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Circulating Transforming Growth Factor-β in Marfan Syndrome

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Background—Marfan syndrome (MFS) is caused by mutations in the fibrillin-1 gene and dysregulation of transforming growth factor-β (TGF-β). Recent evidence suggests that losartan, an angiotensin II type 1 blocker that blunts TGF-β activation, may be an effective treatment for MFS. We hypothesized that dysregulation of TGF-β might be mirrored in circulating TGF-β concentrations.

Methods and Results—Serum obtained from MFS mutant mice ($Fbn1^{C1039G/+}$) treated with losartan was analyzed for circulating TGF-β1 concentrations and compared with those from placebo-treated and wild-type mice. Aortic root size was measured by echocardiography. Data were validated in patients with MFS and healthy individuals. In mice, circulating total TGF-β1 concentrations increased with age and were elevated in older untreated $Fbn1^{C1039G/+}$ mice compared with wild-type mice ($P=0.01$; n=16; mean±SEM, 115±8 ng/mL versus n=17; mean±SEM, 92±4 ng/mL). Losartan-treated $Fbn1^{C1039G/+}$ mice had lower total TGF-β1 concentrations compared with age-matched $Fbn1^{C1039G/+}$ mice treated with placebo ($P=0.01$; n=18; 90±5 ng/mL), and circulating total TGF-β1 levels were indistinguishable from those of age-matched wild-type mice ($P=0.8$). Correlation was observed between circulating TGF-β1 levels and aortic root diameters in $Fbn1^{C1039G/+}$ and wild-type mice ($P=0.002$). In humans, circulating total TGF-β1 concentrations were elevated in patients with MFS compared with control individuals ($P<0.0001$; n=53; 15±1.7 ng/mL versus n=74; 2.5±0.4 ng/mL). MFS patients treated with losartan (n=55) or β-blocker (n=80) showed significantly lower total TGF-β1 concentrations compared with untreated MFS patients ($P\leq0.05$).

Conclusions—Circulating TGF-β1 concentrations are elevated in MFS and decrease after administration of losartan, β-blocker therapy, or both and therefore might serve as a prognostic and therapeutic marker in MFS. (***Circulation*. 2009; 120:526-532.**)

Key Words: aneurysm ■ biomarkers ■ losartan ■ Marfan syndrome ■ TGF-beta

Marfan syndrome (MFS) is a systemic connective tissue disorder that affects ≈1 in 5000 individuals.[1,2] It is inherited as an autosomal dominant trait and is caused by mutations in the gene encoding the extracellular matrix protein fibrillin-1 (*FBN1*).[3] *FBN1* mutations lead to defects in multiple organ systems. Aortic root dilatation is the leading cause of morbidity and mortality in MFS.[1,4] Several studies, based mainly on the analysis and creation of genetically engineered mouse models, have challenged the definition of MFS as a simple structural disorder of the connective tissue.[5–8] It has been shown that many clinical manifestations associated with MFS, including aortic root dilatation, pulmonary emphysema, atrioventricular valve changes, and skeletal muscle myopathy, are induced by altered transforming growth factor-β (TGF-β) activation and signaling.[5–7,9] A mouse model heterozygous for a mutant *Fbn1* allele encoding a cysteine substitution in an epidermal growth factor-like domain of fibrillin-1, $Fbn1^{C1039G/+}$, was shown to develop

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Participating centers for the National Registry of Genetically Triggered Thoracic Aortic Aneurysms and Cardiovascular Conditions (GenTAC) are shown in the Appendix.

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pathological changes in the aorta and aortic root enlargement that closely mimic those seen in humans with MFS accompanied by excessive TGF-β signaling in the aortic root wall.[7] Most importantly, TGF-β antagonism through systemic administration of TGF-β neutralizing antibody prevented the development of pathological changes in the aortic wall and progressive aortic dilatation.[7] TGF-β antagonism also rescued other manifestations of MFS, including muscle regeneration, architecture, and strength, pulmonary alveolar septation, and mitral valve morphology in the mouse model.[5,8] Administration of losartan, an angiotensin II type 1 receptor blocker (ARB) known to antagonize TGF-β signaling through inhibition of TGF-β expression and activation,[10] normalized aortic root growth and dimensions in the *Fbn1*$^{C1039G/+}$ mice and resulted in an aortic wall architecture that was indistinguishable from that of wild-type mice.[7] The therapeutic benefit of losartan was recently replicated in a small pediatric cohort with a severe form of MFS.[11] Losartan, either alone or in addition to β-blocker therapy, led to a reduction in the rate of change in the aortic root diameter compared with β-blocker therapy alone.[11] Taken together, these data suggest that dysregulation of TGF-β, which is an autocrine and paracrine growth factor with involvement in a wide range of biological processes, contributes to the multisystem pathogenesis of MFS.[4,7,11]

