Aortic disease accounts for well >34,000 deaths in the United States annually.¹ Thoracic aortic aneurysms include connective tissue disorders, degenerative/atherosclerotic, inflammatory, mechanical (dissections), and infectious. Marfan syndrome (MFS) is an autosomal dominant systemic connective tissue disorder in which patients typically develop aortic root aneurysms, with dissection and rupture being the leading causes of death. Without surgery, the majority of patients with MFS die prematurely, with an average life expectancy ≤70 years.² Previous studies have demonstrated that the underlying fibrillin-1 gene mutation in MFS increases the activity of transforming growth factor-β.³ Although numerous prospective randomized trials are underway looking at the therapeutic benefit of transforming growth factor-β blockade (losartan) on aneurysm progression, only prophylactic surgical replacement of the entire aortic root increases the life expectancy of patients with MFS. Current guidelines for elective surgery in patients with MFS include aortic aneurysm diameter >5 cm, rapid aortic growth rate (>0.5 cm/y), or symptoms.

Background—Ascending aortic dissection and rupture remain a life-threatening complication in patients with Marfan syndrome. The extracellular matrix provides strength and elastic recoil to the aortic wall, thereby preventing radial expansion. We have previously shown that ascending aortic aneurysm formation in Marfan mice (Fbn1C1039G/+ ) is associated with decreased aortic wall elastogenesis and increased elastin breakdown. In this study, we test the feasibility of quantifying aortic wall elastin content using MRI with a gadolinium-based elastin-specific magnetic resonance contrast agent in Fbn1C1039G/+ mice.

Methods and Results—Ascending aorta elastin content was measured in 32-week-old Fbn1C1039G/+ mice and wild-type (n=9 and n=10, respectively) using 7-T MRI with a T1 mapping sequence. Significantly lower enhancement (ie, lower R1 values, where R1=1/T1) was detected post–elastin-specific magnetic resonance contrast agent in Fbn1C1039G/+ compared with wild-type ascending aortas (1.15±0.07 versus 1.36±0.05; P<0.05). Post–elastin-specific magnetic resonance contrast agent R1 values correlated with ascending aortic wall gadolinium content directly measured by inductively coupled mass spectroscopy (P=0.006).

Conclusions—Herein, we demonstrate that MRI with elastin-specific magnetic resonance contrast agent accurately measures elastin bound gadolinium within the aortic wall and detects a decrease in aortic wall elastin in Marfan mice compared with wild-type controls. This approach has translational potential for noninvasively assessing aneurysm tissue changes and risk, as well as monitoring elastin content in response to therapeutic interventions. (Circ Cardiovasc Imaging. 2014;7:690-696.)

Key Words: aortic aneurysm, thoracic ◼ magnetic resonance imaging ◼ Marfan syndrome

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include increased elastin breakdown and disorganization of the extracellular matrix. Molecular MRI is a novel technique that allows in vivo quantification of numerous biological markers. Recently, an elastin-specific magnetic resonance contrast agent (ESMA) has been described for the noninvasive elastin quantification in the brachiocephalic (murine) and coronary arteries (swine). The ability to noninvasively detect subclinical pathological changes within the aortic wall has great translational potential, possibly assessing the severity of pathological changes within the aneurysm wall and predicting rupture or dissection risk. Moreover, this new imaging modality can be used experimentally or clinically to monitor therapeutic interventions.

Herein, we report a noninvasive method for the assessment of aortic wall elastin content in a Marfan aortic aneurysm model mouse (*Fbn1* C1039G/ +). MRI with ESMA accurately measures elastin bound gadolinium within the aortic wall and detects a decrease in aortic wall elastin in *Fbn1* C1039G/+ mice compared with wild-type (WT) controls.

**Methods**

**Synthesis of the Elastin-Specific Contrast Agent**

LM11174 (Lantheus Medical Imaging, North Billerica, MA), a gadolinium-based ESMA, was used in this study. LM11174 is a low-molecular-weight contrast agent (854.02 Da) with similar structure to BMS753951 (Lantheus Medical Imaging, North Billerica, MA), which has been previously demonstrated to have elastin specificity. Unlike BMS753951, LM11174 has a tetraazacyclododecane-1,1,1,1-tetraacetic acid (DOTA) chelate instead of diethylenetriaminepentaacetic acid (DTPA) to improve gadolinium chelation.

