Loss of Elastic Fiber Integrity and Reduction of Vascular Smooth Muscle Contraction Resulting From the Upregulated Activities of Matrix Metalloproteinase-2 and -9 in the Thoracic Aortic Aneurysm in Marfan Syndrome

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Abstract—Thoracic aortic aneurysm (TAA) is the life-threatening complication of Marfan syndrome (MFS), a connective tissue disorder caused by mutations in the fibrillin-1 gene. TAA is characterized by degradation of elastic fiber, suggesting the involvement of matrix metalloproteinase (MMP)-2 and -9, the activation of which is regulated by TIMP (tissue inhibitor of MMP) types 1 and 2. We hypothesized that MMP-2 and -9 were upregulated during TAA formation in Marfan syndrome, causing loss of elastic fibers and structural integrity. We studied mice, from 3 to 12 months, heterozygous for a mutant Fbn1 allele encoding a cysteine substitution in fibrillin-1 (Fbn1<sup>C1039G/+</sup>), designated as "Marfan" mice) (n = 120), the most common class of mutation in Marfan syndrome. The littermates, Fbn1<sup>+/+</sup> served as controls (n = 120). In Marfan aneurysmal thoracic aorta, mRNA and protein expression of MMP-2 and -9 were detected at 3 months and peaked at 6 months of age, accompanied by severe elastic fiber fragmentation and degradation. From 3 to 9 months, the MMP-2/TIMP-2 ratio increased by 43% to 63% compared with the controls. Dilated thoracic aorta demonstrated increased elasticity but distention caused a pronounced loss of contraction, suggesting weakening of the aortic wall. Breaking stress of the aneurysmal aorta was 70% of the controls. Contraction in response to depolarization and receptor stimulation decreased in the aneurysmal thoracic aorta by 50% to 80%, but the expression of α-smooth muscle actin between the 2 strains was not significantly different. This report demonstrates the upregulation of MMP-2 and -9 during TAA formation in Marfan syndrome. The resulting elastic fiber degeneration with deterioration of the aortic contraction and mechanical properties may explain the pathogenesis of TAA. (Circ Res. 2007;101:000-000.)

Key Words: thoracic aortic aneurysm ● Marfan syndrome ● elastic fiber ● matrix metalloproteinase ● vascular smooth muscle contraction

Marfan syndrome (MFS) is an autosomal dominant disorder of the connective tissue caused by mutations of the gene (FBN1) encoding fibrillin-1.<sup>1,2</sup> Fibrillin-1 is the structural glycoprotein for microfibrils, which act as scaffolding proteins for elastin deposition and formation of elastic fibers in the large artery. Fibrillin-1 has been recently suggested as a negative regulator of the cytokine transforming growth factor (TGF)-β, which controls a variety of cellular processes including proliferation, differentiation, and apoptosis.<sup>1</sup> We have recently shown that in MFS, excessive TGF-β activation and augmented downstream signaling contribute to the multisystem pathogenesis and that antagonism of TGF-β with neutralizing antibody or the angiotensin II type 1 receptor blocker losartan rescues alveolar septation, normalizes muscle function, and prevents thoracic aortic aneurysm (TAA).<sup>3–8</sup>

TAA, dilatation of the supradiaphragmatic aorta, is the most life-threatening cardiovascular complication in MFS, leading to aortic rupture, dissection, and sudden death.<sup>1,9</sup> Development, expansion, and rupture of TAA involve a multifactorial process that is influenced by both cellular and extracellular mechanisms.<sup>9</sup> Degradation of elastic media in the extracellular matrix (ECM) is the consistent histopathological and biochemical feature.<sup>9,10</sup> MMP-2 and -9, known for their roles in degrading elastic fibers, have been suggested to be involved in the pathological vascular remodeling.<sup>10</sup> The balance between MMP-2/-9 and the tissue inhibitor of MMP-1 (TIMP-1)/-2 tightly governs matrix remodeling and is believed to play a central role in the pathogenesis of abdominal aortic aneurysm.<sup>11–15</sup> Activation of TGF-β and the concomitant upregulation of various MMPs and TIMPs have...
been suggested to be associated with the increased apoptosis, impaired progenitor cell recruitment, and abnormal directional migration, which are likely to contribute to aneurysm development in MFS. However, few studies have investigated the regulation of MMP-2 and -9 in TAA in MFS, and contradictory observations regarding to the expressions of MMPs as well as TIMPs have been reported.

