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FOLLOW-UP PAPER

Histological and genetic studies in patients with bicuspid aortic valve and ascending aorta complications[†]

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Abstract

OBJECTIVES: Aneurysm diameter and growing rate does not represent a definite parameter for operation in bicuspid aortic valve (BAV), ascending aortic aneurysm and normal root patients. Thus, we investigated histological and immunohistochemical aspects of different segments of ascending aorta (precisely, aortic root without dilatation, aneurysmatic tubular portion, dissected ascending aorta) and genetic features of patients with BAV and ascending aorta complication (aneurysm or dissection).

METHODS: Aorta tissue samples of 24 BAV patients were examined. The patients comprised of 18 men and 6 women; the mean age was 54.2 ± 14.3 years. All patients underwent composite aortic root replacement (button Bentall operation). Multiple histological sections were prepared from each aortic specimen. The evaluated features included elastic fibre fragmentation, cystic medial change, smooth muscle cell necrosis, medial fibrosis, and the markers of medial apoptosis and the metalloproteinases. Furthermore, genetic risk factors were also investigated.

RESULTS: The same medial degenerative lesions in tissue samples of different aorta segments (precisely of aortic root without dilatation, and aneurysmatic ascending aorta portion) were observed. More significant associations between single nucleotide polymorphisms (−786T/C endothelial nitric oxide synthase enzyme, D/I angiotensin-converting enzyme, −1562C/T metalloproteinase-9 and −735C/T metalloproteinase-2) and aneurysm risk were detected in BAV patients than in controls.

CONCLUSIONS: Based on our histological and genetic data, we underline that a surgical approach in patients with BAV, ascending aortic aneurysm and normal root, should consider not only the diameter of the aneurysmatic aortic portion but also the histological features of the whole ascending aorta and the genetic risk profile.

Keywords: Bicuspid aortic valve • Aneurysm • Dissection

INTRODUCTION

Bicuspid aortic valve (BAV) is one of the most frequent congenital heart defects having a prevalence of 0.5–1.4%. It appears to be sporadically transmitted through families by an autosomal-dominant pathway, with a 3:1 male predominance [1–3]. Pathogenesis is considered as a very complex development process [4–6].

BAV is frequently (10–35% of cases) associated with an enlargement of ascending aorta [7] and its severe complications such as aortic rupture or dissection. Case dissections associated with BAV are about 4% [8]. Different, controversial theories have been suggested to explain the strong association between BAV and ascending aorta aneurysm and dissection [9, 10].

Because ascending aorta dilatation is a widely recognized risk factor for aortic dissection [11], a more aggressive posture towards replacement of the moderately enlarged ascending aorta has been advocated in the most recent American College of Cardiology/American Heart Association guidelines for the treatment of patients with thoracic aortic disease [12].

Some authors propose a more radical resection of the aorta because of the potential development of a late dilatation and dissection in the remaining distal ascending aorta and aortic root with or without a mild dilatation [13, 14].

Based on this evidence, we suggest that the diameter of aneurysmatic aortic portion is not the unique parameter to consider for operation on patients with BAV and aneurysm. Thus, the histological features of complete ascending aortic wall

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(inclusive aortic root without dilatation) and the genetic risk profile should also be considered.

To confirm this hypothesis, we examined histopathological features of different ascending aorta segments (precisely, aortic root without dilatation, aneurysmatic tubular portion, dissected ascending aorta) in aorta tissue samples of 24 BAV patients with ascending aorta complication (aneurysm or dissection) who underwent cardiac surgery between January 2004 and July 2008 in our Cardiac Surgery Unit. Furthermore, the role of genetic component in the susceptibility and risk for ascending aorta aneurysm and dissection in BAV patients was also evaluated.

MATERIALS AND METHODS

Our study received approval from local ethic committees and all participants gave their informed consent. Data were encoded to ensure patient and control protection.

