Recent advances in understanding Marfan syndrome: Should we now treat surgical patients with losartan?

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Objective: Marfan syndrome is a systemic connective tissue disorder caused by mutations in the fibrillin-1 gene. It was originally believed that Marfan syndrome results exclusively from the production of abnormal fibrillin-1 that leads to structurally weaker connective tissue when incorporated into the extracellular matrix. This effect seemed to explain many of the clinical features of Marfan syndrome, including aortic root dilatation and acute aortic dissection, which represent the main causes of morbidity and mortality in Marfan syndrome.

Methods: Recent molecular studies, most based on genetically defined mouse models of Marfan syndrome, have challenged this paradigm. These studies established the critical contribution of fibrillin-1 haploinsufficiency and dysregulated transforming growth factor-beta signaling to disease progression.

Results: It seems that many manifestations of Marfan syndrome are less related to a primary structural deficiency of the tissues than to altered morphogenetic and homeostatic programs that are induced by altered transforming growth factor-beta signaling. Most important, transforming growth factor-beta antagonism, through transforming growth factor-beta neutralizing antibodies or losartan (an angiotensin II type 1 receptor antagonist), has been shown to prevent and possibly reverse aortic root dilatation, mitral valve prolapse, lung disease, and skeletal muscle dysfunction in a mouse model of Marfan syndrome.

Conclusion: There are indicators that losartan, a drug widely used to treat arterial hypertension in humans, offers the first potential for primary prevention of clinical manifestations in Marfan syndrome.

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Marfan syndrome (MFS) is a systemic connective tissue disorder that affects 1 in 5000 individuals.[1] It is inherited as an autosomal dominant trait and displays a variety of clinical manifestations in the ocular, musculoskeletal, and cardiovascular systems. Aortic root aneurysm and subsequent aortic dissection are the leading cause of morbidity and mortality in those with MFS. The majority of fatal events occur in untreated patients in early adult life.[2] Therefore, early diagnosis and advances in surgical treatment, in particular the Bentall procedure and more recently the valve-sparing procedure, have significantly improved life expectancy in MFS.[3] A commonly used medical therapy is beta-adrenergic blockade; however, its efficacy in slowing the aortic root growth and preventing aortic dissection remains controversial.[4] Recent advances in understanding the complex molecular pathogenesis of MFS have challenged the definition of MFS as a structural disorder of the connective tissue. Several studies showed that MFS is more a developmental abnormality with broad and complex effects on the morphogenesis and function of multiple organ systems.[5-8] Such findings present a far more optimistic outlook for patients with MFS, in both pre- and postoperative states, because there is the potential to modify the progression of the disease. A

Abbreviations and Acronyms
AT1 = angiotensin II type 1 receptor
AT2 = angiotensin II type 2 receptor
MFS = Marfan syndrome
TGF = transforming growth factor

recent study showed the efficacy of such therapeutic strategies in a mouse model of MFS.[7] In this review, we describe the most recent progress in understanding the molecular pathogenesis of MFS and discuss its possible clinical implications.

Genetics of Marfan syndrome

MFS is caused by mutations in the gene encoding fibrillin-1 (*Fbn1*). Most mutations in fibrillin-1 occur within repeated epidermal growth factor-like domains, many disrupting one of the cysteine residues that interact via disulfide linkage and determine ternary structure or amino acid residues affecting calcium binding.[9,10] Such perturbations lead to enhanced proteolytic degradation and malfunction of fibrillin-1. To date, more than 800 mutations in *Fbn1* have been reported in individuals with MFS; however, most families with MFS have private mutations.[11] Fibrillin-1, a 350-kDa glycoprotein, is a principal component of the extracellular matrix microfibril.[12] Fibrillin-rich microfibrils are present in all tissues with clinical manifestations of MFS, for example, the ocular system, aortic root, and musculoskeletal system. MFS was originally believed to result from the production of mutant fibrillin-1 that results in structurally weaker connective tissue when incorporated into the microfibrils. This adverse effect seemed to explain many of the typical clinical phenotypes of MFS, including aortic root dilatation, aortic dissection, lens dislocation, and joint laxity. However, it did not fully explain all disease manifestations associated with MFS. For example, why would a weakness in the structure of the connective tissue lead to bone overgrowth, facial features of MFS, thickening of the heart valves, or low muscle mass or fat stores? Recent studies, mainly based on the creation and analysis of genetically engineered mouse models, revealed new and promising insights into the complex pathogenesis of MFS.