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TGF-β is secreted from the cell in the context of a large latent complex (LLC) that includes the active cytokine, its processed N-terminal propeptide (termed *latency-associated peptide*), and 1 of 3 latent TGF-β binding proteins (LTBP1, LTBP3, or LTBP4). The LLC is targeted to the matrix by virtue of interactions between LTBPs and both fibronectin and fibrillins. Current models suggest that fibrillin-1 deficiency leads to failed matrix sequestration of the LLC of TGF-β, with consequent excessive TGF-β activation and signaling. In this light, pathogenic events might correlate with circulating TGF-β concentrations. If so, then systemic administration of losartan in the mouse models of MFS should decrease circulating TGF-β concentrations. If transferable to humans, circulating TGF-β might serve as a promising marker for prognostication and for individualizing therapeutic regimens in MFS.

Methods

All mouse and human protocols were approved by the institutional review board of Johns Hopkins University School of Medicine and MedStar Research Institute, Baltimore, Md.

Mice

The mouse line heterozygous for *Fbn1* mutation C1039G (*Fbn1*$^{C1039G/+}$) has been described previously.[5,6] All analyses were performed after back-crossing this mutation into the C57BL/6J background (>9 generations), allowing valid comparisons between litters. *Fbn1*$^{C1039G/+}$ mice were treated with oral losartan (0.6 g/L in drinking water; n=18) or placebo (n=37) beginning at 7 weeks of age and continuing until they were euthanized; wild-type littermates received only placebo (n=41). A small subgroup of *Fbn1*$^{C1039G/+}$ mice was treated with a higher dose of oral losartan (1.2 g/L in drinking water; n=3). All mice were euthanized with an inhalation overdose of halothane (Sigma-Aldrich, St Louis, Mo). Blood samples from mice were collected from the right ventricle immediately after they were euthanized and were allowed to clot for 2 hours at room temperature before being centrifuged for 20 minutes at 2000*g*. Serum was removed, aliquoted, and stored immediately at −80°C until further analysis.

Human Subjects

Plasma samples and clinical data from 207 patients diagnosed with MFS were collected through the National Registry of Genetically Triggered Thoracic Aortic Aneurysms and Cardiovascular Conditions (GenTAC). Plasma samples from 74 healthy individuals obtained at the National Institute on Aging served as controls. Selection criteria for control patients were as follows: no cardiovascular disease or drugs, no cancer, no inflammatory of autoimmune disorders, no diabetes, and no drugs that could affect the extracellular matrix. Blood samples in MFS and control patients were collected on ice with EDTA used as an anticoagulant. Samples were centrifuged at 3000*g* for 15 minutes at 4°C, and plasma samples were stored immediately at −80°C until further analysis.

Enzyme-Linked Immunosorbent Assay for TGF-β

TGF-β1 concentrations in mice samples were measured by enzyme-linked immunosorbent assay with the Quantikine TGF-β1 immunoassay (R&D Systems, Minneapolis, Minn; catalog No. MB100M). The minimum detectable concentration of TGF-β1 in this assay ranges from 1.7 to 15.4 pg/mL, and there is <1% cross-reactivity with the latent TGF-β1 complex. Total TGF-β1 concentrations were measured by first acid-activating (with 1N HCl) latent TGF-β1 to immunoreactive TGF-β1; free (active) TGF-β1 levels were measured without first acid-activating the sample. The enzyme-linked immunosorbent assay was performed according to the manufacturer's protocol. Briefly, the wells are precoated with monoclonal antibody specific for TGF-β1. Standards, controls, and samples were added and incubated for 2 hours. After a washing, an enzyme-linked polyclonal antibody specific for TGF-β1 was added. After another washing, samples were incubated with a substrate solution for 30 minutes, and the color reaction was stopped by addition of a diluted HCl solution. The optical density of each well was measured immediately with a microplate reader at 450 nm, and the wavelength correction was set to 570 nm. All samples were run in duplicate.

Human EDTA plasma samples were assayed for levels of total TGF-β1 with the use of a commercially available, ruthenium-based electrochemiluminescence platform (Meso Scale Discovery, Gaithersburg, Md) following the manufacturer's recommendations. To be able to measure total TGF-β1, samples were first acid-activated (with 1N HCl) before assaying. All samples were run in duplicate. The lowest level of detection for total TGF-β1 was 4±2.6 pg/mL.