Subjects

Our study included 24 BAV individuals [18 men (75%) and 6 (25%) women; mean age: 54.2 ± 14.3] from Western Sicily. They were registered between January 2004 to July 2008 at the Cardiac Surgery Unit of the Surgery and Oncology Department of Palermo University Hospital. We selected BAV patients with aortic complications [23 (96%) aneurysms and 1 (4%) dissection] and not dilated aortic root. All patients showed qualitatively altered aortic root at the time of operation: thin and weak walls with graining. BAV (stenotic or incontinent) was fibrocalcific in 13 (54%) cases and prolapsed in one (4%) case (Table 1).

Diameter evaluation of ascending aorta was made both preoperatively and in the operating room by transthoracic echocardiography (TTE) and transesophageal echocardiography (TEE) estimations performed as follows: estimating dimensions of aortic annulus, sinuses of Valsalva and proximal ascending aorta (above 2.5 cm of the sinotubular junction) in parasternal long axis; evaluating the ones of aortic arch from suprasternal view. Echocardiography derived sizes were reported as internal diameter size [15]. Sinus of Valsalva mean size was 34 ± 3.0 mm in men and 30 ± 3.0 mm in women (Table 1). Aneurysmatic ascending aorta mean size was 50 ± 5.6 in men and 58 ± 16 mm in women (Table 1). Colour Doppler was used to assess the presence and severity of aortic regurgitation and stenosis. Furthermore, aortic root and ascending aorta diameter sizes were carried out using helical computed tomography image analysis techniques. Relevant medical histories regarding aortic disease were obtained from patients' medical records.

Thus, demographic and clinical features, comorbidity conditions and pharmacological treatments were collected (Table 1). In particular, our patients had suffered from hypertension for a few years. In all cases, hypertension was controlled by beta-blockers.

The surgical procedure used was the button Bentall operation, which is a modification of the original technique described by Kouchoukos *et al.* 1991 [16]. In order to detect histopathological abnormalities, control ascending aortas were obtained from 30 individuals (20 men and 10 women; mean age: 55 ± 11.57 years) who died from causes unrelated to aortic disease and with no sepsis at the time of death, as confirmed by autopsy.

Table 1: Demographics and preoperative clinical characteristics

Variables	Patients (*n* = 24)	Women (*n* = 6)	Men (*n* = 18)	*P*-value
Demographic characteristics				
Age, mean (SD)	54.2 (14.3)	56.3 (13)	53.5 (15)	0.46
Male sex, no .(%)	18 (75%)			
Female sex, no. (%)	6 (25%)			
Body mass index, mean (SD)	27 (3.1)	27.4 (4.8)	26.9 (2.5)	0.92
Size (echocardiographic value)				
Proximal ascending aorta size (mm), mean (SD)	52.3 (9.4)	58 (16)	50 (5.6)	0.15
Sinus of Valsalva size (mm), mean (SD)	33 (3.8)	34 (3.0)	30 (3.0)	0.001
Comorbidity conditions, no. (%)				
Aortic aneurysm familiarity	1 (4)	0 (7.1)	1 (7.9)	1.00
Cardiovascular ischaemic familiarity	9 (38)	2 (35.7)	7 (39.4)	1.00
Smoke	8 (33)	0 (7.1)	8 (44.7)	0.05
Hypertension	18 (75)	6 (92.8)	12 (65.8)	0.28
Dislipidaemia	4 (17)	0 (7.1)	4 (23.7)	0.54
Diabetes mellitus	1 (4)	0 (7.1)	1 (7.9)	1.00
Renal failure	1 (4)	0 (7.1)	1 (7.9)	1.00
Aortic valve pathology, no. (%)				1.00
Normal	10 (42)	3 (46.7)	7 (53.8)	
Prolapse	1 (4)	0 (6.6)	1 (7.7)	
Vascular calcium fibrosis	13 (54)	3 (46.7)	10 (38.5)	
Aortic valve dysfunction, no. (%)				0.26
Normal	3 (13)	2 (29.4)	1 (7.3)	
Faint incontinence	9 (38)	1 (17.7)	8 (41.4)	
Moderate incontinence	1 (4)	0 (5.8)	1 (7.3)	
Severe incontinence	0 (0)	0 (0)	0 (0)	
Faint stenosis	0 (0)	0 (0)	0 (0)	
Moderate stenosis	2 (8)	1 (17.7)	1 (7.3)	
Severe stenosis	9 (38)	2 (29.4)	7 (36.6)	
Atherosclerosis coronary syndrome, no. (%)	11 (46)	4 (64.3)	7 (39.5)	0.36

SD, standard deviation; No., number of patients.

