Endothelial Function, Carotid–Femoral Stiffness, and Plasma Matrix Metalloproteinase-2 in Men With Bicuspid Aortic Valve and Dilated Aorta

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Objectives
This study sought to examine the relationship between proximal aortic dilation and systemic vascular function in men with bicuspid aortic valve (BAV).

Background
Proximal aortic dilation in subjects with BAV is associated with structural and functional abnormalities in the ascending aorta.

Methods
We studied 32 men (median age 31 years [range 28 to 32 years]) with nonstenotic BAV categorized into 2 subgroups according to proximal ascending aorta dimensions (nondilated ≤35 mm and dilated ≥40 mm, respectively). Sixteen healthy men were studied as control subjects. Flow-mediated dilation in response to hyperemia (a marker of endothelial dysfunction) and carotid–femoral pulse wave velocity (an index of aortic stiffness) were assessed, and peripheral blood was sampled for matrix metalloproteinases (MMP-2 and -9) and their tissue inhibitors (TIMP-1 and -2), respectively. Cardiac chamber and aortic dimensions were assessed by echocardiography and cardiac magnetic resonance imaging, respectively.

Results
Despite the similar severity of aortic stenosis, left ventricular mass, and function, men with dilated aortas had blunted brachial flow-mediated vasodilation to hyperemia (5% [interquartile range (IQR) 4% to 6%] vs. 8% [IQR 7% to 9%], p < 0.001), higher carotid–femoral pulse wave velocity (9.3 cm/s [IQR 9 to 10 cm/s] vs. 7 cm/s [IQR 6.9 to 7.4 cm/s], p = 0.001), and significantly higher plasma levels of MMP-2 (1,523 [IQR 1,460 to 1,674] vs. 1,036 [IQR 962 to 1,167], p < 0.001) compared with men with BAV and nondilated aorta. Values for MMP-9, TIMP-1 and -2 levels, and nitroglycerin-induced (endothelium-independent) vasodilation were similar in all 3 groups.

Conclusions
Young men with BAV and dilated proximal aortas manifest systemic endothelial dysfunction, increased carotid–femoral pulse wave velocity, and higher plasma levels of MMP-2. These observations could introduce new targets for screening and perhaps for therapeutic intervention. (J Am Coll Cardiol 2010;55:660–8) © 2010 by the American College of Cardiology Foundation

Bicuspid aortic valve (BAV) is the most common congenital cardiac malformation, affecting as much as 1% to 2% of the population (1). The natural history of BAV includes the development of aortic valvular dysfunction as well as vascular complications such as aortic dilation, aortic dissection, or rupture (2,3). When compared with patients with a trileaflet valve, patients with BAV have larger aortic root dimensions and an increased rate of aortic dilation over time, with consequent higher risk of aortic dissection (4).

However, not all patients with BAV show aortic dilation, and in those who do, the magnitude of dilation is unrelated to the degree of valvar stenosis or regurgitation (4,5). These findings suggest a specific role for altered conduit vessel structure, rather than the nonspecific effect of disturbed transvalvar blood flow. Indeed, structural changes in aortic...
wall composition in BAV subjects have been linked recently to increased arterial stiffness (6). Such changes may be mediated by increased degradation of elastin and collagen by matrix metalloproteinase (MMP)-2, which is expressed in abundance in tissue excised from the proximal aortas of patients with BAV (7–10). Interestingly, MMP-2 overexpression is not confined to the proximal aorta, but also is evident in the pulmonary valve, suggesting a more widespread pathology involving both arteries and valves (7,11).

Abnormalities of endothelial function, due to altered expression of endothelial nitric oxide synthase (eNOS), have also been implicated in the genesis and progression of aortic dilation (12,13). These several observations would suggest that the presence of proximal aortic dilation in such subjects is only one aspect of a more diffuse process resulting in widespread alterations in vascular endothelial function and conduit artery properties, as well as valve formation and degeneration. If so, this constellation of abnormalities should be evident in subjects with BAV and aortic dilation, but absent in subjects with BAV without this dilation. We therefore conducted the present study to test the hypothesis that, in contrast to men with BAV and without aortic dilation, those with BAV and dilation would have a phenotype including increases in plasma MMP-2 concentrations, carotid–femoral pulse wave velocity (cf-PWV) as an index of aortic stiffness, and impaired flow-mediated vasodilation (FMD) in response to hyperemia, a marker of endothelial dysfunction. Findings in BAV subjects were compared with healthy male control subjects.

