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Marfan Syndrome: From Molecules to Medicines*

Harry C. Dietz

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I would like to thank the American Society of Human Genetics for this great honor. To receive professional recognition is always gratifying, but to be earmarked for distinction by a group of people that I so thoroughly admire and respect is truly extraordinary.

Today, I will talk about my journey with Marfan syndrome (MFS). While reflecting about my career during the preparation for this talk, it was overtly clear to me that I have simply been blessed, both by my association with an outstanding institution and, more importantly, with outstanding people. While training as a pediatric cardiologist, I developed a strong bond with a large population of individuals with MFS who were followed at Johns Hopkins. With conviction that I could best help these people through research, I approached Victor McKusick, Clair Fancomano, and Reed Pyeritz regarding potential laboratory opportunities. Despite my lack of research experience—I could guess correctly at the time that A went with T and G with C about 25% of the time (I won't dwell on the fact that this is less success than predicted by chance)—I was welcomed with open arms. If this remarkable response set the foundation for my career, the frame was established by my colleagues at Johns Hopkins, with particular thanks to Dave Valle and Haig Kazazian for providing guidance and inspiration. In keeping with the "career as house" analogy, the incredible young people who have trained in my lab have been the bricks—I will try to highlight some of their brilliant contributions throughout this talk—and the people with MFS and related disorders whom I care for have been the mortar. I must also recognize the neighbors, my collaborators at other institutions. In particular, without the keen insight and scientific generosity of Francesco Ramirez at a vulnerable point in my career, I can assure you that I would not be here today.

When I arrived to the lab in 1990, it was known that elastic tissues in MFS show fragmentation and disarray, with the accumulation of amorphous matrix between fibers. Pioneering work by Lynn Sakai, David Hollister, Reed Pyeritz, and Peter Byers demonstrated a deficiency of the connective-tissue protein fibrillin-1 in patient tissues and abnormalities in fibrillin-1 synthesis, secretion, and/or matrix deposition by cultured cells.[1,2] In 1991, a positional-candidate approach culminated with the knowledge that mutations in the fibrillin-1 gene (*FBN1*) on chromosome 15 cause MFS.[3–5] Fibrillin-1 monomers aggregate to form complex extracellular structures called "microfibrils" that cluster at the margins of maturing elastic fibers during embryogenesis. Early pathogenetic models for MFS focused on structural weakness of the tissues imposed by microfibrillar deficiency and a postulated consequent failure of elastogenesis. This boded poorly for the development of productive treatment strategies. The implication was that children with MFS are born without sufficient elastic fibers and therefore have an obligate structural predisposition for tissue failure later in life. In collaboration with Francesco Ramirez, our subsequent studies, with use of genetically defined animal models of MFS, demonstrated that fibrillin-1 is not needed for elastogenesis, as previously inferred, but, rather, is critical for elastic-fiber maintenance in postnatal life.[6,7] Elastic-fiber breakdown correlated both temporally and spatially with a number of predictable events, including elastic-fiber calcification, local recruitment of inflammatory cells, and increased expression of selected matrix-degrading enzymes (specifically, matrix metalloproteinases [MMPs]−2 and −9) by resident vascular smooth-muscle cells.[8] Nevertheless, models of disease pathogenesis continued to singularly invoke an acquired weakness of the tissues. In retrospect, there are many manifestations of MFS that are difficult or impos-

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sible to reconcile with such models. For example, why should weakness of the tissues cause overgrowth of the bones, myxomatous valve changes, craniofacial dysmorphism, low muscle mass, or reduced fat stores? These findings more plausibly relate to abnormalities of cellular performance (proliferation, migration, and/or programmed death).

