Gene 524 (2013) 304–308

Contents lists available at SciVerse ScienceDirect

Gene

journal homepage: www.elsevier.com/locate/gene

Low transcriptional activity haplotype of matrix metalloproteinase 1 is less frequent in bicuspid aortic valve patients

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ARTICLE INFO

Article history:
Accepted 27 March 2013
Available online 19 April 2013

Keywords:
Aortic valve morphology
Thoracic aortic aneurysm
Polymorphisms
Genetic association study

ABSTRACT

Purpose: Bicuspid aortic valve (BAV) is the most common congenital cardiac anomaly which affects 0.5–2% of the population. It can be associated with other cardiac congenital lesions such as aortic aneurysms or aortic coarctation. Some genetic abnormalities have been suggested as the underlying cause of BAV and aortic root dilatation, but no clear genetic substrate and no specific pathogenic gene variant have already been identified. Increased matrix metalloproteinase activity has been described in the aortic wall of thoracic aortic aneurysms (TAA).

Methods: 87 patients with BAV and 77 controls with normal tricuspid aortic valve were prospectively assessed. We analysed three functional polymorphisms (−1607 1G/2G, −519 A/G, and −340 T/C) in the matrix metalloproteinase (MMP)-1 gene using polymerase chain reaction and restriction fragment length analysis.

Results: We found a haplotype composed of the lower activity allele from each polymorphism (−1607 1G/−519 A/−340 C) significantly less frequent in BAV group ($p = 0.016$; OR [95% CI] = 0.37 [0.16–0.85]), association even more clear when we consider only men ($p = 0.0005$, OR [95% CI] = 0.24 [0.10–0.56]).

We also found a borderline statistical significance in the distribution of the −1607 alleles, being 2G allele more frequent in patients with TAA ($p = 0.053$). This association was stronger and statistically significant when we consider only men ($p = 0.013$; OR [95% CI] = 2.0 [1.16–3.50]). In addition, genotype −1607 2G2G, theoretically the more active transcriptionally, was also significantly more frequent in TAA group, independently of aortic valve morphology.

Conclusions: Our study suggests that specific genotypes of *MMP1* gene could be in part responsible of the complications of BAV pathology, like TAA.

1. Introduction

The prevalence of bicuspid aortic valve (BAV) is about 0.5–2% in the general population and it constitutes the most common congenital cardiac abnormality (Van der Bom et al., 2012). BAV is an important risk factor for early development of aortic valve disease and it can be associated with a high risk of aortic dilatation and dissection (Ferencik and Pape, 2003; Tadros et al., 2009; Tzemos et al., 2008). This association seems to be related to an underlying common congenital defect of valve and vessel structure due to their common embryonic origin (Kappetein et al., 1991; Schaefer et al., 2007). Although genetic abnormalities have been suggested as the underlying cause of BAV and aortic root dilatation, no clear genetic substrate and no specific pathogenic gene variant have already been identified. Furthermore, a familial hereditary form of BAV has been described in about 10–30% of individuals (Cripe et al., 2004) and it is also associated with other genetic disorders such as Turner syndrome (Sachdev et al., 2008). Otherwise, Marfan syndrome and BAV disease share common histopathological findings, including medial degeneration, increased matrix metalloproteinase (MMP) activity and decreased fibrillin-1 in the aortic wall (Fedak et al., 2003).

MMP constitutes a family including 23 zinc-dependent proteolytic enzymes in humans that play an important role in diseases related to the extracellular matrix protein metabolism and aortic wall remodelling, which are important processes in the development of aneurysms and dissections (Nataatmadja et al., 2003). Ascending aortic aneurysms have previously been shown to exhibit increased protein level of

Abbreviations: MMP, matrix metalloproteinase; BAV, bicuspid aortic valve; TAA, thoracic aortic aneurysm; TAV, tricuspid aortic valve.

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http://dx.doi.org/10.1016/j.gene.2013.03.127

MMPs, especially MMP-2 and -9, when compared to controls (Boyum et al., 2004; Fedak et al., 2003; Wilton et al., 2008) and it has also been described an association between some polymorphisms in the *MMP9* gene (−8202 A/G) with thoracic aortic aneurysms and thoracic aortic dissections (Chen et al., 2006). Therefore, genetic variations that affect the protease expression or activity may contribute to the development of valvular or thoracic aortic disease in patients with BAV.

MMP-1 is a collagenase like MMP-8 and MMP-13 and they cleave interstitial collagens I, II and III, although they can digest other extracellular matrix molecules and soluble proteins (Nagase et al., 2006). Few studies have analysed the relationship between MMP-1 and aortic disease. Some authors aimed to determine any association between ascending aortic pathology in patients with BAV and TAV and gene expression of MMP-1, -2, and -9, among others, without finding any difference in the gene expression (Wilton et al., 2008). However, other studies found an increased expression of MMP-1 and MMP-9 in aneurysm and aortic dissection patients compared to controls (Koullias et al., 2004). These controversial data led us to the hypothesis that MMP-1 could be implicated in the pathogenesis of bicuspid aortic valve and the susceptibility to develop aneurysms and dissections.

The aim of this case–control study performed in patients with BAV and in a control group of patients with tricuspid aortic valve (TAV) was to determine whether 3 functional polymorphisms (Pearce et al., 2005; Rutter et al., 1998) in the promoter region of *MMP1* gene (−1607 1G/2G, −519 A/G, and −340 T/C) are associated with BAV and if there is any difference related to the development of valve stenosis, regurgitation, or aortic aneurysms in patients with BAV.