**Mice**

All experiments were performed with 32-week-old male heterozygous C57BL/6 *Fbn1* C1039G/+ mice and C57BL/6 littermate WT controls. The *Fbn1* C1039G/+ breeding pairs were a kind gift from Harry C. Dietz, MD, Johns Hopkins University School of Medicine. All animal protocols were approved by the Administrative Panel on Laboratory Animal Care at Stanford University (http://labanimals.stanford.edu/) and followed the National Institutes of Health and United States Department of Agriculture Association Guidelines for the Care and Use of Animals in Research.

**Histology**

Mice were euthanized by over exposure of isoflurane (2-chloro-2-(difluoromethoxy)-1,1,1-trifluoro-ethane; Baxter Healthcare Corporation, Deerfield, IL), and the ascending aorta was dissected and fixed in 4% paraformaldehyde. To preserve the shape of the aorta, 3% agarose was injected into the aorta via the left ventricle before excision. The aorta was embedded in Tissue-Tek OCT Compound Histomount (Sakura, Torrance, CA). The sample was sliced at 4-μm cross sections and stained with Accustain Elastin Verhoeff’s Van Gieson kit (Sigma Aldrich, St. Louis, MO) according to the manufacturer’s instruction. The aorta was imaged at ×40 magnification using a Leica DM4000B microscope.

**Thickness of Aortic Wall Tunica Media and Percent Area of Medial Elastin**

Cross sections of the aortic wall at the level of the ascending aorta were sectioned at 4-μm thickness and digitally photographed at 8 equidistant areas at ×40 magnification using a Leica DM4000B fluorescence microscope. Areas of elastin were identified by their intense autofluorescence using the fluorescein channels of excitation/emission and exposure times of 2 s. The thickness of the tunica media was identified from internal elastic lamina to external elastic lamina. The percent area of medial elastin was quantified using the Image J software as percentage of highly autofluorescent pixels within the demarcated tunica media using a color threshold mask (hue 80, saturation 0, brightness >100).

**Inductively Coupled Mass Spectroscopy for ESMA Binding in Ex Vivo Aorta**

The ascending aorta of 32-week-old male *Fbn1* C1039G/+ and WT mice was harvested (n=4 and 5, respectively). The aorta was incubated with 10 mmol/L of ESMA at 37°C for 2 hours, washed with PBS, then incubated in PBS for 1 hour to remove unbound ESMA. After washing, the aorta was digested with 70% nitric acid at 60°C overnight. Gadolinium concentration in the tissue was measured using inductively coupled mass spectroscopy (ICP-MS). The gadolinium content was normalized to the weight of the excised aorta.

**MRI Scanning**

In vivo imaging of the mouse aorta was performed using a preclinical 7.0-T actively shielded MRI scanner Discovery MR901 (Agilent Technologies-General Electric Healthcare) with a 2-channel receive-only radiofrequency coil (Rapid MR International, LLC) and radio-frequency transmission from a decoupling quadrature volume coil (Agilent Technologies). Image data were acquired at the Stanford Center for Innovation and In Vivo Imaging (SCI). Animal induction was accomplished with 3% isoflurane anesthesia in medical grade oxygen. A 30-gauge tail vein catheter was inserted for subsequent contrast agent administration at 0.2 mmol/kg. The mouse was placed in the radiofrequency coil for imaging, with 2% isoflurane anesthesia delivered through a nose cone and with respiratory, temperature, and cardiac monitoring (SA Instruments Inc, NY). Mouse body temperature was maintained at 37°C with a feedback-controlled warm air blower. Three-plane fast spoiled gradient echo (FSPGR) localized images were used to prescribe cardiac-gated bright-blood FSPGR images intersecting the ascending aorta. A slice perpendicular to the distal ascending aorta with the maximal diameter was prescribed for precontrast and postcontrast in vivo evaluation of T1 (T1 mapping) and inversion recovery fast gradient echo images. Single diastolic phase inversion recovery fast gradient echo images were acquired with field of view=30 mm, 256×256 matrix, slice thickness=0.9 mm, resolution=117 μm, and scan duration=3 minutes 20 s. The aortic wall signal-nulling inversion time was determined empirically in vivo, by acquiring inversion recovery fast gradient echo images with the following inversion times: 550, 580, 600, 620, 640, 660, 680, and 700 ms. The maximal diameter of the ascending aorta was measured in FSPGR images. Pre- and postcontrast MRI data were acquired before and 30 minutes after injection of the nonspecific gadolinium-DTPA contrast agent, the reported time period to reach peak aortic wall concentration of ESMA contrast agent. T1 mapping quantification was performed at the maximal aortic diameter slice. A 24-hour interval was allowed for clearance of the nonspecific gadolinium-DTPA contrast agent before administration of the targeted ESMA contrast agent and postcontrast MRI. Postcontrast MRI acquisitions were performed 30 minutes after contrast agent injection, the time period to reach peak aortic wall concentration of ESMA.
In this study, the ascending aorta reached 2.30±0.13 mm in diameter (n=10) compared with 1.63±0.04 mm in WT controls (n=11) at 32 weeks of age (P<0.001), as measured by MRI (Figure 1A and 1B). The average vessel wall thickness was greater in Fbn1C1039G/+ (n=5) versus WT (n=5) mice (91.3±5.2 versus 53.5±5.9 μm, respectively; P=0.001). Histological analysis with Elastin Verhoeff’s Van Gieson staining reveals extracellular matrix remodeling within the aortic wall media, with both reduced total elastin content and increased elastin fragmentation in Fbn1C1039G/+ mice compared with WT littermate controls (Figure 2A). Corroborating the qualitative elastin histological findings, the percentage of elastin within the media wall was significantly lower in Fbn1C1039G/+ mice (n=5) compared with WT control (n=6; 19.5±1.6% and 27.8±1.7%, respectively; P=0.018; Figure 2B and 2C). Although we have detected increased vascular smooth muscle cell apoptosis during early aneurysm development (in press, data not shown), no significant apoptotic cell population was seen at 32 weeks of age in this study. In contrast to models of abdominal aortic aneurysms, characterized by media wall macrophage and lymphocyte infiltration, inflammatory cells are not detected in the Fbn1C1039G/+ model.