Elastic fibers are responsible for vessel elasticity, the passive force of mechanical property. Their fragmentation has been widely documented in the aorta of patients with and the mouse model of MFS. In the clinical assessment, reduced aortic distensibility and elasticity, as well as elevated pulse–wave velocity, are predictors of dilatation and dissection. However, the underlying causes related to the

Figure 1. A, RT-PCR result showing the mRNA expression of MMP-2 and -9 in the control arch, control descending, Marfan aneurysmal, and Marfan nonaneurysmal TA. B, Western immunoblots showing the immunoreactivity of MMP-2 and -9. Shown are representative results from 15 mice from the same group same age. Expression of β-actin served as an internal standard. The bar graphs show the ratio of protein expressions of MMP-2/TIMP-2 (C) and MMP-9/TIMP-1 (D). *P<0.05 versus control arch; #P<0.05 versus Marfan nonaneurysmal TA.
loss of integrity of elastic fibers and abnormalities of aortic mechanical properties during the development of TAA have not been elucidated.

Because of their potent elastolytic activity, the mechanism of actions of MMPs in aneurysm formation has largely been attributed to their proteolytic properties in the ECM and the subsequent weakening of the vascular wall. However, a recent study has suggested that MMP-2 and -9 could modulate aortic contraction, which may regulate the overall tensile strength and the structural integrity of the aortic wall. The contractile function of smooth muscle cells (SMCs) contributes to the active force of blood vessels. However, their role in biomechanical property of the aorta has not yet been studied during aneurysm formation in MFS.

In the present study, we compared the activation of MMP-2 and -9 in the aneurysmal parts of the thoracic aorta from a mouse model of MFS6–8,19,26 with those from the nonaneurysmal parts or wild-type littermates. We have concluded that MMP-2 and -9 are upregulated in TAA, a process that is associated with the pronounced degeneration of elastic fibers and the alterations in aortic elasticity. The contractile function of SMCs was markedly impaired, implicating the deterioration of the mechanical properties of the aortic wall during the formation of TAA in MFS.

Materials and Methods

Experimental Animals and Tissue Preparation

Heterozygous (Fbn1<sup>C1039G</sup>+) mice were bred with wild-type females to generate equal numbers of control (Fbn1<sup>+/+</sup>) and Marfan mice,6–8,19,26 which were housed in the institutional animal facility. Mice at ages 3 (n = 60), 6 (n = 70), 9 (n = 70), and 12 (n = 40) months were euthanized. Dilatation of thoracic aorta (TA) was observed at 3 months of age and followed by a severe aneurysm formation in the ascending TA and the aortic arch. A 2% sudden death between 6 and 7 months was recorded. From each Marfan mouse, both aneurysmal and nonaneurysmal TAs were dissected. From the controls, TA segments were dissected from the descending part and the corresponding aneurysmal part in Marfan mice (ie, ascending TA and aortic arch) (Figure I in the online data supplement, available at http://circres.ahajournals.org).