One hundred and twenty-eight matched controls [61 (47%) men and 67 (53%) women; mean age: 61.08 ± 5.83 years] were also enrolled to perform genotype analyses. They were in good health, according to their clinical history and blood tests (complete blood cell count, erythrocyte sedimentation rate, glucose, urea nitrogen, creatinine, electrolytes, C-reactive protein, liver function tests, iron and proteins). None had any other major cardiac risk factors, with the exception of smoking (23%). Furthermore, TTE imaging examinations confirmed the absence of ascending aorta aneurysms, BAV and aortic valve dysfunction in all controls. We selected a very homogenous population. As a matter of fact, all patients and controls belonged to the same ethnic group, as their parents and grandparents were born in Western Sicily.

Aortic specimens and histopathological assays

Full aortic segments were collected from resected aortic wall of 24 patients at the time of surgery and fixed in 10% neutral buffered

formalin for 24 h and then processed for routine paraffin embedding. Multiple histological sections from each sample were prepared and stained with haematoxylin–eosin, Weigert-van Gieson, Alcian-PAS for microscopic examinations. Histopathological abnormalities of aortic wall media were graded and defined according to the definitions and grading systems used by Bechtel *et al*. [17].

Serial sections of these tissues were also used for immunohistochemical staining. The aortic wall was mainly evaluated for the following histological features: elastic fibre fragmentation and collagen fibre network, presence of necrosis, apoptosis, amounts of metalloproteinase-9 (MMP-9) and inflammatory cell infiltration.

Immunohistochemical assays

Immunohistochemical analyses were performed on 5 μm-thick paraffin-embedded sections. The deparaffinized sections were treated for 20 min in a microwave oven in 10 mM citrate buffer of pH 6 or Tris–EDTA of pH 9. Sections were then incubated for 1 h with specific monoclonal antibodies against MMP-9 (Clone 15W2, NCL-MMP9 439, Novocastra Laboratories Ltd, UK, 1:50), or isotype-matched controls at appropriate dilutions. After washing in TBS 1X (Tris-buffered solution), staining was performed by biotinylated link antibody and streptovidin labelled with PEROXIDASE KITS (Dako, North America, Inc, USA) and it was detected using AEC (3-amino-9-ethylcarbazole) substrate chromogen. After that, the counterstaining of cells and tissue sections was performed using aqueous haematoxylin (Novocastra Laboratories Ltd).

Tunel testing

We performed TdT (Terminal deoxynucleotidyl Transferase)-mediated X-dUTP (deoxyuridine triphosphate nucleotides) nick end-labelling (TUNEL) reaction ('*In situ* cell death detection kit', Roche Diagnostics S.p.A, Milano, Italy) on full-thickness aortic wall paraffin sections (5 μm). Tissues were deparaffinized and then permeabilized with PBS, 0.1% sodium citrate/0.1% Triton X-100. Specimens were then incubated with TdT and fluorescein-labelled dUTP in a humidified atmosphere for 1 h at 37°C. *In situ* apoptosis staining was revealed by using an AP converter. DNA strand breaks were detected using the 5-bromo-4chloro-3-indolyl-phosphate (BCIP/NBT, Dako, Italy) substrate chromogen. Tissues were subsequently counterstained with eosin under light microscopy.

Semi-quantitative evaluation of MMP-9 by immunohistochemical assays

A semi-quantitative evaluation of MMP-9 amount in aortic specimens of 24 patients was performed during the course of immunohistochemical assays. Staining was classified as faint, moderate or severe.

Genotyping

DNA samples of 24 cases and 128 matched controls were extracted from peripheral blood samples collected in tripotassium EDTA and purified using a QIAamp Blood DNA Maxi kit (Qiagen, Dusseldorf, Germany). Samples were genotyped for 10 single nucleotide polymorphisms (SNPs) located in the promoter and coding regions of selected genes (Table 2). Two procedures were used, such as restriction fragment length polymorphism–PCR (RFLP–PCR) and single specific primer (SSP)–PCR.