**Methods**

**Subject selection.** Subjects were identified from the Toronto Congenital Cardiac Centre for Adults (Toronto, Ontario, Canada) computerized database. Inclusion criteria were BAV previously identified by echocardiography and with concomitant coronary artery disease risk factors, aortic coarctation, and significant valvular disease, 26 subjects (21 male and 5 female) with dilated aortic root were identified. Five subjects declined to take part in the study. Similarly, after applying inclusion and exclusion criteria as well as willingness to participate in the study, only 16 of 90 men were selected from the normal aortic dimensions root group. The final cohort therefore included 16 men with dilated aortic and 16 men with normal aortic root dimensions. Thus, in total, 32 otherwise healthy men with BAV and without significant valvular disease were recruited to form the study population. A healthy control group (n = 16 men) with similar age range (18 to 40 years) as the BAV subjects and without family history of hypertension or aortic aneurysms was recruited. The University Health Network Research Ethics Board approved the study, and written consent was obtained in all subjects.

**Experimental protocol.** All studies took place early in the morning, and subjects attended after an overnight fast (8 h). Additionally, no subject ingested coffee, tea, or any other vasoactive substance for at least 12 h before the study day. After 15 min of resting, brachial blood pressure (BP) and heart rate were measured in the left arm with an automated digital oscillometric sphygomanometer (Omron, HEM 773-ACCAN, Omron Corporation, Ontario, Canada). Three readings separated by 1-min intervals were taken, and the mean value was used for the analysis. After heart rate and BP determinations, all subjects underwent echocardiography, applanation tonometry, brachial ultrasound, and peripheral blood sampling during the same visit.

**Echocardiography.** Echocardiographic imaging was performed in all subjects using a commercially available system (Philips iE-33, Philips Medical System, Andover, Massachusetts). The BAV was confirmed by 2 observers based on established criteria (15). Aortic regurgitation and stenosis were quantitated according to current guidelines (16,17), and aortic valve area was calculated using continuity equation. The presence of coarctation of the aorta was assessed using the suprasternal view with both pulsed- and continuous-wave Doppler. End-diastolic aortic root measurements were taken at the level of the sinus of Valsalva, ascending aorta, and aortic arch, as previously described (18). Real-time 3-dimensional imaging (RT3DE) was also performed to obtain digital images for volumetric reconstruction. Thus, left ventricular (LV) end-diastolic and end-systolic volumes, ejection fraction, and mass were calculated using commercial software (3D-Qlab, Philips Ultrasound, Andover, Massachusetts) as previously de-
scribed by Mor-Avi et al. (19). All measurements were made off-line by the same author (M.J.) blinded to the results of other investigations.

**Aortic stiffness assessment: applanation tonometry.** The cf-PWV was determined by electrocardiograph-gated carotid–femoral applanation tonometry (Millar Instruments, Houston, Texas) with available software (SphygmoCor, AtCor Medical, version 8.0, Sydney, Australia) by the same operator (N.T.) blinded to the echocardiographic data. To determine cf-PWV, pressure waveforms were recorded at 2 sites sequentially; carotid–femoral for aortic pulse wave velocity (20). The distance traveled by the pulse wave between the carotid artery and femoral artery was measured with calipers to reduce the influence of body contours. Wave transit time was calculated by the system software, using the R-wave of a simultaneously recorded electrocardiogram as a reference frame. The cf-PWV was calculated as the ratio of the distance traveled by the pulse wave and the foot-to-foot time delay between the pulse waves, expressed in m/s (21). Recordings were taken when a reproducible signal was obtained with high-amplitude excursion (usually 2 screens or 10 consecutive beats to cover a complete respiratory cycle are needed for subsequent analysis). The average of 10 successive measurements was used for analysis. This procedure was repeated 3 times per subject. Carotid tonometry was also used to noninvasively estimate central aortic pressure and central (carotid) augmentation index as previously described (20).