We considered TGF-β1 results (in mice and in human samples) valid when recovery (expected concentration divided by calculated concentration multiplied by 100) of the standards/calibrators was 100±20%, the coefficient of variation was <20%, intra-assay coefficient of variation was <10%, and the interassay coefficient of variation was <20%. A run was considered valid when >85% of the samples were within these specifications.

Aortic Root Diameters: Echocardiography

Transthoracic echocardiograms (n=39) were performed on awake, nonsedated mice with the use of the VisualSonics Vevo 660 V1.3.6 imaging system and a 40- or 60-MHz transducer (model RMV603, VisualSonics, Inc, Toronto, Ontario, Canada). The aorta was imaged in the parasternal long-axis view, and 3 measurements were obtained at the level of the sinuses of Valsalva (SOV) by an observer blinded to genotype and treatment arm. All echocardiographic studies were performed by 2 individuals with extensive experience with mouse echocardiography.

In patients with MFS, aortic root dimensions (SOV) were obtained through transthoracic echocardiography by multiple echocardiographers. All echocardiographic data were collected in the GenTAC

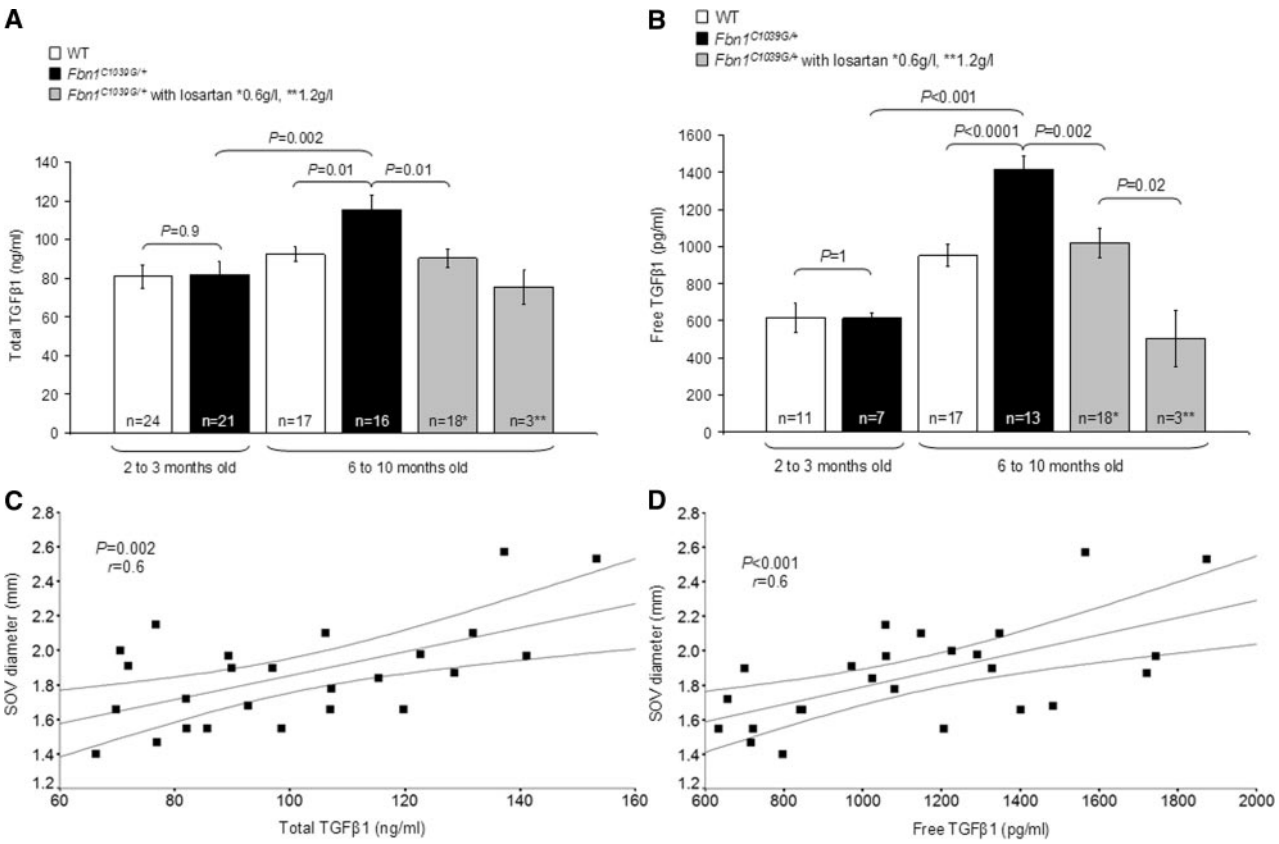

Figure 1. Circulating TGF-β1 concentrations in *Fbn1*$^{C1039G/+}$ and wild-type mice and their correlation with the aortic root size. A, Mean total TGF-β1 serum concentrations showing increased concentrations with age and significantly higher circulating total TGF-β1 levels in 6- to 10-month-old *Fbn1*$^{C1039G/+}$ mice compared with age-matched wild-type (WT) and losartan-treated *Fbn1*$^{C1039G/+}$ mice. B, Mean free TGF-β1 serum concentrations changed in the same manner as seen with total TGF-β1. A subgroup of *Fbn1*$^{C1039G/+}$ mice was treated with a higher dose of losartan (1.2 g/L in drinking water instead of the standard dose of 0.6 g/L), which resulted in a further reduction in circulating total and free TGF-β1 concentrations. C, Correlation was observed between circulating total TGF-β1 concentrations and the SOV diameters in untreated *Fbn1*$^{C1039G/+}$ and wild-type mice (regression prediction lines with mean value and 95% confidence interval). D, Correlation was observed between free TGF-β1 concentrations and SOV size in untreated *Fbn1*$^{C1039G/+}$ and wild-type mice (regression prediction lines with mean value and 95% confidence interval).

registry before measurements of circulating TGF-β1 concentrations were performed. Measurements of the maximal aortic root diameter were taken by the leading-edge technique, consistent with the current American Society of Echocardiography guidelines.[12] A *Z* score, which represents the standard deviation from the mean aortic diameter normalized for the patient's body surface area and age, was calculated from each echocardiographic measurement with the use of standard algorithms.

Statistical Analysis