For genotyping +896A/G (Asp299Gly, rs4986790), +1196C/T (Thr399Ile, rs4986791), Toll-like receptor 4 (TLR4) SNPs and Δ32 C-C chemokine receptor type 5 (CCR5) deletions (rs333), procedures previously described were used [18–21].

For identification of −1562C/T MMP9 (rs3918242), −1306C/T (rs243865) and −735C/T MMP2 (no rs available designation) SNPs, RFLP–PCR procedure was used, followed by restriction cleavage with *Shp*I, *Acc*I and *Hin*fI (New England Biolabs, USA), respectively, and separation of DNA fragments by electrophoresis. For genotyping +894G/T (Glu208Asp, rs1799983) and

Table 2: Genes (accession number), SNPs (accession number) and substitutions investigated in the study

Genes	SNPs	Biological effect
TLR4 (NM-138554.1)	+896A/G (Asp299Gly; rs4986790) +1196C/T (Thr 399Ile; rs4986791)	Determining a single amino acid substitution in the extracellular receptor domain and hence, a blunted innate/inflammatory response to both foreign pathogens and endogenously generated inflammatory ligands
CCR5 (NM-00579)	Deletion 32 (rs333)	A non-functional allele resulting from a 32-bp deletion in exon 4 (CCR5_32) determines a loss of expression of functional CCR5 receptor
MMP9 (NM-004985)	−1562C/T (rs3918242)	Located within a putative binding site for a regulator factor of the gene transcription, determining an increased expression of MMP-9 enzyme
MMP2 (NM-001121363.1)	−1306C/T (rs243865) −735C/T (rs 2285053)	They are located within a putative binding site for a regulator factor, Sp1, of the gene transcription, determining an increased expression of MMP-2 enzyme
eNOs (NM-000594)	894G/T (Glu208Asp; rs 1799983)	Determining a single amino acid substitution (Glu/Asp-208), associated with a reduced basal production of eNOs and an increased amount of plasmatic homocysteine
	−786T/C (rs2070744)	SNP located in the promoter region determining a reduced gene transcription
	4a/4b VNTR repeat	A VNTR repeat of 27 bp in a intron region associated with an altered production of eNOs
ACE (NM-152830.1)	D/I SNP (rs1799752)	Determining changes in the plasmatic and cellular concentration of ACE enzyme. The D allele is associated with a high risk and susceptibility of vascular disorders

TRL, Toll-like receptor 4; CCR, C-C chemokine receptor type 5; MMP-2 and -9, metalloproteinase 2 and 9; eNOs, endothelial nitric oxide synthase enzyme; ACE, angiotensin-converting enzyme; VNTR, variable number tandem repeats.

−786T/C (rs2070744) SNPs in endothelial nitric oxide synthase enzyme (eNOs) gene, RFLP–PCR was also executed, followed by restriction cleavage with *Ban*I and *Nae*I (New England Biolabs), respectively, and separation of DNA fragments by electrophoresis. Meanwhile, an SSP–PCR was assessed for genotyping 4a/4b eNOs and D/I (rs1799752) angiotensin-converting enzyme (ACE) SNPs.

Statistical analysis

All analyses were performed with R and Excel software. Fisher tests were conducted to compare all demographic and clinical features, comorbidity conditions and pharmacological treatments according to gender, and also to verify the hypothesis of association between elementary lesions and valvular dysfunction. To verify the hypothesis of a relationship between the size of an aneurysm and an elementary lesion, non-parametric Kruskal–Wallis tests were executed; this is due to the strong asymmetry of the size distributions. To analyse significantly the relationships between quantitative variables, the Wilcoxon rank sum test was employed. Allele and genotype frequencies were evaluated by gene count. Data were tested for finding out the consistency between observed and expected genotype frequencies, according to Hardy–Weinberg equilibrium, by χ^2-tests. Significant differences in frequencies among groups were calculated by using χ^2-test and appropriate tables (3×2, 2×2 tables, etc. wherever appropriate). Furthermore, odds ratios (OR) with 95% confidence intervals (CI) and their significance were calculated.