A breakthrough in our understanding of the pathogenesis of MFS came while studying lung disease in mouse models. The prior assumption was that air-space widening simply reflected stress acting on a biomechanically fragile tissue. The expectation was that the mice would show normal lungs at birth, with gradual evidence of tissue destruction and inflammation, analogous to traditional destructive emphysema. Instead, we saw diffuse widening of the distal air space at birth due to failure of septation of the prealveolar saccule, with no associated destructive or inflammatory changes.[9] In some manner, a deficiency of the structural protein fibrillin-1 was impeding the developmental signal for this morphogenetic event. We reasoned that perhaps this related to altered regulation of and signaling by the TGFβ family of cytokines. This hypothesis was based on the earlier observation that the fibrillins show significant homology to the latent TGFβ-binding proteins (LTBPs).[10–12] It was known that TGFβ is secreted form the cell in the context of a large latent complex (LLC) composed of the mature cytokine, a dimer of its processed N-terminal propeptide (latency-associated peptide [LAP]), and one of three LTBPs. It was also known that the LLC binds to the matrix, but the binding partner was unknown, as was the significance of this event. We reasoned that perhaps microfibrils bind to LTBPs, a hypothesis that was biochemically validated by Lynn Sakai, Dan Rifkin, and colleagues,[13,14] and that microfibrillar deficiency (as in MFS) would result in inadequate matrix sequestration, with consequent promiscuous activation of TGFβ. Enid Neptune and Pam Frischmeyer, in my lab, found increased free TGFβ in the fibrillin-1–deficient lung in association with reduced LAP (suggesting excessive activation rather than production of TGFβ) and increased activity of a transgenic TGFβ reporter allele.[9] Finally, we showed that systemic administration of TGFβ-neutralizing antibody rescued lung septation in mouse models of MFS, providing evidence of a cause-and-effect relationship.[9] Our subsequent experiments showed that the same process underlies other phenotypic manifestations of MFS. Connie Ng and Dan Judge showed that myxomatous changes of the mitral valve correlate with increased TGFβ signaling, increased output of TGFβ-responsive genes (including collagens), excessive cellular proliferation, and reduced apoptosis in valve leaflets.[15] Once again, TGFβ-neutralizing antibody rescued phenotype.[15] Ronni Cohn went on to show that the low muscle mass and muscle weakness in MFS reflects failure of muscle regeneration due to TGFβ-induced failure of satellite cells to proliferate and differentiate in response to injury or physiologic signals for hypertrophy.[16] Short-term administration of TGFβ-neutralizing antibody restores satellite-cell performance, muscle regeneration, steady-state muscle architecture, and muscle strength.

The aortic wall in mouse models of MFS shows the predictable sequence of elastic-fiber fragmentation, aortic-wall thickening with excess matrix deposition (including collagens, elastin, and proteoglycans), and excessive expression of MMP-2 and MMP-9. Vacular smooth muscle cells show nuclear enrichment of phosphorylated Smad2 (p-Smad2), a direct marker of TGFβ signaling, and increased output of TGFβ-responsive gene products, such as connective-tissue growth factor (CTGF).[17] In a randomized and blind trial of TGFβ-neutralizing antibody, Jennifer Habashi and Dan Judge saw a reduced rate of aortic-root growth and improved aortic-wall architecture—including improved elastic-fiber maintenance, reduced thickness of the aortic wall, and decreased collagen deposition—in mouse models.[17]

In an attempt to become more clinically relevant, we asked, "Is there a drug?" and, even better, "Is there an FDA-approved drug?" that might mimic the effects of TGFβ-neutralizing antibody. Our attention turned to losartan, an angiotensin II type 1 receptor (AT1) blocker (ARB) that reduces blood pressure, something that we believe is good for people with aortic enlargement, and that also had been shown to reduce fibrosis, at least in part through antagonism of TGFβ, in animal models of chronic renal disease.[18,19] In a blind and placebo-controlled trial in mice, Jennifer Habashi and Dan Judge showed that losartan provided remarkable protection, with normalized aortic-root growth and aortic dimension and with aortic-wall architecture that was indistinguishable from that seen in wild-type mice[17] (fig. 1). Evidence suggests that AT1 blockade reduces expression of TGFβ ligands and receptors and also limits the production of potent activators of TGFβ, such as thrombospondin-1.[20–22] Remarkably, losartan also rescued other manifestations of MFS in mouse models, including pulmonary alveolar septation[9] and muscle regeneration.[16] In these tissues, we have observed direct (reduced pSmad2) and indirect (reduced collagen and CTGF expression) evidence of substantial TGFβ antagonism in vivo in response to losartan.

Taken together, these data left us with the firm convictions that (1) most of the multisystem manifestations of MFS relate to excess TGFβ signaling, (2) TGFβ antagonism is a productive treatment strategy for MFS, and (3) Marfan mouse models could provide a valuable resource for investigating the roles of TGFβ in tissue development and homeostasis, with relevance to more-common and nonsyndromic presentations of relevant phenotypes, and could be utilized to assess the therapeutic value of other strategies aimed at TGFβ antagonism.