Reverse Transcription–Polymerase Chain Reaction

Total RNA was extracted using TRizol reagent (Invitrogen Life Technologies, Carlsbad, Calif). RNA (1 μg) was subjected to reverse transcription (Gene Amp PCR System 9700, Applied Biosystems) and PCR. Gene-specific primers for MMP-2, MMP-9, and β-actin have been reported.27

Western Immunoblotting

Protein samples (40 μg) were electrophoresed in 8% SDS-PAGE, followed by immunoblotting with specific antibodies (Calbiochem, San Diego, Calif) against MMP-2, MMP-9, TIMP-1, and TIMP-2.27

Gelatinolytic Zymography

Enzyme contents of MMP-2 and -9 in the aortic homogenate (3 μg) were analyzed by gelatinolytic zymography as described previously.27

Mechanical Properties of the Aorta

"Vessel elasticity" was deduced from the stress–strain curves. In a small vessel myograph (AS Danish Myotechnology, Aarhus, Denmark), a 2-mm aortic segment was stretched by increasing the distance between the 2 stainless wires (=increase in length of vascular SMC) and held at each length for 3 minutes. Initially, 2 wires were adjusted to L<sub>o</sub>, at which the vessel was not stretched. The inside circumference of the aortic segment was measured as twice the distance between 2 wires, plus the wire circumference, plus 2 wire radii (2 × 20 μm). The distance between the 2 wires was then increased by 100 μm, and the new length was denoted as "L." The developed force (mN) was divided by the surface area (=inside circumference of the segment × length of the segment) of the aorta segment (mm<sup>2</sup>) to calculate the wall stress (mN/mm<sup>2</sup>). The procedure was repeated until the vessel was unable to maintain its tone. The stress at which rupture occurred was reported as “breaking stress.” The ΔL/L<sub>o</sub> and the wall stress were fitted on an exponential curve. “Passive force” was measured by repeating the above procedures in a calcium-free Krebs solution prepared by replacing CaCl<sub>2</sub> with 320 μmol/L EGTA to eliminate SMC contractility. “Total force” was determined by assessing the active contractility at each level of stretch in response to depolarization (80 mmol/L potassium chloride, KCl). To study the “reversibility of vessel contractility” after stretching, TA was stimulated with 80 mmol/L KCl and 3 μmol/L phenylephrine at the optimal tension (ΔL/L<sub>o</sub> is approximately equal to 2.0, a value that gives the maximal force generation in response to KCl and phenylephrine),28 then stretched to ΔL/L<sub>o</sub> = 2.0, 3.0 and 4.0 for 3 minutes, and restored to optimal tension. Contraction was induced, and the percentage of developed force change was calculated.

Isometric Force Measurement

Two-millimeter aortic segments were mounted isometrically in a small vessel myograph. Phenylephrine, endothelin-1, and serotonin (10<sup>-10</sup> to 10<sup>-7</sup> mol/L) were added cumulatively, generating concentration–response curves. The negative logarithm (pD<sub>2</sub>) of the concentration of agonist giving half-maximum response (EC<sub>50</sub>) was assessed (pD<sub>2</sub> = −log[EC<sub>50</sub>]).26

Movat’s Staining

Four-millimeter aortic segments were formalin fixed and embedded in paraffin. Because of the limited samples from each mouse, perfusion fixation was not performed. Three-micrometer cross-sections were prepared and stained with modified Movat’s pentachrome. Image acquisition and processing were performed using a Nikon Microphot microscope (Nikon Inc). Images were captured by a SPOT digital camera (Diagnostic Instrument Inc) and analyzed with ImageProPlus5 software as described.27
Immunohistochemical Analysis

Seven-micrometer cross-sections were immunostained with primary mouse monoclonal antibody against α-smooth muscle (α-SM) actin (clone 1A4; dilution 1:400) and secondary horse anti-mouse antibody (Vector Laboratory, Burlingame, Calif; dilution 1:400). Immunoreactive staining was detected by an ABC kit (Vector Laboratory). Nuclei were counterstained with Mayer's hematoxylin. The area (percentage of total aortic media) of α-SM actin–positive immunostaining (brown) was quantified with ImageProPlus5 software. Normal mouse IgG was used as the negative control.

Materials

All other reagents were of the highest molecular grade and were purchased from Sigma (Oakville, Canada).