Continuous variables were presented as mean±SEM. Comparisons of circulating TGF-β1 concentrations among the groups were conducted by 1-way ANOVA. If significance was found for group effect, pairwise comparisons between the groups were made with the unpaired *t* test. Associations between circulating TGF-β1 concentrations and SOV diameters were studied with the use of the Pearson correlation and linear regression analysis and illustrated in scatterplots with confidence bands on a regression line. For comparisons of nonparametric data, the χ^2 test was used. Two-sided *P* values of <0.05 were considered to indicate statistical significance for all statistical tests and models. SPSS statistical software 9.0 was used for all analyses.

The authors had full access to and take full responsibility for the integrity of the data. All authors have read and agree to the manuscript as written.

Results

Mice

One-way ANOVA showed high group effects for circulating total and free TGF-β1 concentrations ($P=0.002$, $P<0.0001$). Circulating total TGF-β1 concentrations increased with age in *Fbn1*$^{C1039G/+}$ and wild-type mice (Figure 1A and 1B). Total TGF-β1 was higher in samples obtained from 6- to 10-month-old *Fbn1*$^{C1039G/+}$ mice compared with age-matched wild-type mice ($P=0.01$; n=16; mean±SEM, 115±8 ng/mL versus n=17; mean±SEM, 92±4 ng/mL; Figure 1A). Losartan-treated *Fbn1*$^{C1039G/+}$ mice had lower total TGF-β1 concentrations compared with age-matched *Fbn1*$^{C1039G/+}$ mice treated with placebo ($P=0.01$; n=18; 90±5 ng/mL versus n=16; 115±8 ng/mL; Figure 1A). Circulating total TGF-β1 in losartan-treated *Fbn1*$^{C1039G/+}$ mice and age-matched wild-type mice were indistinguishable ($P=0.8$; Figure 1A). A subgroup of *Fbn1*$^{C1039G/+}$ mice (n=3) treated with a higher dose of losartan (1.2 g/L in drinking water) showed a further reduction of circulating TGF-β1 concentrations (Figure 1A). Changes in circulating free TGF-β1 levels mirrored the changes seen in total TGF-β1 (Figure 1B). Correlation was

Table. Patient Baseline Demographics

Variables	MFS Patients (n=207)	Controls (n=74)	*P*
Age, y*	37.5±1.2	48.7±1.5	<0.0001
Sex, male:female	98:78	26:48	0.003
Body mass index*	24.6±0.5	25±0.4	0.6
Aortic root dimension*†			
SOV, cm	3.8±0.1	...	
Z score‡	3.2±0.2	...	
Previous aortic root surgery, n (%)	77 (37)	0	
No cardiovascular drug therapy, n (%)	53 (25)	74 (100)	
Medication, n (%)			
Losartan§	55 (26)	0	
β-Blockers	80 (39)	0	
ACE inhibitors‖	12 (7)	0	
Other#	7 (3)	0	

*Data are presented as mean±SEM unless otherwise indicated.
†Data from patients without previous aortic root surgery.
‡SD from the mean aortic diameter normalized for the patient's body surface area and age.
§Forty-five patients with combined losartan and β-blocker therapy.
‖Ten patients with combined ACE inhibitor and β-blocker therapy.
#ARB therapy other than losartan, calcium channel blocker, or combination of losartan, ACE inhibitor, and β-blocker.

observed between circulating total TGF-β1 and free TGF-β1 concentrations in matched samples (n=43; r=0.6, P<0.0001).