**Endothelial function assessment: FMD.** Brachial ultrasound studies were performed with subjects supine in a quiet, temperature-controlled (22°C to 24°C) environment after applanation tonometry. Systemic endothelial function was studied noninvasively by quantifying brachial artery responses to endothelium-dependent (post-ischemic reactive hyperemia) and independent (sublingual glyceryl nitrate [GTN]) stimuli using high-resolution brachial ultrasound (22). Briefly, the arterial diameter was determined by B-mode ultrasound using a high-frequency (7- to 12-MHz) linear-array transducer (Philips, Medical System, Andover, Massachusetts). The brachial artery was scanned above the antecubital fossa in the longitudinal plane. After a resting baseline scan was recorded, hyperemia was induced by deflating a BP cuff that had been placed above the antecubital fossa and inflated to a pressure of >200 mm Hg for 4.5 min. A second image was acquired 30 s before cuff deflation and continued for 2 min thereafter. Doppler-derived flow measurements were obtained during the first resting scan and again during the first 15 s of reactive hyperemia. A recovery scan was obtained 15 min after the hyperemic stimulus. Sublingual GTN (400 µg) was then administered, and a final scan for measurement of diameter and Doppler flow images was recorded over the subsequent 5 min. Images were recorded by a Lab View–based (National Instruments, Austin, Texas) data acquisition software platform for subsequent computer-assisted analysis using a custom program. The program operator remained blinded.
Blood sampling and enzyme immunoassay. Peripheral venous blood samples for MMP-2 and -9 and their tissue inhibitors (TIMP-1 and -2, respectively) were drawn at least 30 min after endothelial function assessment. Each sample was centrifuged for 10 min at 4°C. The serum was then separated into aliquots and stored at −80°C pending simultaneous analysis of collagen turnover markers as described below. All plasma MMP and TIMP levels were measured with 2-site sandwich enzyme-linked immunosorbent assays (GE Healthcare Bio-Sciences, Quebec, Canada). The MMP-2 assay (RPN 2617, sensitivity 0.37 ng/ml) detects the proform of MMP-2 and that complexed with TIMP-1; the MMP-9 assay (RPN 2614, sensitivity 0.6 ng/ml) detects the proform of MMP-9 and that complexed with TIMP-2; the TIMP-2 assay (RPN 2611, sensitivity 1.25 ng/ml) detects both free TIMP-2 and that complexed with MMPs. Triplicate measurements were performed according to the manufacturer’s instructions, and the intra-assay, interassay coefficients of variation were <10% for all assays. Additional blood samples were obtained for fasting cholesterol, triglycerides, high-density lipoprotein, low-density lipoprotein, glucose, creatinine, and electrolytes.

Cardiac magnetic resonance (CMR) imaging. All subjects with BAV underwent CMR imaging to assess aortic dimensions and to exclude the presence of aortic coarctation. To maintain blindness of observation, subjects with BAV underwent CMR examination within 1 month after the aforementioned vascular studies. Magnetic resonance scans were performed using a 1.5-T CV/I magnetic resonance imager (Signa, General Electric Medical Systems, Waukesha, Wisconsin) and a dedicated 4-channel phased-array cardiac coil. Aortic annulus was measured at 3-chamber long-axis and coronal double-oblique planes of the heart. Aortic root (measured at the level of sinuses of Valsalva) and sinotubular junction dimensions were measured at the 3-chamber long-axis and coronal double-oblique planes. Ascending aorta diameter was measured at the level of the bifurcation of the pulmonary artery at the axial plane. Finally, aortic arch dimensions and the descending aorta (at the level of the diaphragm) were assessed at the sagittal oblique plane. All image sequences used electrocardiogram gating and cine gradient echo with steady-state free precession (fast imaging employing steady-state), and measurements were taken at end-diastole. The CMR studies, blinded to echocardiography and other results, were analyzed.

Reproducibility. Echocardiographic as well as vascular procedures (applanation tonometry and reactive hyperemia) were repeated in 15 subjects (8 control subjects, 7 with BAV) 1 week later. The intraclass correlation coefficients between the first and second measurements on the 15 subjects for cf-PWV and central pulse pressure were 0.6 and 0.5, respectively, and 0.4 for FMD. The reproducibility of the measured variables was comparable to that in previous studies (23).