Statistics

Data were reported as mean±SEM. Statistical analysis and stress–strain exponential curves were prepared using GraphPad Prism software (San Diego, Calif). Two-way Student’s t test and 1-way ANOVA were used for comparisons between multiple groups. Statistical significance was defined as probability value of <0.05.

Results

Upregulation of MMP-2 and MMP-9 in TAA

Compared with the control arch, control descending and Marfan nonaneurysmal TA, pronounced mRNA expressions of MMP-2 and MMP-9 in the aneurysmal TA were observed in the early stage of disease progression (3 to 6 months). Besides, there was a trend of increase in MMP-9 mRNA in the aneurysmal TA from 3 to 9 months, which accompanied by an upregulated protein expression of the active form (Figure 1A and 1B). In the aneurysmal TA, expression of MMP-2 mRNA gradually decreased with age, but active...
protein increased from 3 months on (Figure 1B). In the nonaneurysmal TA, expressions of mRNA (3 to 6 months) and protein (6 to 9 months) of both MMPs were less compared with the aneurysmal part but remained elevated compared with the control descending TA.

TIMP-1 has preferential inhibitory capability against MMP-9, whereas TIMP-2 at high concentrations, selectively inhibits MMP-2 activation. We observed a significant increase in the MMP-2/TIMP-2 ratio in the Marfan aneurysmal TA at 3, 6, and 9 months by 43%, 63%, and 49%, respectively, compared with the control ascending TA. The ratio of MMP-9/TIMP-1 at 9 and 12 months was reduced by 22% and 30%, respectively. Compared with the nondilated TA, aneurysmal part had augmented ratios of MMP-2/TIMP-2 and MMP-9/TIMP-1 from 6 to 12 months (Figure 1C and 1D).

Similar to the mRNA expression, the Marfan aneurysmal TA had elevated MMP-2 and -9 enzyme content in the early stage (3 to 6 months) compared with the age-matched control ascending arch, control descending TA, and the nondilated Marfan TA (Figure 2).

**Loss of Elastic Fiber Integrity in Aneurysm**

MMP-2 and -9 proteolytically degrade elastic fibers and cause extensive vascular remodeling in TAA. On
Elastic fiber provides the elasticity of blood vessels, and its fragmentation and disarray of elastic fiber dominated in the Marfan aneurysmal TA (Figure 3C), and at 6 months, the elastic fiber-covered area was 69% of that in the controls. The degeneration of elastic media became more pronounced with age. At 9 months, the elastic fiber-covered areas in the aneurysmal TA were decreased by 40% compared with the control aortic segments (Figure 3E). Occasionally, disorganization or discontinuous segments of elastic fibers were visible along the control aorta after 9 months (Figure 3A and 3B), but elastin content remained constant (≈50% of the total medial area) during aging.

**MFS Differentially Regulates Elasticity in Aneurysmal and Nonaneurysmal Aorta**

Elastic fiber provides the elasticity of blood vessels, and its degeneration, particularly at 6 and 9 months (Figure 3E), would alter the aortic elasticity in MFS. From 6 months onward, the slope of stress-strain curve from Marfan aneurysmal TA decreased compared with that of the control arch, indicating increased elasticity. In the nonaneurysmal TA, stress–strain curves were left-shifted, and the elasticity markedly decreased compared with the aneurysmal TA and the control descending TA (Figure 4A and 4B). The elasticity measurements at 3 months were not significantly different between the 2 strains (data not shown).