Aortic root diameters (SOV) were smaller in 6- to 10-month-old wild-type mice compared with age-matched untreated *Fbn1*$^{C1039G/+}$ mice (P<0.0001; n=15; mean±SEM, 1.69±0.02 mm versus n=10; mean±SEM, 2.23±0.11 mm). Losartan treatment in *Fbn1*$^{C1039G/+}$ mice led to smaller SOV dimensions compared with age-matched untreated *Fbn1*$^{C1039G/+}$ mice (P<0.0001), and losartan-treated *Fbn1*$^{C1039G/+}$ mice and age-matched wild-type mice showed indistinguishable SOV dimensions, as shown previously.[7] SOV diameters in untreated *Fbn1*$^{C1039G/+}$ and wild-type mice correlated with circulating total TGF-β1 (r=0.6, P=0.002) and circulating free TGF-β1 concentrations (r=0.6, P<0.001; Figure 1C and 1D).

Human Subjects

Baseline demographics for patients diagnosed with MFS (n=207) and control individuals (n=74) are provided in the Table. MFS patients were younger, and there were more male subjects within the MFS group compared with the controls. Previous aortic root surgery and cardiovascular drug therapy, including losartan, other ARBs, β-blockers, and angiotensin-converting enzyme (ACE) inhibitors, occurred only in people with MFS.

One-way ANOVA showed high group effects for circulating total TGF-β1 concentrations (P<0.0001). Circulating total TGF-β1 concentrations were elevated in MFS patients without cardiovascular drug therapy compared with the control individuals (P<0.0001; n=53; mean±SEM, 15±1.7 ng/mL versus n=74; mean±SEM, 2.5±0.4 ng/mL; Figure 2A). MFS patients treated with losartan (n=55; of those, 45 patients received a combination of losartan and β-blocker therapy) showed significantly lower total TGF-β1 concentrations compared with untreated MFS patients (P=0.05; 11±1.4 ng/mL versus 15±1.7 ng/mL; Figure 2A). Similarly, total TGF-β1 concentrations were lower in MFS patients treated only with β-blockers (P=0.03; n=80; 11±1.2 ng/mL versus 15±1.7 ng/mL; Figure 2A). A subgroup of MFS patients treated with ACE inhibitors (n=12; of those, 10 patients received a combination of ACE inhibitor and β-blocker therapy) showed a tendency toward lower total TGF-β1 concentrations (P=0.1; 12.7±2.8 ng/mL versus 15±1.7 ng/mL; Figure 2A). MFS patients with losartan, β-blocker, or ACE inhibitor therapy showed significantly higher circulating TGF-β1 levels compared with the control individuals (P<0.0001). Correlations between circulating total TGF-β1 concentrations and SOV diameters or *Z* scores, respectively, in MFS patients were not significant (Figure 2B). Circulating total TGF-β1 concentrations were neither sex nor age dependent in people with MFS and healthy control individuals (Figure 2C and 2D).

Discussion

Recent studies have established the critical contribution of dysregulated TGF-β signaling to the progression of disease in MFS.[5–8] Mouse models have validated TGF-β antagonism as a productive treatment strategy for both the cardiovascular and systemic manifestations of MFS, including the use of losartan, and early experience suggests that this protection may extend to people with MFS.[7,11] Despite significant progress regarding the pathogenesis and treatment of MFS, many obstacles remain. In a small observational study of children with severe MFS treated with losartan, all showed at least some reduction in aortic root growth compared with their progression on prior medical therapy, but the degree of response was variable.[11] Although widespread adoption of the practice of performing aortic root surgery once the dimension exceeds 5.0 cm in adults has greatly reduced mortality due to aortic dissection, meaningful guidelines for children are lacking, and death due to root dissection continues to be observed in both age groups. Even among patients with successful prophylactic aortic root replacement, there is risk of dissection of other aortic segments, prominently of the proximal descending thoracic aorta, and this occurs in many patients without prior dilatation.