Ascending aortic specimens were harvested from Fbn1C1039G/+ and WT mice, incubated with ESMA (Figure 3A) ex vivo,
and gadolinium concentrations measured with ICP-MS. Demonstrating elastin-specific binding, the Fbn1<sup>C1039G/+</sup> ascending aortic wall (n=4) had significantly less bound gadolinium than WT (n=5; 0.21±0.03 and 0.39±0.03 μg/mg, respectively; P=0.027; Figure 3B). Because 32-week-old Fbn1<sup>C1039G/+</sup> mice have sizeable aneurysms with reduced elastin content, we anticipated that a difference in aortic wall elastin content would be detectable with MRI using a gadolinium-based ESMA.

**MRI of the Murine Ascending Aortic Wall**

To better define this new contrast agent (ESMA with DOTA chelate), the relaxivity of unbound and bound ESMA was determined at 7 T. In the unbound study, R1 values linearly correlated with ESMA concentration (Figure 3C and 3D). We also measured the relaxivity of bound ESMA after incubating the aorta with different concentrations of ESMA. The R1 values measured by MRI were significantly associated with the gadolinium content of the aorta (P<0.001; Figure 3E and 3F).

The distal ascending aorta, perpendicular to the vessel axis, was visualized by MRI in both Fbn1<sup>C1039G/+</sup> and WT mice (Figure 4A). Contrast agent specificity was initially confirmed after scanning each mouse with (1) no contrast agent, (2) gadolinium-DTPA, and (3) ESMA. MRI data showed reduced elastin-specific contrast visualized in the Fbn1<sup>C1039G/+</sup> ascending aorta compared with WT mice (Figure 4B), corroborating reduced elastin content noted with Elastin Verhoeff’s Van Gieson histological staining and elastin quantification. The increased aortic diameter of the Fbn1<sup>C1039G/+</sup> mouse is also apparent in this figure.

After visually confirming a difference in contrast agent uptake, we measured aortic wall T1 values for a quantitative estimate of bound ESMA. To define the aortic wall borders in a mCine-IR pulse sequence, the FSPGR bright-blood image was superimposed on the mCine-IR image and the region of interest outlined (Figure 5A). As expected, low precontrast R1 values were observed in both Fbn1<sup>C1039G/+</sup> (n=9) and WT (n=10) aortic walls (0.63±0.04 and 0.71±0.05 s<sup>-1</sup>, respectively; P=0.25). Injection of nonspecific gadolinium-DTPA or ESMA significantly increased R1 values in both Fbn1<sup>C1039G/+</sup> (n=9) and WT (n=10) ascending aortas (P=0.007 and P<0.001, respectively). After injection of nonspecific gadolinium-DTPA, a statistically equivalent increase in R1 values was detected in both Fbn1<sup>C1039G/+</sup> and WT aortic walls. A control scan 24 hours after nonspecific gadolinium-DTPA injection confirmed that the contrast agent was washed out (Fbn1<sup>C1039G/+</sup> R1 value, no contrast agent versus 24 hours after gadolinium-DTPA injection, 0.63±0.07 and 0.63±0.05 s<sup>-1</sup>, respectively, P=0.98, n=6). Importantly, imaging after ESMA injection showed a significantly smaller increase in R1 values for Fbn1<sup>C1039G/+</sup> compared with WT ascending aortic wall (n=10; 1.15±0.07 and 1.36±0.05 s<sup>-1</sup>, respectively; P=0.045; Figure 5B), consistent with reduced aortic wall elastin content in Marfan mice.

