant differences remained for levels of BV/TV after adjustment for sex, age, presence of bicuspid aortic valve and glomerular filtration rate (p=0.021), maintaining the trend, but without being significant, for BMD levels (p=0.073).

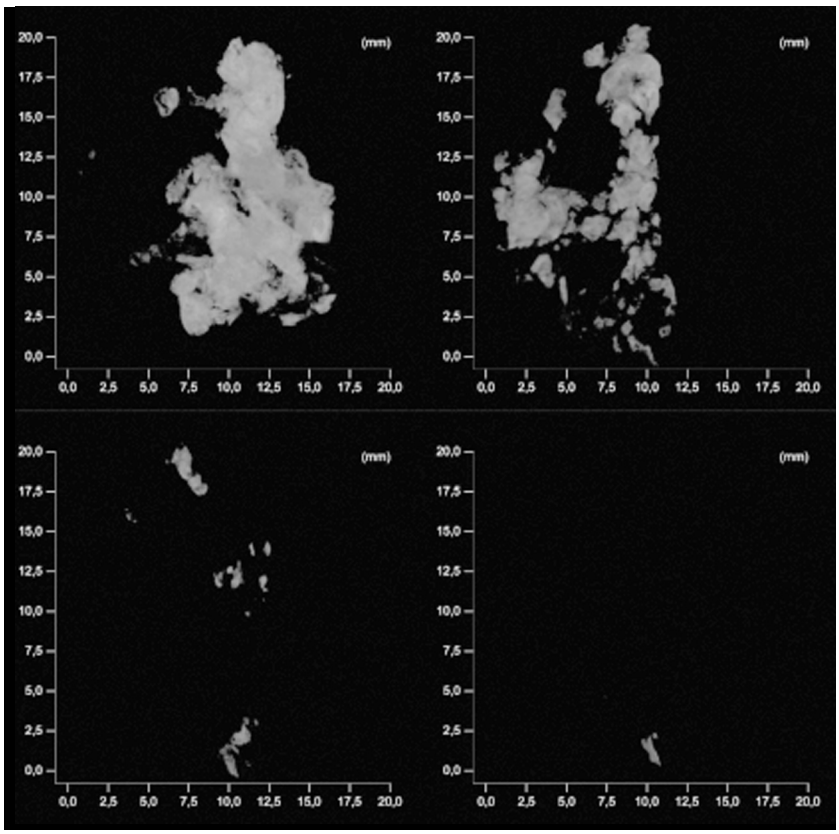
Discussion

This study is the first to describe an association between a polymorphism of MMP1 gene and the amount of calcium in the aortic valves. The literature contains various associations of variants of this gene with other cardiovascular conditions[11-13]. Among these variants, one of the most studied is the 1G>2G polymorphism in the promoter region of the gene, for the insertion allele confers greater transcriptional activity[14] which can have effects on the cell and therefore in the body. Thus, a significantly increased risk of atherosclerosis in the carotid artery in individuals carrying the 2G allele[15] and a greater presence of this allele in patients who had suffered ischemic stroke was observed[16]. However, until now associations of this polymorphism and calcific aortic valve disease had not been described, although the MMPs have been known to play an important role in their physiopathology[17,18].

Two important aspects in the development of disease are calcified aortic valve inflammation and extracellular matrix remodeling[5]. Both are modulated by valve interstitial cells (VIC), which pass from a rest state in which they retain tissue homeostasis, to an activated state which take myofibroblasts[19]. Activated VIC respond to inflammation by secreting, among other factors, MMPs, which will contribute to the accumulation of disorganized fibrous tissue, to keep the valves in a state of chronic inflammation and induce osteoblastic differentiation of VIC. The latter event would promote and accelerate calcium deposition, which would result in reduced function of the valve[20].

The interaction between cells and the extracellular matrix that contains them is essential for the physiology and functionality of the valve tissue and affects the VIC phenotype[3]. The extracellular matrix of the heart valves is made up to 90% collagen and, in fact, excessive deposition of protein, accompa-

Figure 1. Images obtained after analysis by computerized micro-tomography (microCT) of the aortic valve cusps. 4 Examples of valve cusps are shown with varying degrees of calcification



nied by an altered alignment of fibers increases tissue stiffness[21]. Several studies have shown the crucial role of collagen in calcification of aortic valves[22-24]. Specifically, in vitro culture of porcine aortic valve cusps of collagenase treated with increased collagen and simultaneously a decrease in the amount of other components of the extracellular matrix such as hyaluronic acid has been observed[23]. Also, an increase was detected in both proliferation and apoptosis of VIC, which expressed markers associated with a myofibroblast phenotype (alpha-smooth muscle actin) and osteoblast (alkaline phosphatase, osteocalcin and bone sialoprotein) resulting in increased tissue mineralization[23].