RESULTS

Clinical data

Table 1 reports demographic and clinical features, comorbidity conditions and pharmacological treatments of all BAV patients. Considering the elevated number of men affected by BAV associated to aneurysms (18 vs. 6 women), comparisons of all demographic and clinical features, comorbidity conditions and pharmacological treatments were performed depending on gender. No statistically significant differences were detected with the exception of smoking (8 males vs. 0 female, $P = 0.05$ by Fisher test).

Hospital mortality and short-term outcome

Hospital mortality—defined as in hospital mortality or death within the postoperative 30 days—was 0%. Bleeding occurred in just 1 case. One patient required prolonged ventilation. The average intensive care stay unit was 2.5 ± 2 days, and the mean hospital stay was 10 ± 2 days.

Histological and immunohistochemical observations

Histological and immunohistochemical assessments evidenced the presence of media-degenerative lesions in tissue samples of the aortic wall both of aneurysmatic or dissected tubular portion and aortic root without dilatation. In particular, we observed: elastic fragmentation of grade I in five cases (21%), grade II in 13 cases (54%) and grade III in six cases (25%); medionecrosis of grade I in three cases (13%), grade II in 11 cases (46%) and grade III in 10 cases (41%); cystic necrosis of grade I in four cases (17%), grade II in 13 cases (54%), grade III in seven cases (29%); medial fibrosis: grade I in 17 cases (70%), grade II in three cases (13%), grade III in one case (4%) (Fig. 1).

No significant associations were observed between aortic valve dysfunction, with or without cuspid pathological lesions, and medial change severity (P-value > 0.05 by Fisher test; data not shown). Apoptosis of media smooth muscle cells (SMCs) was found in 23 cases (96%); it was plurifocal in 18 cases (75%), and focal in five cases (21%). The research of collagenases in aortic media revealed the presence of MMP-9 in all cases with a different concentration (faint: three cases, 13%; moderate: nine cases, 37%; severe: 12 cases, 50%) and localization (intramyocitic pattern; interstitial pattern; intramyocitic and interstitial pattern).

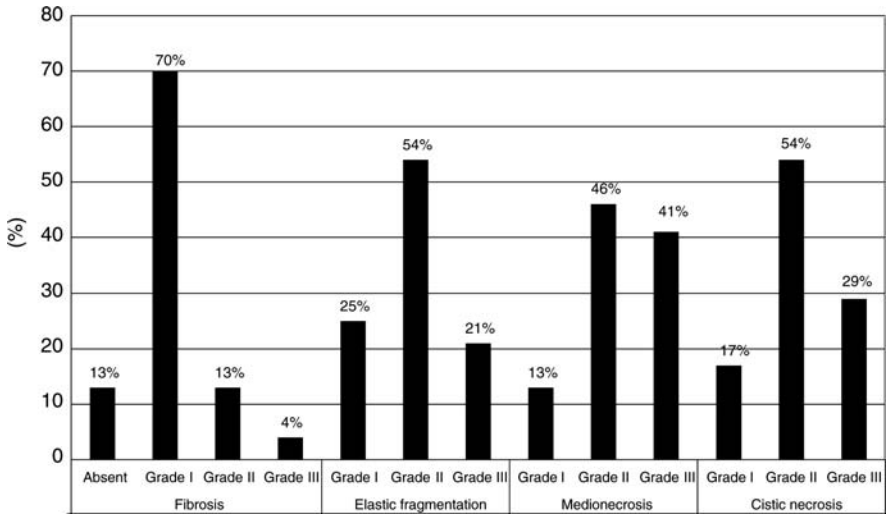

Figure 1: Media-degenerative lesion distribution in BAV patients with aortic complications (aneurysm and dissection).