The increase in elasticity in Marfan aneurysmal, TA might not be beneficial; instead, it might suggest weakening of vessel wall. We, therefore, investigated the impact of stretching on the contractility of TA from control arch and Marfan aneurysmal part. In both groups, at 9 months, distending at ΔL/L₀=2.0 for 3 minutes increased the contractility. However, at ΔL/L₀=3.0, Marfan aneurysmal TA lost 13.0% and 24.8% contraction stimulated by KCl and phenylephrine, respectively, whereas in the controls, it potentiated contractility by 36.5% and 14.3%. At ΔL/L₀=4.0, contractility in Marfan TA was markedly suppressed by 24.0% and 51.5% (Figure 4C). Nevertheless, the increased elasticity in the aneurysmal part did not offer protection against breakage. The breaking stress of Marfan aneurysmal TA at 6 months was 31% less than that of the control aorta (control arch=41.3±3.2; control descending=47.2±6.7; Marfan=28.5±1.7 mN/mm²) and 54% less than that of the nonaneurysmal part (Figure 5).

**Reduced Contractility of SMCs in the Aneurysmal Aorta**

Contraction of vascular SM has been suggested to regulate the integrity of tensile strength of aortic wall and likely contributes to the formation of aneurysm. From 3 months onward, vasoconstriction in response to KCl-induced depolarization remarkably reduced in Marfan aneurysmal TA by 61% to 84% when compared with age-matched controls (Figure 6). At 6 and 9 months, the contractility of dilated TA was only 27% to 36% of that in the nonaneurysmal TA. Vasoconstriction in response to receptor-mediated stimulation was also studied. From age 9 months, the pEC5₀ values of phenylephrine in Marfan aneurysmal TA (6.76±0.07) was significantly less than those from controls (7.26±0.07). The maximal force (E₉₀) induced by 3 μmol/L phenylephrine in the Marfan aneurysmal TA from 3 months onward was ≈50% of the controls (Figure 6B). We also stimulated vasoconstriction with serotonin and endothelin-1, and Marfan aneurysmal TA developed less force regardless of the means of stimulation compared with the nonaneurysmal TA and controls.

To further examine the contractile function of SMCs in the aneurysmal TA, we measured the active force in response to depolarization. Control TA between 3 and 9 months generated active force over a range of strain, ΔL/L₀ from 0.0 to 3.2 (Figure 6C through 6E). This range was gradually reduced in the Marfan aneurysmal TA and shifted to 2.6 at 9 months. Furthermore, the active force generated in the Marfan aneurysmal TA was markedly reduced at all age groups.

**Preserved α-SM Actin in the Aneurysmal Aorta**

Decreased expression of α-SM actin has been suggested to be associated with the impairment of vessel contraction. To elucidate the possible mechanism of reduced contractility in Marfan aorta, we performed immunohistochemistry to label SMC α-actin in the aortic media. Almost all SMCs from both control and Marfan aorta stained positively with an antibody against α-SM actin (Figure 7). For all age groups, the aortic segments from the 2 strains did not demonstrate a significant

![Figure 5. Measurement of breaking stress. At 6 months, breaking stress of Marfan aneurysmal TA (black) was 31% and 40% less compared with the control arch (white bars) and control descending TA (checker bars) and 54% less compared with the Marfan nonaneurysmal TA (striped bars) (n=10 to 15). *P<0.05 versus control; #P<0.05 versus nonaneurysmal TA.](Image)
difference in α-SM actin–stained area, which compromised of ~90% of the total medial field in each groups.

**Discussion**

Using a well-defined genetic mouse model of MFS, we elucidated the relationship between MMP regulation and TAA development during the continuum of disease progression. We have shown a pronounced aneurysm formation in the ascending and the arch of thoracic aorta, which was accompanied by (1) upregulation of MMP-2 and -9 in mRNA and protein expression, (2) differential regulation of ratios of TIMP-1/MMP-9 and MMP-2/TIMP-2, (3) loss of integrity of elastic fibers consistent with weakening of vessel wall and reduction of breaking stress, and (4) a remarkable reduction

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**Figure 6.** Isometric force measurement. Maximal force generated in the dilated and nondilated TA from 3 to 9 months in response to 80 mmol/L KCl (A) and 3 μmol/L phenylephrine (B) (n=10 to 12). \*P<0.05 versus control; \#P<0.05 versus nonaneurysmal TA. Measurement of active force in Marfan aneurysmal TA. Active force, the difference between total and passive force, was compared between Marfan aneurysmal (triangle) and control (square) TA at 3 (C), 6 (D), and 9 (E) months of age (n=3 to 7). \*P<0.05 versus control; \#P<0.05 versus nonaneurysmal TA.
in aortic SM contraction. These findings present plausible molecular mechanisms for the development and expansion of TAA in MFS, which could contribute to the deterioration of mechanical properties and structural integrity of the aortic wall (Figure 8).