Mouse models of Marfan syndrome

Several mouse models with different mutations in the *Fbn1* gene have been developed.[13,14] Many of these mouse lines display typical manifestations of MFS, including aortic root aneurysm and subsequent aortic dissection, mitral valve thickening and prolapse, lung emphysema, and long-bone overgrowth. First insights into the pathogenesis of MFS came from a mouse strain with a mutant allele producing structurally normal fibrillin-1 protein, but at 15% of the normal level.[15] Heterozygous mice showed no abnormalities throughout life, whereas homozygous mice died of aortic dissection between 3 and 6 months of age. Histologic analyses revealed that these mice had a normal content and architecture of elastic fibers at birth. The first detectable change in the aortic media was the absence of connecting filaments, which are bundles of microfibrils that connect elastic fibers to adjacent cells.[16] The loss of these connections between vascular smooth muscle cells and elastic fibers is associated with increased secretion of extracellular matrix proteins and matrix-degrading enzymes, including matrix metalloproteinases 2 and 9. Later events, including increased elastolysis and the recruitment of inflammatory cells, lead to structural collapse of the aortic wall, aortic root dilatation, and subsequent aortic dissection.[17,18] Concordant changes were found in aortic root specimens from patients with MFS.[19] Judge and colleagues[6] found that transgenic overexpression of fibrillin-1 harboring a missense mutation, in the context of 2 normal *Fbn1* genes, was insufficient to produce clinical features of MFS in mice. In contrast, mice heterozygous for a comparable *Fbn1* missense mutation showed aortic changes similar to those seen in other mouse lines and people with MFS.[6] These data suggest that a decrease in the amount of normal fibrillin-1, independent of the production of mutant protein, plays a critical role in the pathogenesis of MFS. Matyas and colleagues[20] reached similar conclusions through human genetic studies. Hutchinson and colleagues[21] described a family with MFS in whom phenotypic severity correlated inversely with the expression level of non-mutant fibrillin-1. These data raised the possibility that boosting fibrillin-1 expression in patients with MFS could be a therapeutic strategy.

Regulatory role of fibrillin-1 and transforming growth factor-beta

Recent studies showed that extracellular microfibrils containing fibrillin-1 not only have an important structural function but also interact with and regulate the transforming growth factor (TGF)-beta family of growth factors. Fibrillin-1 shares a high degree of homology with the latent TGF-beta binding proteins.[22] TGF-beta is secreted in the context of a large latent complex that includes 1 of 3 latent TGF-beta–binding proteins. The latent TGF-beta–binding protein component of the large latent complex targets the complex to the matrix and interacts directly with fibrillin-1. These findings led to the hypothesis that fibrillin-1 may participate in the regulation of TGF-beta signaling, with known influences on cellular proliferation, differentiation, synthetic repertoire, and survival in a variety of cell types. Neptune and colleagues[5] revealed such an interaction when investigating lung tissue from newborn mice deficient in fibrillin-1. These mice showed a widening in the distal airspaces at birth with no evidence of inflammation or destruction. Therefore, they hypothesized that such lung

lesions may be due to primary failure of alveolar septation during development, as opposed to simple mechanical failure of a structurally deficient tissue.[5] Similar pulmonary lesions have been reported in patients with MFS for years but were attributed to the effects of increased physiologic stress acting on a "weak" tissue. Neptune and colleagues further revealed that changes in the lung tissue were associated with excess free TGF-beta and increased intracellular TGF-beta signaling. Most important, prenatal administration of TGF-beta–neutralizing antibodies significantly reduced TGF-beta activity in the tissue and resulted in a dose-dependent increase in alveolar septation.[5] These findings suggested that TGF-beta has a crucial role in regulating alveolar septation and that up-regulation of growth factor activity may contribute to disease progression in MFS. Neptune and colleagues hypothesized that a similar pathogenetic mechanism could underlie other manifestations of MFS. Ng and colleagues[23] addressed changes in mitral valves from mice deficient in fibrillin-1 and revealed postnatally acquired alterations, including an increase in valve length and thickness. These findings correlated with increased cell proliferation, increased collagen production, decreased apoptosis in valve leaflets, and excessive TGF-beta activation and TGF-beta signaling. Again, prenatal administration of TGF-beta–neutralizing antibodies led to a significant reduction in TGF-beta activity and improved postnatal valve architecture.