Around this time, Bart Loeys (one of the most exceptional physician-scientists that I have encountered) and I recognized a novel autosomal dominant aortic-aneurysm syndrome (Loeys-Dietz syndrome [LDS {MIM 609192}]) characterized, in 10 families, by the triad of hypertelorism,

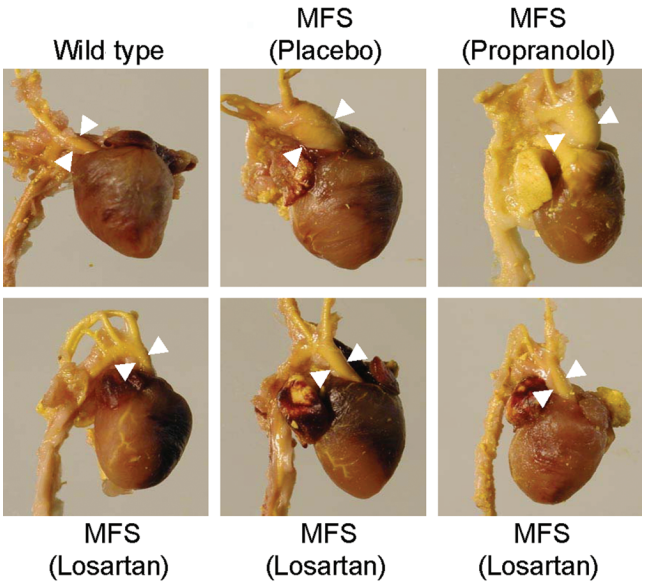

Figure 1. Representative images of heart and thoracic aorta from wild-type mice and mice harboring an MFS-associated missense mutation in the *Fbn1* gene. Note the dilated ascending aorta (*arrowheads*) in Marfan-affected mice that received either placebo or the β-adrenergic receptor–blocking agent propranolol (the current standard of care for people with MFS). In contrast, mutant mice that received losartan had normal ascending aortic dimensions. This figure was modified from the work of Habashi et al.[17]

bifid uvula/cleft palate, and arterial tortuosity with ascending aortic aneurysm and dissection[23] (fig. 2). This syndrome also presents with multiple other findings, including craniosynostosis, Arnold Chiari type I malformation, pectus deformity, scoliosis, arachnodactyly, club feet, patent ductus arteriosus, atrial septal defect, bicuspid semilunar valves, and aneurysms/dissections throughout the arterial tree.[23] Evaluation of a larger series of patients confirmed that the previously reported triad remains the most specific finding for this diagnosis.[24] This larger study also confirmed the increased incidence of additional findings, including developmental delay, hydrocephalus, congenital hip dislocation, dural ectasia, spondylolisthesis, cervical spine dislocation or instability, submandibular branchial cysts, osteoporosis with multiple fractures at a young age, and defective tooth enamel. When present, developmental delay did not always associate with either craniosynostosis or hydrocephalus, suggesting that learning disability is a rare primary manifestation. None of the patients had ectopia lentis, and dolichostenomelia, a finding that is typical in MFS, was both rare (18%) and subtle.

On the basis of the central role of TGFβ signaling in cardiovascular, skeletal, and craniofacial development, the genes encoding the TGFβ receptor (*TGFBR1* and *TGFBR2*) were considered as candidate genes. A prior report had suggested that heterozygous loss-of-function mutations in *TGFBR2* phenocopy MFS,[25] a phenotype with significant overlap with LDS (aortic-root aneurysm, arachnodactyly, pectus deformity, dural ectasia, and scoliosis). In our initial analysis of 10 patients with the classically severe presentation of LDS (including typical craniofacial features [i.e., LDS-I]) we found six mutations in *TGFBR2* and four in *TGFBR1*.[23] In a subsequent analysis of 40 patients with systemic features of both vascular Ehlers-Danlos syndrome (including joint laxity, easy bruising, dystrophic scars, translucent skin, organ rupture, and rupture of the gravid uterus) and LDS (e.g., arterial tortuosity and aneurysms throughout the arterial tree that tend to tear or rupture at small dimensions and at a young age) but less severe or absent craniofacial features (i.e., LDS-II), 12 had mutations in the *TGFBR* genes.[24] To date, we have identified *TGFBR1* or *TGFBR2* mutations in 71 probands with either type of LDS. With rare exceptions, these mutations involve the missense substitution of evolutionarily conserved residues in the serine-threonine kinase domain of either TGFβ receptor. However, a splice-site mutation in the extracellular domain and a nonsense mutation in the penultimate exon, predicted to derive a stable transcript but a protein product lacking the terminal half of the kinase domain, led to LDS phenotypes that are indistinguishable from those associated with missense mutations.