Genotype distributions and allele frequencies of the ten SNPs

Comparing genotype distributions and allele frequencies of 10 SNPs selected among 24 BAV patients and 128 matched controls, some significant differences were observed for following SNPs: −786T/C eNOs, D/I ACE, −1562C/T MMP-9 and −735C/T MMP-2 (Table 3). In particular, we observed that −786T/T eNOS genotype was significantly represented in 24 BAV patients than 128 matched controls ($P = 0.02$ by χ^2-test 3 × 2 table). Furthermore, out of 128 patients, nobody presented −786C/C genotypes. In contrast, 4% and 33% of control group were carriers of mutant variant, associated with a decreased expression of eNOs molecule as indicated in Table 2. In addition, −786T allele was significantly over-represented in patients with respect to controls ($P = 0.01$ by χ^2-test with Yates's correction, 2 × 2 table; OR = 0.17 (0.04–0.7) $P = 0.006$ by Fisher's exact test).

A significant difference in genotype distribution of D/I ACE SNP was found among 24 BAV patients and 128 matched controls ($P = 0.01$ by χ^2-test 3 × 2 table). Accordingly, D ACE variant was significantly over-expressed in patients compared with controls ($P = 0.02$ by χ^2-test with Yates's correction, 2 × 2 table; OR = 3.3 (1.6–6.5) $P = 0.0005$ by Fisher's exact test).

Furthermore, we found that −1562T/T MMP-9 was significantly represented in 24 BAV patients than in 128 matched controls ($P = 0.0005$ by χ^2-test 3 × 2 table; Table 3). Accordingly, −1562T MMP-9 allele was significantly over-represented in patients with respect to controls ($P = 0.00001$ by χ^2-test with Yates's correction, 2 × 2 table; OR = 5.9 (2.6–13.5) $P < 0.0001$ by Fisher's exact test). The same results were obtained for −735C/T MMP-2. Precisely, −735T/T genotype was significantly represented in 24 BAV patients than in 128 matched controls ($P = 0.0005$ by χ^2-test; 3 × 2 table; Table 3). Accordingly, −735T MMP-2 allele was significantly over-represented in patients with respect to controls ($P = 0.0001$ by χ^2-test with Yates's correction, 2 × 2 table; OR = 8.5 (2.5–28.2) $P = 0.0007$ by Fisher's exact test).

Long-term outcome

Median follow-up time was 4.5 years. No patient required ascending aortic reoperation. None has died. They manage normal daily activities without any limitations.

DISCUSSION

A high incidence of both ascending aorta aneurysm and Stanford type A dissection and a high risk for aorta-related death (rupture or dissection) have been evidenced in BAV patients [8]. The high tendency for ascending aortic aneurysm or Stanford type A dissection observed in BAV patients seems due to histopathological abnormalities of the aorta wall. This evidences and the 11 times higher incidence of Stanford type A dissection in BAV patients than in the normal tricuspid aortic valve population, as recently reported, underline that the intricate problem within the aortic wall in BAV patients might involve the ascending aortic portion, as well as the non-dilated aortic root. According to our study, although based on a limited sample size, histological and immunohistochemical data evidenced aorta wall abnormalities (a small amount of elastic tissue and a very significant apoptosis and amount of MMP-9 collagenase) in BAV patients, both in non-dilated aortic root and aneurysmatic ascending aorta. These features suggest high risk even in the case of non-dilated aortic root

Table 3: Genotype distributions and allele frequencies of −786eNOs, D/I ACE, +896TLR4, −1562C/T MMP-9, −735C/T MMP-2 SNPs in 24 BAV patients and 128 matched controls (2 × 2 comparisons between the different groups with odd ratio (OR) and 95% confidence interval)

Candidate genes	Reference SNP number	Candidate SNPs	Patients ($n = 24$) (%)	Matched controls ($n = 128$) (%)	P (3 × 2, 2 × 2 table)	OR (95% CI)
eNOS	rs2070744	−786T/T	22 (92%)	81 (63%)	0.02	0.17 (0.04–0.7), $P = 0.006$
		−786T/C	2 (8%)	42 (33%)		
		−786C/C	0 (0%)	5 (4%)		
		−786T	46 (96%)	204 (80%)	0.01	
		−786C	2 (4%)	52 (20%)		
ACE	rs1799752	I/I	6 (25%)	70 (55%)	0.01	3.3 (1.6–6.5), $P = 0.0005$
		D/I	1 (4%)	1 (1%)		
		D/D	17 (71%)	57 (44%)		
		I	13 (27%)	141 (55%)	0.02	
		D	35 (73%)	115 (45%)		
MMP-9	rs3918242	−1562C/C	15 (63%)	116 (90%)	0.0005	5.9 (2.6–13.5), $P < 0.0001$
		−1562C/T	5 (21%)	9 (8%)		
		−1562T/T	4 (16%)	3 (2%)		
		−1562C	35 (73%)	241 (94%)	0.00001	
		−1562T	13 (27%)	15 (6%)		
MMP2	(no rs designation available)	−735C/C	18 (75%)	123 (96%)	0.0005	8.5 (2.5–28.2), $P = 0.0007$
		−735C/T	5 (21%)	5 (4%)		
		−735T/T	1 (4%)	0 (0%)		
		−735C	41 (85%)	251 (98%)	0.0001	
		−735T	7 (15%)	5 (2%)		