Statistical analysis. Data are expressed as median and interquartile range. Comparisons of demographic and clinical characteristics among the 3 groups were performed using a Kruskal-Wallis test. Comparisons between echocardiographic and CMR parameters and serum markers for subjects with BAV disease with and without dilated aortic roots were performed using the Mann-Whitney U test. Also, similar comparisons among healthy subjects with BAV disease without dilated aortic roots were performed using the Mann-Whitney U test. In the BAV cohort, univariate pairwise Pearson correlations were used to compare MMP-2 and -9, FMD (hyperemic response expressed as percentage change from baseline) versus cf-PWV and ascending aorta diameter, respectively. Statistics were performed with SPSS version 15.0 (SPSS Inc., Chicago, Illinois). The level of significance for pairwise comparisons was adjusted when multiple comparisons were performed (p = 0.05/2 = 0.025).

Results

Baseline demographic, hemodynamic, and echocardiographic parameters. There were no significant between-group differences in age, body surface area, renal function, blood electrolytes level, fasting lipid profile, peripheral (brachial) BP, LV size, mass, or function (Table 1). There was an equal proportion of subjects with anteroposterior (horizontal) aortic leaflet orientation in both groups with BAV (12 subjects in each group). Although subjects with significant aortic stenosis were excluded from this study, those with BAV had higher peak aortic transvalvular gradients and smaller aortic valve areas compared with healthy volunteers, reflecting the abnormal flow characteristics associated with BAV (Table 2). The LV wall thickness, cavity dimensions, volumes, and mass were no different among all groups (Table 2). The ratio of LV wall thickness to LV diastolic dimension was similar in BAV subjects with and without aortic dilation, although their ratios were higher than in control subjects, likely a result of their smaller aortic valve areas. By design, Group A had larger ascending aortic dimensions by both echocardiography and CMR (Tables 2 and 3, respectively). On CMR evaluation, Group A had significantly larger dimensions throughout the aortic tree consistent with more extensive aortic involvement (Table 3).

Vascular response to reactive hyperemia and nitroglycerin. All groups (including healthy volunteers) had similar baseline brachial artery diameters, but in Group A both percentage and absolute changes in FMD after reactive hyperemia were significantly blunted (Table 4). In contrast, there
was no difference in reactive hyperemia parameters between Group B and the healthy control group (Fig. 2). Nitroglycerin-induced FMD, representing endothelium-independent responsiveness, did not differ among all groups.

Arterial stiffness and central pulse pressure. Despite similar peripheral (brachial) BP in all groups, there was a trend toward higher central systolic and wide pulse pressure in those with dilated proximal aortas (Table 1). Similarly, cf-PWV was significantly higher in those with dilated aortas (Fig. 2), indicating decreased compliance of the descending thoracic and abdominal aortas (Table 4). This was confirmed by the finding that those with dilated proximal aortas had lower pulse pressure amplification and a higher augmentation index than the other 2 groups (Tables 1 and 4). Also, in the BAV cohort, cf-PWV correlated significantly with CMR-assessed ascending aortic dimensions ($r = 0.73$, $p < 0.001$). A post hoc multivariate analysis was performed to evaluate the relationship between ascending aortic dimensions and cf-PWV while controlling for potential con-
foundling effects of mean arterial pressure and age. The dependent variable cf-PWV was transformed to better meet the requirements for normally distributed data. The relationship between ascending aortic dimension and cf-PWV was again confirmed (p = 0.001) when there was adjustment for mean arterial pressure and age in this multivariate linear regression model.

**Plasma MMPs.** Plasma levels of MMP-2 were significantly higher in the dilated aorats group compared with the nondilated group and control subjects (Fig. 2). In the same group, there was a trend toward higher plasma levels of TIMP-2 that did not reach statistical significance after adjustment for multiple comparisons (Table 4) (p = 0.03). The MMP-9 plasma levels and those of its tissue inhibitor (TIMP-1) were similar between and within all groups (Table 4). There were statistically significant univariate correlations between MMP-2 and FMD (r = −0.571, p = 0.01) as well as MMP-2 and cf-PWV (r = 0.76, p < 0.001). Similarly, MMP-2 correlated significantly with ascending aorta diameter (r = 0.53, p = 0.002). In contrast, no significant correlation was identified between MMP-9 and any of the above variables. Finally, there was a significant difference in the MMP-2 to TIMP-1 ratio (p < 0.001), which is a surrogate of overall enzyme bioactivity, between Group A (median 16, interquartile range 12 to 18) and Group B or control subjects (median 10, interquartile range 9 to 11 for both Group B and control subjects).