In the absence of empirical data, the dose of losartan or other medications currently used in patients with MFS is based largely on dosing regimens for hypertension. Although the weight-based dosing for losartan used in mice was considerably higher than that for people, metabolic differences between species preclude extrapolation.[7] Consideration of dosing may be particularly important because ultrahigh doses of ARBs and other medications have been suggested to have enhanced efficacy in treating other TGF-β–related diseases in both mice and people.[7,8,11] Furthermore, natural genetic variation in people, including genes encoding regulators of drug metabolism or the renin-angiotensin system, can influence both the effective level of and intrinsic response to ARBs, ACE inhibitors, and β-blockers. A similar "one size fits all" philosophy for surgical management is intuitively

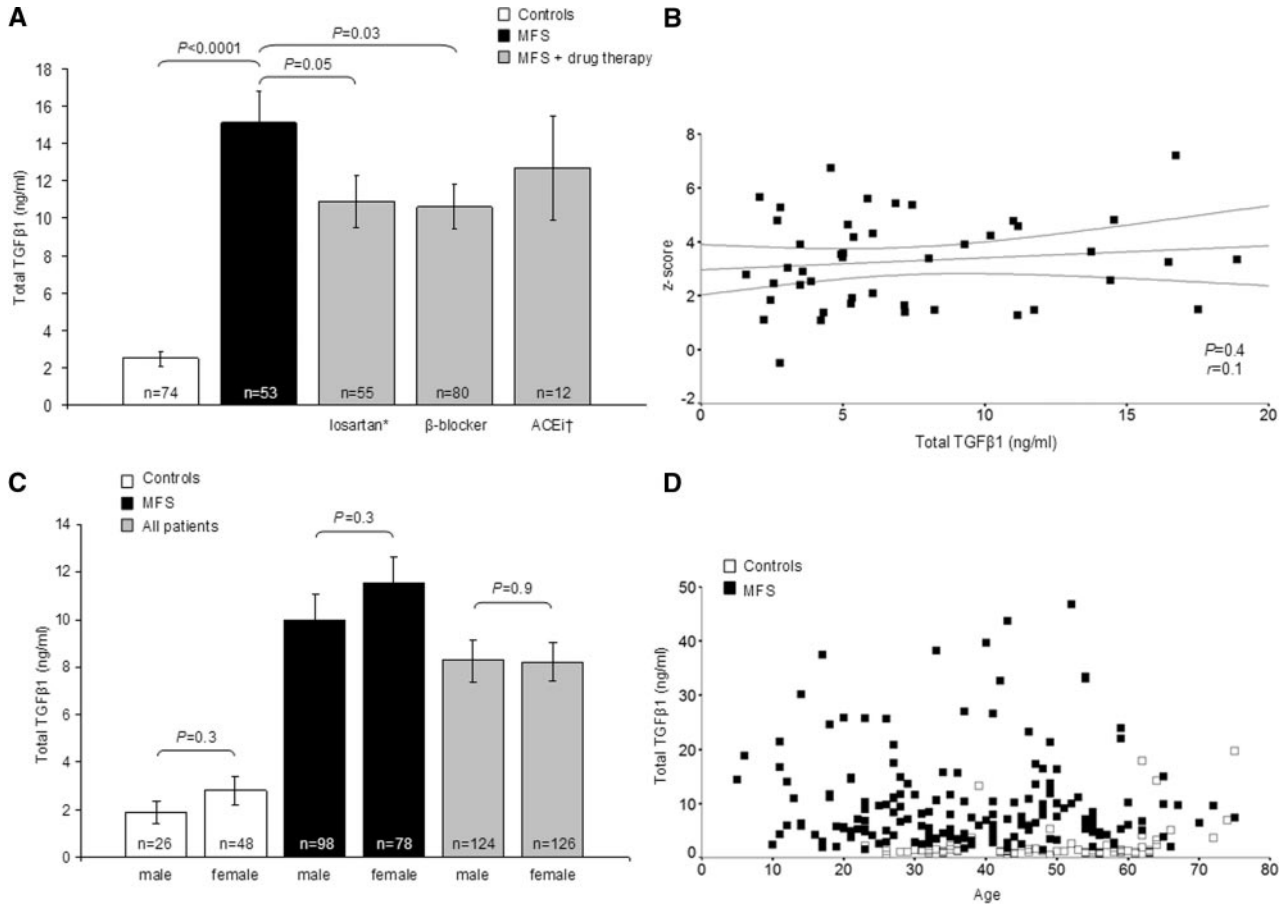

Figure 2. Circulating total TGF-β1 concentrations in human samples, their correlation with *Z* scores in MFS patients, and the association with sex and age, respectively. A, Mean total TGF-β1 plasma concentrations were elevated in MFS patients without cardiovascular drug therapy compared with healthy control individuals. MFS patients with losartan and/or β-blocker treatment showed a significant decrease in circulating total TGF-β1 levels compared with untreated MFS patients, and those with ACE inhibitor (ACEi) therapy showed a tendency toward lower total TGF-β1 concentrations. *Forty-five patients with combined losartan and β-blocker therapy; †10 patients with combined ACE inhibitor and β-blocker therapy. B, Correlation between circulating total TGF-β1 concentrations and *Z* scores in MFS patients without previous aortic root surgery was not significant (regression prediction lines with mean value and 95% confidence interval). In addition, no significant correlation was observed in MFS patients without previous aortic surgery and no cardiovascular drug therapy (n=12, r=0.1, P=0.7). C, Mean total TGF-β1 concentrations showed no significant difference between male and female subjects in healthy control individuals and people with MFS. D, No correlation was observed between total TGF-β1 concentrations and age in controls and those with MFS (r=0.3; r=0.07).

limited. Taken together, these observations highlight the need for an informative prognostic and therapeutic marker in MFS. The lack of robust phenotype-genotype correlations in MFS suggests that *FBN1* genotype fails to accommodate this need.