All genotypes were in Hardy–Weinberg equilibrium. eNOS, endothelial nitric oxide synthase enzyme; ACE, angiotensin-converting enzyme; MMP, metalloproteinase.

for a possible rupture and dissection. Our results lead us to propose that, in BAV patients, the diameter of an aneurysmatic aorta and its growing rate do not represent the only parameters to be considered for surgical strategy. Furthermore, genetic data should be considered. Significant associations and a very high OR between −786T/C eNOs, D/I ACE, −1562C/T MMP-9 and −735C/T MMP-2 SNPs and risk for BAV were detected in the patients than in controls. Because of the biological effects of these SNPs, their significant overexpression in patients suggests the capacity of determining a major fragility of the aorta wall, consequently associated with a major risk to develop severe complication, such as aorta dissection and/or rupture. MMP-9 and MMP-2 overexpression determines an excessive elastic tissue fragmentation with a consequent impairing of the aorta wall's mechanical properties. It is possible that the loss of aortic wall integrity predisposes SMCs to a higher risk of damage and death, with the consequent loss of the latter (medionecrosis) and replacement with mucoid basophil substance (cystic necrosis).

Some authors recommend composite aortic root replacement as the appropriate surgical strategy to treat and prevent these aorta diseases in BAV patients. This surgical procedure has been advocated to prevent ascending aortic or root dilatation [22, 23]. Therefore, the complete replacement of both the aortic root and the ascending aorta segment might eliminate the risk of rupture and dissection in a very large number of BAV patients. On the other hand, Etz *et al*. [14] found that the Bentall operation is associated with superior long-term survival and a lower rate of aortic re-operation in BAV patients. Recently, Park *et al*. demonstrated that a separate valve and graft remains an appropriate procedure for some patients with BAV and ascending aortic dilatation without significant root enlargement. Composite root replacement is a well-established procedure but it is technically more challenging and carries risks of coronary osteal complications, including bleeding, kinking and formation of false aneurysm. Owing to the frequency of aortic valve replacement (AVR) for BAV, any unnecessary incremental increase in risk globally applied will have a powerful clinical impact [24]. McKellar *et al*. [25], in a study of 1286 patients undergoing AVR for BAV without ascending aorta replacement, concluded that, despite a true risk for aortic events after AVR for BAV, the occurrence of aortic dissection is low and any incremental surgical risk imposed by prophylactic replacement of ascending aorta must be equally low.

Based on our results, we would suggest that the use of a surgical procedure based on the complete removal of ascending aorta including aortic root with or without dilatation might be reasonable in BAV patients with a high risk of rupture and dissection, especially in BAV patients with a thin aortic root wall at the time of operation and a particular genetic risk profile. However, our suggestion is based only on histological and genetic features. Clinical data are required to support them. A longitudinal study of both procedures, or a larger series on both the techniques would be needed to clear out the issue of the replacement, or not, of the aortic root.

In conclusion, BAV is frequently associated with an enlargement of the ascending aorta and its severe complications such as aortic rupture or dissection. The diameter of aneurysm and the growing rate does not represent a definite parameter for operation. Surgical strategy should also consider the structure of the complete ascending aortic wall (the grade of medial degeneration) and genetic risk factors as demonstrated by our study.

Study limitations

The principle limitation of this study is the limited sample size and the rarity of BAV pathology. Besides, clinical recommendations require clinical data to support them.

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