Aortic root aneurysm and effect of losartan

Mice heterozygous for a mutant *Fbn1* allele (C1039G/+) develop aortic root dilatation as early as 2 weeks of age with progression throughout life.[6] The aortic wall in young *Fbn1* mice is already thickened, and elastic fibers are fragmented and disarrayed.[7] Furthermore, there is excessive free TGF-beta and TGF-beta signaling in the aortic root tissue, which is also found in aortic specimens from patients with MFS.[7] Habashi and colleagues[7] hypothesized that TGF-beta may play a causal role in aortic root dilatation, as previously shown for lung lesions and mitral valve changes in MFS. Postnatal treatment of fibrillin-1–deficient mice with TGF-beta neutralizing antibody led to a reduction in TGF-beta signaling in the aortic tissue and significantly reduced elastic fiber fragmentation, thickening of the aortic wall, and aortic root growth.[7] Taken together, the data showed that increased TGF-beta signaling contributes to the process of aortic root dilatation in MFS and that TGF-beta antagonism may represent a productive treatment strategy. Unfortunately, the application of TGF-beta–neutralizing antibodies in patients with MFS is not yet practical. Losartan, an angiotensin II type 1 receptor (AT1) antagonist, came to attention because of its known effect in antagonizing TGF-beta in human and animal models of chronic renal insufficiency and cardiomyopathy.[24,25] Losartan is a Food and Drug Administration-approved medication and is widely used medication to decrease blood pressure, a desirable effect in individuals with MFS and aortic root aneurysm. Habashi and colleagues[7] undertook a blinded randomized study comparing the efficacy of losartan with propranolol, which is representative of beta-blocking agents widely used in patients with MFS, and placebo. The doses of beta-blockade and losartan were titrated to achieve a comparable blood pressure response. Treatment was started beginning at 7 weeks, after echocardiographic documentation of aortic root dilatation. Fibrillin-1–deficient mice treated with propranolol showed a reduction in the aortic root growth rate during a 6-month period compared with the placebo mice; however, the growth rate was still significantly greater than in wild-type mice.[7] However, mice treated with losartan could not be distinguished from wild-type mice by any parameter, including absolute aortic root size, aortic root growth rate, aortic wall thickness, or histologic architecture (Figures 1 and 2).[7] Habashi and colleagues[7] concluded that propranolol may diminish the aortic root growth in MFS but does not prevent progressive deterioration of the aortic wall, which corresponds to clinical observations in patients with MFS. Most important, losartan maintained the aortic wall structure in *Fbn1* mice and fully prevented aortic root dilatation. One could argue that losartan may achieve a superior protection over propranolol by virtue of increased blunting of the hemodynamic shear stress on the aortic root. However, this was not the case because doses of losartan and propranolol were titrated to achieve comparable hemodynamic effects, and isolated antagonism of TGF-beta with neutralizing antibody provided similar protection. In addition, losartan antagonized TGF-beta in the aortic wall, an event seen in mice treated with TGF-beta–neutralizing antibody but not in mice treated with propranolol, and further improved manifestations of MFS in the lungs and skeletal muscle, which could not be related to altered hemodynamics.[7]

The mechanisms through which AT1 blockade antagonizes TGF-beta in the aortic wall are not known in detail. Everett and colleagues[26] showed that signaling through the AT1 receptor increases expression of TGF-beta ligands and receptors. AT1 signaling also stimulates the proliferation of vascular smooth muscle cells and vessel-wall fibrosis, although this may depend on the specific vascular segment.[27] Recent work from Daugherty and colleagues[28] showed that signaling through the angiotensin II type 2 receptor (AT2) antagonizes many of the effects promoted by AT1 signaling in the formation of abdominal aortic aneurysm. Indeed, the use of a selective AT2 blocker increased both the incidence and the severity of abdominal aortic aneurysms in ApoE null mice after angiotensin II infusion, whereas AT1 blockade prevented aneurysms. Therefore, selective AT1 antagonism, as achieved with losartan, may be preferable to