In the mouse model of MFS, we detected upregulation of MMP-2 and -9 in mRNA and protein expression in the aneurysmal TA during disease progression. Consistent with a previous study, increased TIMP-1 and -2 protein levels were observed during aneurysm formation in MFS. The novelty of the present study is the demonstration of the differential regulation of TIMP-1 and -2 in MFS. In the dilated TA, the greater ratio of MMP-2/TIMP-2 would favor elastinolysis especially in the early stage, whereas a slight reduction of...
MMP-9/TIMP-1 ratio might be a compensatory mechanism to protect from extensive vascular remodeling at the end-stage lesion (Figure 1C and 1D). We also showed that the upregulation of MMP generally correlated with the extent of loss of elastic content and elastic fiber architecture during TAA formation (Figures 1 through 3). In patients with dilatation or dissection of the ascending TA associated with MFS, fragmentation of elastic fibers, fibrosis with collagen production and apoptosis of SMCs are commonly observed.3–5 Mouse models of fibrillin-1 deficiency suggest that aneurysm does not result from failure of elastogenesis,20,31 Therefore, the disintegration of elastic lamellae could be owing to the increase in the elastinolytic activity of MMP-2 and -9.

The underlying mechanisms of excessive MMP activation in MFS during TAA formation could be multifactorial and involve interplay between genetic predisposition, inflammatory responses, as well as hemodynamic factors (Figure 8).9 The fibrillin fragments in the aortic tissue in MFS could upregulate MMPs32 suggesting the possibility of a vicious cycle whereby the constant presence of fibrillin-1 fragments could lead to increased MMP production, which in turn could generate more fibrillin-1 fragments. The pronounced disintegration of elastic fibers in the aneurysmal aorta (Figure 3C) may stimulate macrophage chemotaxis and contribute to the inflammatory infiltrates,33 which could further enhance the local activities of proteolytic enzymes. Aneurysm development and marked degeneration of elastic fibers, particularly observed in the ascending TA and the aortic arch (supplemental Figure IB and IC), implicated that mechanical stress could activate MMPs.6,19,34 The loss of connecting filaments between SMCs and elastic fibers17 would result in changing the SMC phenotype and activating MMPs. Increased expression of osteopontin in the media of dilated TA indicated the transition of SMCs from the contractile to the synthetic phenotype also preceding increased secretion of MMPs and elastinolysis in MFS.17,35 Furthermore, our recent investigation confirmed the augmented TGF-β activation and signaling in MFS contribute to disease pathogenesis.3–11 Excessive TGF-β expression is in association with MMP upregulation,4,5,36–38 and MMP-2 and -9 have been identified as latent TGF-β activators.39 Therefore, the increased proteinase-mediated TGF-β release could further reinforce the proposed vicious cycle in the development of aneurysm (Figure 8).

Increased MMP-2 and -9 have been demonstrated in the aortic media of patients and a mouse model of MFS.4,5,16,17 In contrast, reduction of MMP-2 in human thoracic aortic samples has also been reported.16 The controversial result could be explained by the following reasons. (1) Relatively small groups of MFS and non-MFS patient samples (usually less than 10) were recruited in those studies. (2) Most of the studies highly focused on the most severe end-stage lesions and paid less attention to the early stage. (3) A wide range of ages of MFS patients (between 10 and 80 years of age). As indicated by the data from the present study, the pathogenesis of TAA in MFS with respect to the molecular and functional properties of the aorta varied during aging; combining both pediatric and adult patients in the same study would disturb the results and data interpretation.