MMP-1, also known as fibroblast collagenase, degrades interstitial collagen types I, II and III. Consequently, its increased activity would promote the destruction of collagen, osteoblast differentiation and calcification. The results obtained in this study support this reasoning, associating the highest amounts of calcium in valves with those individuals carrying the allele resulting in increased transcription of the gene and, consequently, a greater amount of MMP-1 protein. Viewed another way, the lack of the protective effect brought about in patients less transcript (provided by the less active allele) facilitating the development of calcification in the aortic valve.

A limitation of our study is the small number of patients included and the fact that it is a cross-sectional study. However, an association with 1G>2G polymorphism has been affirmed, suggesting the protective effect of the 1G allele will necessitate studying larger samples and other population groups in order to ascertain whether this finding could be used in the future as a predictor of calcification and aortic stenosis.

Competing interests: The authors declare that they have no conflict of interest.

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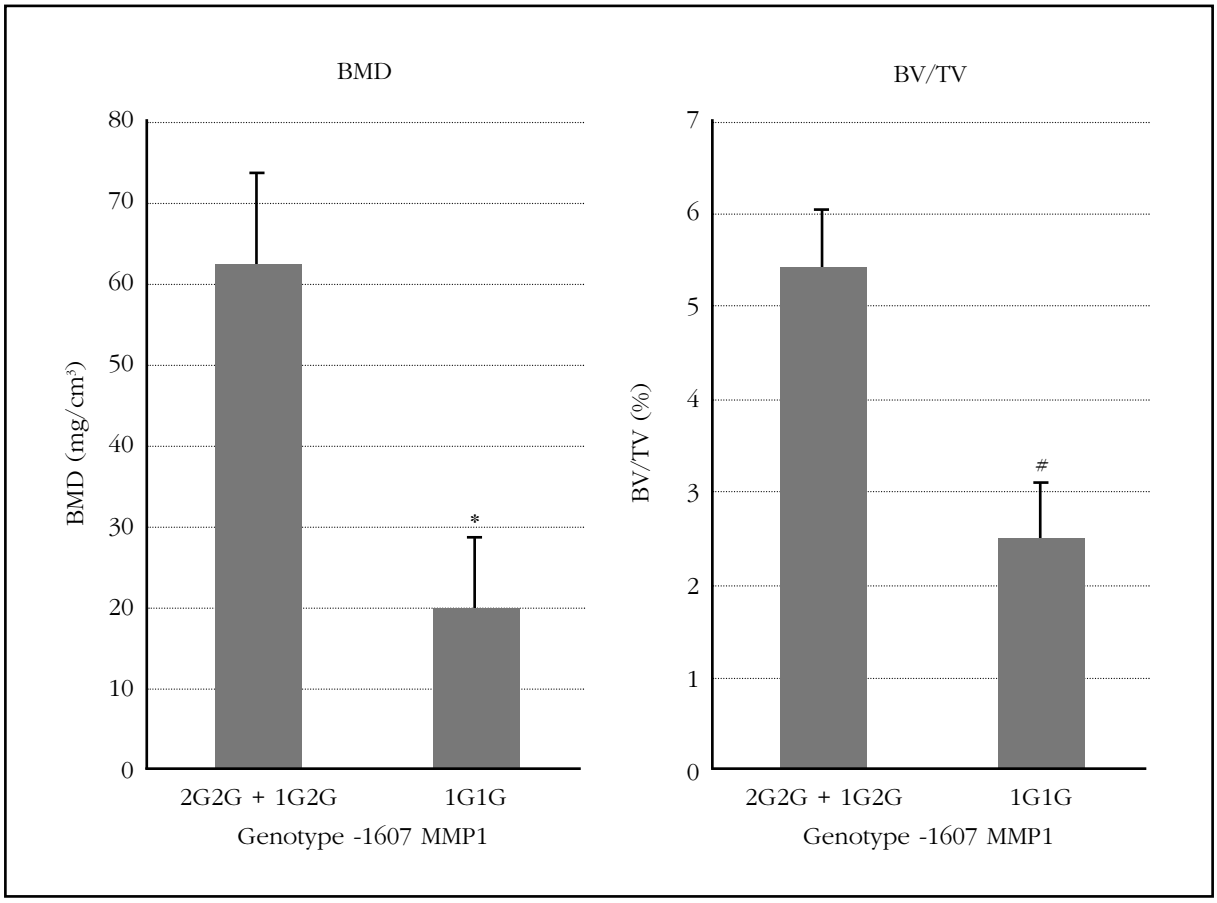
Table 2. Values of BMD and BV/TV for each genotype polymorphism of MMP1 at the -1607 position

Genotype -1607 MMP1	BMD (mg/cm³)	BV/TV (%)
2G2G (N=13)	66.72±65.31	5.87±3.76
1G2G (N=20)	59.79±63.22	5.16±3.51
1G1G (N=12)	20.08±29.59	2.52±2.03
Total (N=45)	51.20±58.96	4.66±3.46
p-valor	0.095	0.033

The data are represented as mean ± standard deviation. P-value obtained by ANOVA.

Figure 2. Values of BMD and BV/TV for MMP1 -1607 genotypes as a model of recessive inheritance for the 1G allele. Average ± standard error is shown. *p=0.004; #p=0.002



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