2. Material and methods

2.1. Patients

We prospectively assessed 87 consecutive Caucasian patients, all of a region in northern Spain (Asturias), with BAV who were referred to our institution (45 with previously known BAV who were submitted for follow-up or after valve replacement, and 42 as a "de novo" diagnosis). Selection of the patients was made by two observers through an echocardiography as having two separate functional leaflets or after valve replacement once valve morphology was confirmed by the surgeon. Patients with suboptimal echocardiographic images and with any doubt in the number of valve leaflets were excluded. Patients with previous or a suspicious diagnosis of Marfan syndrome (Loeys et al., 2010), Ehlers–Danlos syndrome (Beighton et al., 1998), or other connective tissue disorders associated with aortic disease were also excluded.

Simultaneously, the control group consisted of 77 consecutive Caucasian patients from the same region who were submitted by a heart murmur or with a previous valve disease, and with TAV confirmed by echocardiogram as having three separate functional leaflets or after surgical removal of the valve.

In both cases and controls, we followed guidelines and criteria for the diagnosis of ascending aortic aneurysm, coarctation, aortic regurgitation and stenosis (Baumgartner et al., 2009, 2010; Hiratzka et al., 2010; Lancellotti et al., 2010) by echocardiographic analysis and, in case of aortic aneurysms, previous dissection and aortic coarctation, those conditions were confirmed by magnetic resonance or computed tomography. Aortic aneurysm was defined as a permanent localized dilatation of an artery having at least a 50% increase in diameter compared with the expected normal diameter of the artery in question (Hiratzka et al., 2010; Lancellotti et al., 2010).

Patients were considered to have a positive family history of BAV or aortic disease when these conditions were confirmed in, at least, a first degree relative. Written informed consent was obtained from all patients and the study was approved by the Ethical Committee of Clinical Investigation of Asturias, Oviedo, Spain.

2.2. MMP1 genotyping

DNA was obtained from 10 mL of peripheral blood following a salting-out method (Miller et al., 1988). The three polymorphisms (−1607 1G/2G [rs11292517], −519 A/G [rs1144393], and −340 T/C [rs514921]) were genotyped through PCR restriction fragment length polymorphism as previously described (Roman-Garcia et al., 2009).

2.3. Statistical analysis

The SPSS Statistical Package (v.17.0) was used for the standard statistical comparisons. Categorical data at baseline were compared with a χ^2 test, which was also used to compare allele and genotype frequencies for each polymorphism and the deviation from Hardy–Weinberg equilibrium. Fisher's exact test was used when appropriate. SHEsis online programme (http://analysis.bio-x.cn/myAnalysis.php) was used to estimate haplotype frequencies in both groups and for their statistical comparisons (Li et al., 2009; Shi and He, 2005). Haplotypes with frequency less than 0.05 were not considered in the analysis. A p-value < 0.05 was considered statistically significant in all tests.

3. Results

3.1. Clinical parameters of patients

Clinical characteristics of patients, both cases with BAV and controls with TAV, are presented in Table 1. We found statistically significant higher frequencies in the presence of aortic regurgitation, ascending aortic aneurysms, and coarctation in the BAV group compared with TAV group. Familial disease was also more frequent in BAV group but it did not reach statistical significance.

3.2. Genetic association study

In the analysis of the three *MMP1* polymorphisms we found that all of them fit the Hardy–Weinberg equilibrium in all the groups we generated (data not shown). We did not find statistical differences between allelic nor genotypic frequencies' distribution of the three polymorphisms between BAV and TAV patients (Table 2). However, we found the haplotype 1G/A/C (i.e., 1G allele from rs11292517, A allele from rs1144393, and C allele from rs514921) significantly less frequent in BAV group (Table 3) that is even more clear when we consider only men: 18.3% in TAV and 5.1% in BAV ($p = 0.0005$, OR [95% CI] = 0.24 [0.10–0.56]).

We also tried to find an association of these polymorphisms with other characteristics of our population, like the presence of thoracic aortic aneurysms (TAA), stenosis, regurgitation and coarctation. We only found a borderline statistical significance in the distribution of

Table 1
Clinical and demographic characteristics of the 164 patients, both cases with bicuspid (BAV) and controls with tricuspid aortic valve (TAV).

	TAV (n = 77)	BAV (n = 87)	p-Value
Mean age (years ± SD)	69.9 ± 10.2	50.4 ± 15.4	<0.001
Male	48 (62.3)	69 (79.3)	0.016
Hypertensives	49 (63.6)	22 (25.3)	<0.001
Smokers	28 (36.4)	28 (32.2)	0.57
Dyslipidemia	34 (44.2)	26 (29.9)	0.058
Diabetics	23 (29.9)	9 (10.3)	0.002
Thoracic aortic aneurysm	6 (7.8)	39 (44.8)	<0.001
Coarctation	0 (0)	7 (8.05)	0.015
Aortic regurgitation	6 (7.8)	44 (50.6)	<0.001
Aortic stenosis	36 (46.7)	42 (48.3)	0.85
Aortic surgery	31 (40.3)	49 (56.3)	0.048
Familial history	2 (2.6)	9 (10.3)	0.062

Numbers are n (%).