### Table 4 Vascular and Humoral Parameters

<table>
<thead>
<tr>
<th>Variables</th>
<th>Group A (n = 16)</th>
<th>Group B (n = 16)</th>
<th>Control (n = 16)</th>
<th>p Value (Group A vs. Group B)</th>
<th>p Value (Group A vs. Control)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brachial artery diameter (mm)</td>
<td>4.0 (4.0–4.2)</td>
<td>4.0 (3.7–4.2)</td>
<td>3.9 (3.4–4.3)</td>
<td>0.10</td>
<td>0.53</td>
</tr>
<tr>
<td>Absolute change in FMD</td>
<td>0.2 (0.1–0.3)</td>
<td>0.3 (0.2–0.3)</td>
<td>0.3 (0.2–0.3)</td>
<td>0.02</td>
<td>0.02</td>
</tr>
<tr>
<td>Percentage change in FMD (%)</td>
<td>5.0 (4.6)</td>
<td>8.0 (7.8–7.8)</td>
<td>8.0 (7–11)</td>
<td>0.001</td>
<td>0.001</td>
</tr>
<tr>
<td>Percentage change in NTG (%)</td>
<td>10 (9–11)</td>
<td>10 (8–11)</td>
<td>11 (8–12)</td>
<td>0.54</td>
<td>0.43</td>
</tr>
<tr>
<td>cf-PWV (m/s)</td>
<td>9.3 (9–10)</td>
<td>7 (6.9–7.4)</td>
<td>7 (6.9–7.2)</td>
<td>0.001</td>
<td>0.001</td>
</tr>
<tr>
<td>Central augmentation index (%)</td>
<td>25 (17–31)</td>
<td>14 (11–20)</td>
<td>13 (11–18)</td>
<td>0.01</td>
<td>0.01</td>
</tr>
<tr>
<td>MMP-2 (ng/ml)</td>
<td>1,523 (1,460–1,674)</td>
<td>1,036 (962–1,167)</td>
<td>1,022 (985–1,124)</td>
<td>0.001</td>
<td>0.001</td>
</tr>
<tr>
<td>MMP-9 (ng/ml)</td>
<td>39 (32–46)</td>
<td>40 (32–68)</td>
<td>39 (22–64)</td>
<td>0.20</td>
<td>0.46</td>
</tr>
<tr>
<td>TIMP-1 (ng/ml)</td>
<td>104 (86–102)</td>
<td>99 (86–110)</td>
<td>102 (93–107)</td>
<td>0.50</td>
<td>0.61</td>
</tr>
<tr>
<td>TIMP-2 (ng/ml)</td>
<td>94 (84–122)</td>
<td>79 (73–95)</td>
<td>82 (73–100)</td>
<td>0.03</td>
<td>0.90</td>
</tr>
</tbody>
</table>

Group A indicates men with bicuspid aortic valve and dilated proximal ascending aorta; Group B those without proximal aortic dilation. Values are expressed as median (interquartile range).

cf-PWV = carotid femoral pulse wave velocity; FMD = flow-mediated vasodilation; MMP = matrix metalloproteinase; NTG = nitroglycerin; PWV = pulse wave velocity; TIMP = tissue inhibitors of matrix metalloproteinase.

**Discussion**

In this study of young men with BAV in which the comparison groups had similar baseline characteristics, we demonstrated that those with dilated proximal ascending aorta manifested increased cf-PWV, systemic endothelial dysfunction, and higher plasma MMP-2 levels compared with BAV subjects without dilated ascending aorta or control subjects. These observations provide novel and important evidence that dilated ascending aorta in subjects with BAV is accompanied by generalized vascular (arterial) and humoral abnormalities.