Despite gaps in our understanding of the precise mechanism by which fibrillin-1 deficiency correlates with increased TGF-β signaling, current data are consistent with a model in which LLC that fails to be sequestered by the extracellular matrix is more bioavailable for or prone to activation in a protease-dependent (eg, matrix metalloproteinases or plasmin) or -independent (eg, through the action of thrombospondin-1 or selected integrins) manner.[13–16] Curiously, the levels of selected TGF-β activators (including matrix metalloproteinase-2, matrix metalloproteinase-9, and thrombospondin-1) and ligands have been shown to be elevated in the tissues of patients with MFS, including the aortic wall.[17] It remains to be determined whether this is the result of positive autoregulation by TGF-β or either proximal or parallel events. Nevertheless, blockade of the angiotensin II type 1 receptor with ARBs has been shown to diminish the expression of TGF-β, its receptor, and potential activators, including thrombospondin-1 and matrix metalloproteinases, providing multiple potential mechanisms of protection from TGF-β–induced pathogenic events.[7] In agreement with this paradigm, we now report that a genetically defined mouse model of MFS that faithfully recapitulates most aspects of the disease, including progressive aortic root dilatation, shows elevated circulating levels of both latent and active TGF-β. Furthermore, circulating TGF-β levels show close correlation with aortic root dimension in mice that have or have not been treated with losartan, diminish on treatment in a dose-dependent manner, and are fully normalized in mice that show a robust therapeutic response.

Although the use of circulating TGF-β levels to individualize patient counseling and management is appealing because the assay is monitoring a central and direct effector of disease progression, it is essential to gain a better understanding of how and why events in tissues and markers in the circulation correlate. The simplest hypothesis posits that

complexed TGF-β and free TGF-β simply leach into the circulation because of failed matrix sequestration and increased activation of the LLC, respectively. It is also formally possible that increased circulating active TGF-β contributes to altered cellular and tissue performance. This seems unlikely, however, given the relatively high pericellular and matrix concentrations of TGF-β that result from direct secretion compared with that in the circulation. The description of a patient with hemi-MFS, as a result of discrete lateral somatic mosaicism, also argues against a major contribution of circulating TGF-β to the disease phenotype.[18]

In agreement with our results, the ability of ARBs to lower circulating TGF-β levels has been observed in other disease processes, and perindopril was shown to lower elevated circulating TGF-β levels in a small group of patients with MFS.[19] Although we observed a significant increase in the amount of total TGF-β in MFS patients versus controls, the levels of free TGF-β were extremely low in both groups, precluding a meaningful comparison. This is not surprising because human latent TGF-β1 has a half-life of ≈90 minutes, whereas free TGF-β1 has a half-life of only 2 minutes.[14,15] It is possible that modified handling or processing of human samples would allow for valid measurements.