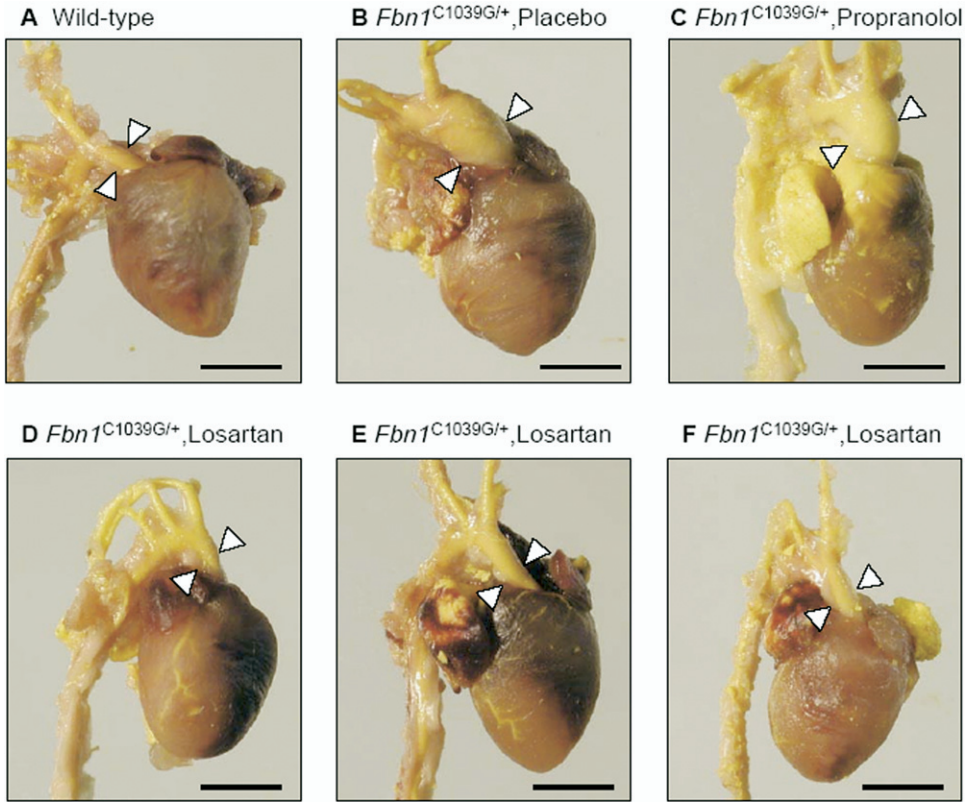

Figure 1. Inspection of ascending aortic dimension (*arrowheads*) after latex injection in representative wild-type (A) and *Fbn1* (C1039G/+) mice treated prenatally with placebo (B), propranolol (C), or losartan (D–F). Scale bars (A–F) = 4 mm. From Habashi JP, Judge DP, Holm TM, Cohn RD, Loeys BL, Cooper TK, et al. Losartan, an AT1 antagonist, prevents aortic aneurysm in a mouse model of Marfan syndrome. *Science.* 2006;312:117-21. Reprinted with permission from the American Association for the Advancement of Science.

dual AT1/AT2 blockade as achieved with angiotensin-converting enzyme inhibitors. However, the positive effect of losartan on TGF-beta expression may also depend on a variety of unknown feedback and signal-cascade mechanisms.

Should we now treat patients who have Marfan syndrome with losartan?

The current data indicate that patients with MFS are likely to benefit from being treated with losartan. As such, it may be tempting to treat all patients who have MFS with losartan, given the safety profile of this Food and Drug Administration-approved medication. However, we believe that a cautious approach is needed because data from *Fbn1*-targeted mice, although promising, are preliminary. More studies are needed to address important questions. For example, will the losartan effect be consistent and significantly better than that achieved with a beta-blocker over the long term? Will there be unanticipated side effects in patients with MFS? What is the consequence of stopping losartan? This is an important question given the contraindication to the use of angiotensin receptor blockers during pregnancy. Studies addressing the efficacy of beta-blockade in MFS have concluded that the therapy is successful in a subset of individuals.[4,29] Overall, medicated patients showed slower aortic root growth, fewer cardiovascular end points, and improved survival. Such therapeutic benefit has been observed in all age groups, including young children. It is important to point out, however, that beta-blockade does not stop or reverse aortic root dilatation but typically slows the aortic root growth in patients with MFS. Beta-blockers have no effect on other clinical manifestations of MFS, and 10% to 20% of patients are intolerant to beta-blockers because of asthma, depression, or fatigue.[1] In contrast, losartan may offer the first potential treatment for the primary prevention of phenotypes associated with MFS, including life-threatening aortic root dilatation and aortic dissection, mitral valve changes, lung disease, and skeletal myopathy. A multicenter randomized clinical trial comparing losartan with beta-blocker therapy in children and young adults with MFS