Pre-clinical studies have suggested a role of endothelial factors in the genesis of aortic dilation in BAV. In a transgenic model, eNOS-deficient mice were pre-disposed to development of BA and aortic dilation (12). Similarly, aortic endothelial cells from patients with BAV undergoing aortic valve or root replacement had significantly lower levels of eNOS protein expression than those patients with trileaflet aortic valves (13). In this pathological study, an inverse correlation between the level of eNOS expression and maximum aortic diameter was noted only in BAV patients (13). In the present study, we observed a significant blunting of endothelium-dependent vasodilation to reactive hyperemia only in the group of BAV with dilated proximal aortas, whereas those with preserved aortic size had evoked nitric oxide (NO)–dependent responses similar to those of healthy volunteers. Our study extended the results of the previously mentioned animal and pathology studies by providing further evidence of impaired NO bioactivity in BAV subjects with dilated proximal aorta manifested as blunted endothelial–dependent vasodilation of conduit arteries.

Genetic down-regulation of eNOS expression, and thus of NO bioactivity, increases MMP-2 expression. Indeed, NO is known to attenuate basal secretion of pro–MMP-2 in endothelial cells (24), and inhibition of coronary NO synthase has led to increased MMP-2 activities (25). Recent studies have postulated a pivotal role of MMPs, especially...
MMP-2 and -9, in the pathogenesis of aortic aneurysms (26,27); MMP-2 is activated by membrane-bound type-1 MMP and inhibited by TIMP-1, has substrate specificity for elastin and fibrillar collagen (28), and is found in the normal and aneurysmal aorta in association with membrane-bound type-1 MMP and TIMP (26). A recent study has reported increased (>2-fold) MMP-2 activity in excised aortas of patients with BAV compared with those with 3 leaflets, whereas MMP-9 levels remain unchanged between both groups (8). Similarly, in our study we found significantly higher plasma levels of MMP-2 only in subjects with BAV and dilated proximal aorta. In contrast, MMP-9, TIMP-1, and TIMP-2 were no different among all subjects with BAV and control subjects. It is possible that systemic overexpression of MMP-2 is responsible for the intrinsic abnormalities such as matrix disruption and smooth muscle cell loss within the aortic media commonly seen in patients with BAV and dilated aortas (29). The MMP-2 systemic overexpression through negative remodeling of the aortic media may also account for the extensive aortic involvement observed in BAV and progressive aortic dilation despite aortic valve replacement (30). Higher concentrations of MMP-2, perhaps not counteracted by a proportional increase in TIMP, have the potential to negatively affect the composition and geometry of the vessel wall leading to decreased vascular compliance and increased aortic wall fragility. Indeed, an imbalance between the proteolytic activity of MMPs and their endogenous inhibition has been recently implicated as a possible cause of aortic aneurysm formation in patients with BAV. This imbalance seems to be secondary to an increased expression of aortic wall MMP-2 genes (31), which leads to increased MMP-2 release and MMP-2/TIMP-1 ratio (32).

Structurally, the aortic conduit is heterogeneous with respect to the relative composition of elastic–collagen fiber ratio through its length. Alteration of this equilibrium will lead to increased wall stiffness, of which the most representative model is represented by aging. Similarly, functional conduit vessel abnormalities mediated perhaps through NO dysregulation could also contribute to increased aortic stiffness. A recent study has reported that aortic pulse wave velocity was inversely correlated with FMD in the brachial artery, demonstrating the relationship between endothelial function and large artery stiffness in vivo (33). Abnormal aortic root and ascending aortic stiffness in patients with BAV have been previously reported using echocardiography and CMR (6,34). A reduction in ascending aortic wall elasticity in patients with BAV has been associated with severity of aortic regurgitation and degree of LV hypertrophy (6). In agreement with the previously mentioned studies, we also found increased aortic stiffness in BAV patients, albeit confined to those with proximal aortic dilation in the absence of significant aortic valve dysfunction or LV hypertrophy. Importantly, using a study design that minimizes the potential confounding effects of age, BP, LV dimensions, and sex, the present study extended the results of prior studies by demonstrating that increased aortic