It is intriguing that MFS patients treated with β-adrenergic receptor blocking agents (β-blockers) showed a significant decrease in circulating levels of TGF-β. Although prior studies have linked β-adrenergic and TGF-β signaling, the mechanistic details are not well established. Patients with dilated cardiomyopathy–associated fibrosis showed decreased expression of TGF-β1 and its target genes encoding types I and III collagens on treatment with β-blockers.[20] Treatment of Marfan mouse models with atenolol reduced TGF-β expression and signaling, although this effect appeared restricted to early age groups.[21] In agreement with this finding, Marfan mice treated with propranolol showed a significant reduction in aortic root size compared with placebo-treated animals. However, there was no apparent effect on TGF-β signaling in the aortic wall in older animals, and the extent of long-term protection was far less than that achieved with losartan.[7] The finding that TGF-β can positively stimulate its own expression and activation and can increase β-adrenergic receptor density and signaling in the cardiovascular system[22] highlights the potential for both auto- and cross-induction of these signaling cascades. The extent to which circulating TGF-β levels serve as a valid surrogate for critical pathogenic events in the tissues of MFS patients may prove both dynamic and context dependent. The emerging view is that β-blockers can reduce TGF-β expression, whereas ARBs reduce both expression and activation. In this light, the reduction in circulating TGF-β seen while a patient is on β-blockers or ARBs may herald protection in tissues or stages in disease progression in which TGF-β signaling is limited by TGF-β expression levels. In other contexts in which excessive TGF-β activation is sufficient to achieve pathogenic signaling thresholds, the prognostic value of a drop in circulating TGF-β levels may be unique to patients receiving ARBs. This important issue clearly warrants further study.

We did not observe close correlation between circulating TGF-β and aortic root size or *Z* score in people with MFS. There are many possible explanations. First, surrogate markers in human samples often show a much higher variability than seen in mice with the same genetic background and standardized environment. Second, our study mainly focused on adults and was enriched for people with milder disease and who were receiving medical therapy that could modify pathogenic events, including TGF-β levels. Finally, the aortic root is likely a minor contributor to circulating TGF-β levels compared with other tissues that are affected in MFS, including skeletal muscle, skin, and lung. Although there is high concordance between the severity of aortic and systemic disease in the various inbred mouse strains with *Fbn1* mutations and in people at the extremes of disease severity, this is not the case in people with more moderate presentations of MFS. In this light, the high correlation between circulating TGF-β levels and aortic performance in mice might be recapitulated in defined subsets of patients. Even if there is little intrinsic prognostic value to a snapshot measurement of circulating TGF-β in people with MFS, there remains a high probability of obtaining important information for individual trends observed during the progression of disease and in response to therapy.

Appendix

GenTAC participating centers are as follows: Johns Hopkins University, Kathryn W. Holmes, MD, Harry C. Dietz, MD, Williams Ravekes, MD, Kira Lurman, RN; University of Texas–Houston, Dianna M. Milewicz, MD, PhD, Claire Noll, MS, CGC; Baylor College of Medicine, Scott A. LeMaire, MD, Irina Volguina, PhD; Oregon Health and Science University, Cheryl L Maslen, PhD, Howard K. Song, MD, PhD, Victor Menashe, MD, Jessica D. Kushner, MS, CGC; University of Pennsylvania, Reed E. Pyeritz, MD, PhD, Joseph E. Bavaria, MD, Megan Morales; Weill Medical College of Cornell University, Craig T. Basson, MD, PhD, Richard Devereux, MD, Jonathan W. Weinsaft, MD, Deborah McDermott, MS, CGC; University of Michigan, Kim Eagle, MD; National Heart, Lung, and Blood Institute, H. Eser Tolunay, PhD, Patrice Desvigne-Nickens, MD, Mario P. Stylianou, PhD, Megan Mitchell, MPH; RTI International, Barbara L. Kroner, PhD, Donald Brambilla, PhD, Tabitha Hendershot, Danny Ringer, Meg Cunningham, Mark Kindem.

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Disclosures

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CLINICAL PERSPECTIVE

Recent evidence suggests that transforming growth factor-β (TGF-β) antagonism through losartan may be an effective treatment for Marfan syndrome (MFS). In the absence of empirical data, the dose of losartan or other medications currently used in people with MFS is based largely on dosing regimens for hypertension. Consideration of dosing may be particularly important because natural genetic variation in MFS can influence effective levels of and intrinsic response to drug therapy. This highlights the need for an informative biomarker—prognostic, therapeutic, or both—in MFS. We report that a genetically defined mouse model of MFS, which recapitulates many manifestations of the disease including progressive aortic root dilatation, shows elevated circulating TGF-β concentrations. Furthermore, circulating TGF-β levels show close correlation with aortic root dimension in mice, diminish on treatment with losartan in a dose-dependent manner, and are fully normalized in mice that show a robust therapeutic response. As in the mouse model, we observed elevated circulating TGF-β concentrations in people diagnosed with MFS, and those levels decreased after treatment with losartan or β-blocker. However, we did not observe a close correlation between circulating TGF-β levels and the aortic root size or Z scores in humans. The use of circulating TGF-β to individualize patient counseling and management is appealing because the assay is monitoring a central and direct effector of disease progression. Even if there is little intrinsic prognostic value to a snapshot measurement of circulating TGF-β, observing TGF-β over time in the individual patient might yield important information on progression of the disease, response to therapy, or both.