The consequences of the degeneration of elastic media on vessel elasticity were different between the dilated and the nondilated TA. The abnormality in vessel mechanical properties in MFS was demonstrated by the dramatic reduction of elasticity in the nondilated part and an increase in elasticity in the dilated TA as compared with the controls (Figure 4). The reduced elasticity in MFS nonaneurysmal TA could be explained by the disorganization of elastic fibers (Figure 3). Elastin is the major component that confers elastic and viscoelastic properties on the aorta over most of the physiological pressure range.18,40 In MFS, fibrillin-1 abnormalities alter the structural integrity of elastin and thus the formation of elastic fibers, which provides vessel elasticity.1,2,18 The broken elastin array is highly prone to calcification,17 further reducing arterial elasticity. In the clinical assessment of patients with MFS, reduced aortic distensibility and elasticity are predictors of dilatation and dissection.23,24

Severe fragmentation and degeneration of elastic fibers dominated in the aneurysmal TA, as compared with the localized minimal elastic disruption in the nondilated TA (Figure 3C and 3D). A lower breaking stress in the dilated TA at 6 months (Figure 5) implicating the ease of dissection and rupture was likely attributed to the elevated MMP-2 and -9 expression as well as the high MMP-2/TIMP-2 ratio (Figures 1 and 2), which has been reported in the acute phase of abdominal aortic dissection.12 The increase in elasticity in the dilated TA might result in the weakening of the vessel wall. Stretching the aorta far above the optimal length (L0) caused a pronounced loss of contractility (Figure 4C), indicating the permanent damage in the ECM, SMCs, as well as their interrelationship in the aortic media. The increased elasticity might implicate that a slight increase in pressure (analog to stress) causes a pronounced increase in diameter (analog to strain), thereby causing aneurysm formation.

TAA is characterized by destruction of ECM components which provide structural support of the aorta. However, it has been suggested that the integrity of aortic SM contractile function may also contribute to the structural integrity and the tensile strength of the aortic wall.25 The present study is the first to elucidate the contraction of SMC in MFS and show that aortic contraction (Emax values) and sensitivity to agonists (EC50 values) (Figure 6A and 6B) were highly suppressed in the aneurysmal TA. The decrease in the ΔL/L0 range in which active force is generated in the dilated TA also suggested that at high distention, the association between SMCs and ECM might be disrupted (Figure 6C through 6E). The active and tonic contraction of SMCs is believed to limit the tendency of the aorta to dilate in response to pulsatile force generated with each cardiac cycle.25 Therefore, the inhibition of vascular contraction in MFS may contribute to the progressive aortic dilatation and aneurysm formation.

The reduced contraction in the aneurysmal aorta in MFS unlikely resulted from the paucity of α-SM actin expression (Figure 7).30 Other mechanisms should contribute to the remarkable reduction in contractility in the MFS aneurysmal aorta compared with the nonaneurysmal and control aorta. For instance, the upregulation of MMP-2 and -9 in the dilated TA (Figures 1 and 2) could suppress phenylephrine- and KCI-induced aortic contraction through inhibiting calcium
In conclusion, our results indicate that the progressive vascular wall remodeling, elastic fiber degeneration, and deterioration of aortic mechanical properties and SMC contractility, all tightly associated with the upregulation of MMP-2 and -9, were consistent and characteristic features of the development of TAA in MFS. The present study provides insight on the pathogenesis of TAA and its temporal relationship with MMP activation. Therapeutic strategies aimed at modifying MMP activation may prevent the progression of TAA and the eventual aortic rupture in MFS.

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Disclosures
None.

References


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Representative pictures showing the TA from (A) control, and Marfan at (B) 3 and (C) 6 months of age. Scale bar=0.25 cm. (D) Diagram illustrating sample preparation and experimental procedures using TA segments from control and Marfan mice.