**Aortic Wall Elastin Content Assessed With MRI and ICP-MS**

To correlate R1 values with actual bound gadolinium-labeled ESMA agent within the aortic wall, the amount of gadolinium within the ascending aorta was quantified with ICP-MS. We observed less gadolinium in Fbn1<sup>C1039G/+</sup> ascending aorta compared with WT (0.033±0.003 and 0.045±0.002 μg/mg, respectively; P=0.007), confirming the reduced aortic wall elastin content observed by MRI (Figure 5C). R1 values are associated with ICP-MS values (R²=0.69; P=0.006; Figure 6).

To validate further that our in vivo R1 measurements are accurately detecting aortic wall elastin content, the ascending aorta was excised after imaging and inserted into 3% agarose. The excised aorta was subsequently imaged with the mCine-IR pulse sequence, and no significant difference in the R1 value was detected when comparing in vivo and ex vivo ascending aortas (ratio of in vivo to ex vivo R1: 1.04±018; P=0.76).

**Discussion**

Although MRI with ESMA has been reported to noninvasively quantify coronary artery plaque burden and brachiocephalic vascular remodeling in both large and small animal models, respectively, the ability to detect extracellular matrix remodeling during aneurysm formation in a Marfan mouse model remains unknown. Although Makowski et al¹⁹ recently presented preliminary data that the elastin-specific contrast agent ESMA can be used in a murine abdominal aortic aneurysm model, the chemically induced abdominal aortic aneurysm models (angiotensin II
Apo E–deficient, extraluminal CaCl₂, and intraluminal porcine pancreatic elastase infusion have several limitations and may not accurately reflect processes involved in human abdominal aortic aneurysm development. The angiotensin II model is characterized by an initial abdominal aortic dissection that subsequently enlarges over time, whereas abdominal aortic aneurysm in the CaCl₂ and porcine pancreatic elastase models are induced with a corrosive chemical agent. In the present study, we make use of a well-established Marfan mouse model (heterogeneous for an Fbn1 allele encoding a cysteine to glycine substitution) that reproducibly develops ascending aortic aneurysms, thus recapitulating the pathology observed in human MFS. We think this genetic model system is an excellent experimental tool to evaluate whether elastin deficit as assessed by MRI with ESMA may (1) predict future aneurysm growth and (2) monitor therapeutic interventions. In this study, using Marfan (Fbn1<sup>C1039G/+</sup>) mice, we have demonstrated that MRI coupled with ESMA allows visualization and assessment of elastin content within the ascending aortic aneurysm wall. Moreover, corroborating our histological data, it shows that elastin content is decreased compared with WT mice and inversely related to aneurysm size.

Here, we report for the first time to our knowledge that MRI can be used to assess elastin content within the thoracic aortic wall in a Marfan ascending aortic aneurysm mouse model. Histology specimens illustrate reduced total elastin content in the Fbn1<sup>C1039G/+</sup> aortic wall media, resulting from thinning of elastin fibers, as well as increased elastin fragmentation. Consistent with the histological analysis, we show that MRI with ESMA can detect reduced total elastin content in Marfan ascending aneurysms compared with WT control aorta. Although the pioneering work of Makowski et al<sup>11</sup> previously established that this molecular imaging technique can detect enhanced elastin levels during brachiocephalic artery plaque development,
this study demonstrates the method’s sensitivity in a disease model where the pathology has less than normal elastin levels. However, because the elastin level quantitative difference detected was small when comparing Marfan versus control aorta, it remains to be seen whether this agent will have translational capability. Importantly, we have yet to determine whether ESMA with DOTA chelate binds fragmented elastin fibers in addition to intact elastin nor the biological importance of this information. Makowski et al.11 validated with electron microscopy that to intact elastin nor the biological importance of this information. Makowski et al.11 validated with electron microscopy that the gadolinium in ESMA with DTPA chelate binds elastin only, although in a model system with abundant intact elastin. The contrast agent used in this study was recently changed to DOTA, which is considered safer and more suitable as a chelator for gadolinium-based MRI contrast agents than DTPA. Moreover, DOTA has been reported to have slower kinetics of dissociation, greater specificity than DTPA for gadolinium,12 and less nephrotoxicity in patients with renal dysfunction.12,20