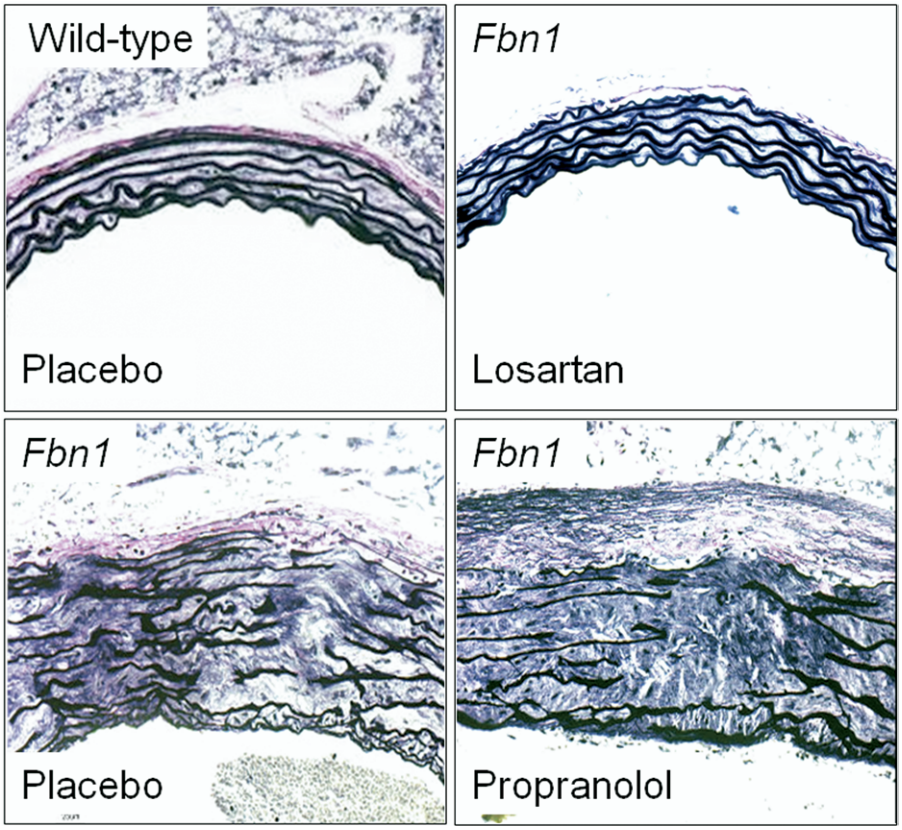

Figure 2. Postnatal treatment with losartan, propranolol, or placebo. Verhoeff–van Gieson staining: Elastic lamellae are intact and the aortic wall is of normal thickness in wild-type and losartan-treated *Fbn1* (C1039G/+) mice. In contrast, placebo- and propranolol-treated *Fbn1* mice have fragmented and disarrayed elastic fibers, and the aortic wall is thickened.

and aortic aneurysm has recently been started (coordinated through the Pediatric Heart Network of the National Heart, Lung, and Blood Institute of the National Institutes of Health) and will aim to answer many of these questions, including the long-term efficacy of losartan and possible side effects in this particular subset of patients. The study aims to recruit at least 600 patients in a 3-year time period. It is also critical to determine whether there is the potential to reverse damage, for example, aortic root dilatation and mitral valve changes associated with MFS. Early indicators in mouse models suggest that this may be true. Less can be said about the potential for losartan therapy to protect segments of the aorta that have already been damaged by chronic dissection. Will antagonizing the profibrotic and/or anti-inflammatory effects of TGF-beta limit the quality of scar formation or promote further destruction through inflammatory cell recruitment, or will it limit disease progression by decreasing the expression of matrix-degrading enzymes, among other potential beneficial effects? As people with MFS are living longer, a later predisposition for dilatation and dissection of other aortic segments, prominently the proximal descending aorta, is becoming apparent. Can we expect a similar therapeutic effect of losartan? Clearly, more research is needed to address these important issues.

Up-regulation of TGF-beta seems to be a final common pathway for aortic aneurysm in many disease states. Therefore, if losartan is effective in treating clinical manifestations in patients with MFS, this could prove a valid treatment strategy for other aneurysm syndromes, including patients with TGF-beta receptor mutations (Loeys–Dietz syndrome). This would be particularly important because Loeys–Dietz syndrome is associated with aggressive vascular disease, including aneurysms throughout the arterial tree and dissection at young ages and at relatively small vascular dimensions that do not confer risk in MFS.[30,31] Despite the promising nature of the results presented in this review, it remains clear that caution and careful study are needed to productively transfer these data from bench to bedside. Such efforts may be rewarded by an improved management of patients with MFS. If and how these promising findings will influence future cardiovascular surgical practice remain to be elucidated.

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