**Figure 2** Individual Data Points of the Study Population

Individual data points representing (A) matrix metalloproteinase (MMP)-2 (ng/ml) plasma concentrations; (B) pulse wave velocities (m/s); and (C) flow-mediated vasodilation (percent change) in subjects with dilated (Group A) and nondilated proximal aortas (Group B) and control subjects. Red lines indicate mean value.
stiffness in subjects with BAV and dilated aorta was accompanied by endothelial dysfunction and increased plasma concentration of MMP-2. This novel finding in BAV subjects that implies a mutual interdependence between aortic stiffness dimension, endothelial dysfunction, and plasma MMP-2 levels, along with a recent study reporting that descending thoracic aortic distensibility is the strongest predictor of progressive descending thoracic aortic dilation in patients with Marfan syndrome (35), underscores the need for early identification and surveillance of aortic wall compromise. Finally, in contrast to the trend toward widening central pulse pressure with higher pulse wave velocity, we did not observe a concomitant widening of peripheral (brachial) pulse pressure in these same subjects with increased pulse wave velocity. Our findings differ from those of recent studies examining a large population sample of normotensive and hypertensive patients (36,37) in which pulse pressure related inversely to the aortic root diameter. There are several explanations for this apparent disparity. First, we examined a distinct patient population with structurally and functionally abnormal aortic wall properties in contrast to patients with hypertension. Giving support to this concept is the fact that when patients with structurally altered aortic wall (Marfan patients with dilated ascending aortas) were recently studied, central but not brachial pulse pressure correlated well with aortic stiffness (38). Second, it is conceivable that the higher central arterial stiffness found in subjects with BAV and dilated aortas may have resulted in loss (or at least attenuation) of the stiffness gradient between central and peripheral arteries. This could have yielded a lower pulse pressure amplification and likely an increased central pulse pressure augmentation, which was observed in this study (Tables 1 and 4) (39). Because of pulse pressure amplification between central and peripheral arteries, it may be misleading to rely on brachial pulse pressure as a surrogate for aortic or carotid pulse pressure, particularly in young subjects (39).

Study limitations. We included only male patients to circumvent the confounding effect of estrogen on the vascular endothelium. Similarly, patients with significant aortic stenosis were also excluded. Whether or not women and patients with stenotic BAV manifest similar findings requires further study. Also, by design, we excluded BAV subjects with mild proximal aortic dilation (diameters between 35 and 40 mm).

The use of RT3DE rather than CMR in the determination of LV volume indexes may appear to be a limitation. However, LV volume indexes determined by RT3DE are more accurate and reproducible than conventional 2-dimensional echocardiographic methods and compare well with CMR-obtained measurements in early validation studies (19). Furthermore, in a recent study in which comparable scanning protocols were used for both RT3DE and CMR, RT3DE measurements were in close agreement with CMR reference with an underestimation of end-diastolic and -systolic volumes by only 5 and 6 ml when datasets were analyzed by the same investigators (40). In addition, the use of RT3DE also would eliminate the confounding effect of time because echocardiographic, FMD, cf-PWV, and venous blood data are obtained at the same visit without movement of the patient. Because RT3DE and CMR were performed at different times, some degree of variation between measurements was inevitable at the time of either image acquisition or subsequent measurement.

We used applanation tonometry to estimate aortic stiffness in these subjects and did not directly evaluate the ascending aorta. Although assessment of the ascending aorta by CMR might have provided additional information, a recent expert consensus recognized cf-PWV as the simplest, most robust, and most reproducible noninvasive method of determining arterial stiffness in population studies (39). Future studies will be needed to evaluate local changes in aortic stiffness, perhaps using methods capable of segmental aortic stiffness interrogation such as those offered by CMR.

We did not extend our blood sampling to other plasma vascular markers, such as endothelin, which could also have affected MMP bioactivity. Finally, we assumed that plasma concentration of MMPs and their tissue inhibitors parallels that found on vascular tissue. Although we cannot exclude a discrepancy between plasma and local tissue concentrations, we recognize the overwhelming clinical utility and practicability of assessing plasma rather than tissue MMP levels.

Clinical implications and future studies. Our findings provide evidence of a widespread functional and structural aortic involvement in BAV patients with proximal aortic dilation, supporting the need for detailed and perhaps more extensive vascular surveillance in those patients. The identification of multifactorial interplay in the genesis of aortic wall abnormalities in BAV suggests the possible involvement of a genetic component to environmental factors. Perhaps future studies featuring genetic screening in those patients might shed more light in the pathogenesis of aortic dilation. Also, future studies must include women as well as patients with BAV and a wider range of proximal aortic dimensions. Lastly, assessment of endothelial function and aortic pulse wave velocity has been shown to predict future development of atherosclerotic cardiovascular disease in a variety of patient populations with established cardiovascular risk factors (41,42). Whether our findings could also be translated to those without established cardiovascular disease will require further prospective studies.

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