As noted, this method has translational potential for humans. Aortic elastin content could be assessed noninvasively, and the information used as a diagnostic and prognostic tool. The goal for prophylactic surgery in patients with aortic root/ascending aneurysms is to prevent life-threatening aortic dissection or rupture. Indications that trigger surgical intervention include symptoms, large aortic dimension, or rapid growth rate, commonly measured with echocardiography, computed tomography, or MRI scans. The added information potentially revealed from MRI assessment of aortic elastin may give insight into the composition and structural integrity of the aortic wall and provide more information into aneurysm risk. More specifically, quantifying aortic wall elastin levels can potentially detect aortas at risk for early dissection, thereby identifying patients who should be watched more closely or receive surgical intervention earlier than indicated by aortic dimension alone.

MRI-based molecular imaging techniques have also been successfully used to study several pathological disease processes, including vessel wall plaque burden/vulnerability and clinical oncology.21 In particular, contrast agents have been designed to enhance fibrin, vascular cell adhesion molecule 1, macrophages, lectinlike oxidized-1, and matrix metalloproteinasises.22 In addition to molecular imaging, the advantages of MRI compared with computed tomography when studying aortic disease include (1) absence of radiation, especially important for patients requiring surveillance studies; (2) ability to generate images with high spatial resolution; (3) capability to obtain better information in studies when nephrotoxic contrast administration is not advised (patients with renal compromise); and (4) capacity to evaluate physiology, as well as anatomy (age of mural thrombus, evaluation of true to false lumen interactions).

To confirm that vessel wall elastin levels can be quantified with this agent, ICP-MS gadolinium concentrations were compared with MRI R1 values. After identifying a significant linear correlation, we concluded that elastin content can be accurately estimated noninvasively by MRI using ESMA. Although no correlation study has been published on risk of rupture compared with aortic wall elastin content, this study is now feasible and could make MRI a powerful diagnostic tool for assessing risk of aortic aneurysm rupture. In vivo elastin visualization also has great potential in evaluating the efficacy of a treatment strategy. Although aortic diameter is important, identifying subclinical pathological changes may allow earlier treatment modifications. Finally, experimentally, this technique allows elastin quantification without having to euthanize the animals early.

This study has several limitations. Although we report that ESMA can be used to study pathological changes in ascending aneurysms in a murine model, this agent needs to be investigated further in large animals and eventually confirmed in humans. It is also important to remember the limitations of murine disease models. Although the basic developmental and signaling pathways are conserved between human and mouse, there are differences in the proteomes that may not accurately represent human pathology. Moreover, genetically manipulating mice can lead to inappropriate levels of a protein (too high or low levels of transforming growth factor-β, in this model) or alter nearby genes, thus complicating interpretation of the data.

Figure 5. In vivo MRI R1 value measurements. A, R1 measurements with modified cine inversion recovery (mCine-IR) and fast spoiled gradient echo (FSPGR) data. The corresponding FSPGR image was overlaid to define the aortic lumen in the mCine-IR image. Region of interest (ROI) was drawn defining the aortic lumen in the mCine-IR image. B, R1 values of the ascending aortic wall in 32-week-old wild-type (WT; n=10) and Fbn1<sup>C1039G/+</sup> (n=9) mice. The average R1 value from precontrast, post–gadolinium (Gd)-DTPA, and post–elastin-specific magnetic resonance contrast agent (ESMA) scans is compared in WT and Fbn1<sup>C1039G/+</sup> mice. C, In vivo gadolinium content in the ascending aorta of 32-week-old WT (n=9) and Fbn1<sup>C1039G/+</sup> (n=7) mice. Values are expressed as mean±SEM (*P<0.05).

Figure 6. The relationship between R1 values and gadolinium (Gd) content. R1 values in the ascending aorta of wild-type (n=9) vs Gd content. In vivo R1 values measured by MRI with elastin-specific magnetic resonance contrast agent (ESMA) linearly correlate with Gd content measured by inductively coupled mass spectroscopy (ICP-MS; P=0.001).
In conclusion, this study illustrates that MRI with ESMA can noninvasively measure elastin content in the aortic wall in a Marfan mouse model. This method for the assessment of the aortic wall elastin content has the potential to become an adjunctive clinical approach for determining which patients may benefit from early surgical intervention and evaluating the effect of treatment in both clinical and basic science situations. Importantly, this technique may ultimately have application in patients with a variety of aortic diseases.

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References

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