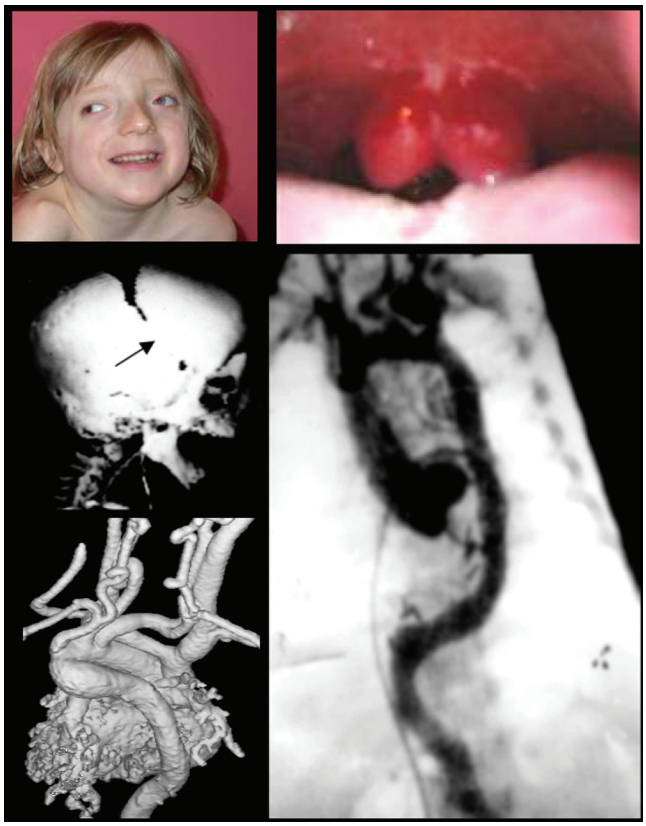

Figure 2. Features of Loeys-Dietz syndrome. Craniofacial features include hypertelorism, bifid uvula, and craniosynostosis (*arrow*). Cardiovascular features include arterial tortuosity and aneurysms throughout the arterial tree. This figure was modified from the works of Loeys et al.[23,24]

There are no differences in phenotype between individuals with mutations in *TGFBR1* and *TGFBR2* and no apparent phenotype-genotype correlations that explain the distinction between LDS-I and LDS-II. Furthermore, there are no apparent differences between the mutations that we and others have found in patients with LDS versus those reported as causing typical MFS or familial thoracic aortic aneurysm and dissection (FTAA).[23–31] Indeed, we have found many of the identical mutations reported as causing MFS or FTAA in patients with typical LDS-I or LDS-II.[23,24]

In the initial description of *TGFBR2* mutations causing MFS, it was observed that expression of mutant receptors in cells that were naive for TGFβ receptors could not support TGFβ signaling.[25] Furthermore, there was no apparent dominant negative interference on the function of coexpressed wild-type receptor. These data were interpreted to infer haploinsufficiency and consequent reduced TGFβ signaling as the relevant pathogenetic mechanisms.[25] In keeping with this hypothesis, one of the original MFS-like patients was shown to harbor a translocation breakpoint within the *TGFBR2* gene. Complicating this hypothesis, however, is the observation of a distinct paucity of nonsense or frameshift mutations in either of the TGFβ receptor genes in patients with LDS or related phenotypes. Furthermore, the only reported nonsense mutation occurs at the very distal margin of the penultimate exon.[24] As opposed to more-proximal nonsense mutations, this context is not predicted to induce nonsense-mediated mRNA decay (NMD) and clearance of the mutant transcripts.[32] As a result, most, if not all, mutations in the TGFβ receptor genes associated with vascular phenotypes are predicted to give rise to a mutant receptor protein that has the ability to traffic to the cell surface and bind extracellular ligand but that specifically lack the ability to propagate the intracellular TGFβ signal. Furthermore, a model that singularly invokes decreased TGFβ signaling would be difficult to reconcile with the substantial evidence that many aspects of MFS, including those that overlap with LDS, are caused by too much TGFβ signaling in animal models and can be attenuated or prevented by TGFβ antagonism in vivo.

We reasoned that perhaps experiments exploring TGFβ signaling in cells that express only mutant receptors were not informative for the situation in vivo when patients are heterozygous for these mutations and that perhaps diminished but not absent function of TGFβ receptors initiates chronic and dysregulated compensatory mechanisms that result in too much TGFβ signaling.[23] Indeed, the study of fibroblasts derived from heterozygous patients with LDS failed to reveal any defect in the acute-phase response to administered ligand and showed an apparent increase in TGFβ signaling after 24 hours of ligand deprivation and a slower decline in the TGFβ signal after restoration of ligand.[23] An even more informative result was the observation of increased nuclear accumulation of pSmad2 in the aortic wall of patients with either MFS or LDS and increased expression of TGFβ-dependent gene products such as collagen and CTGF.[23] Taken together, these data demonstrate increased TGFβ signaling in the aortic wall of LDS-affected patients in a context that is directly relevant to tissue development and homeostasis in vivo. Whereas the basis for this observation remains incompletely understood, it seems possible that dysregulation of signaling requires the cell-surface expression of receptors that can bind TGFβ ligands but that can't propagate signal due to a deficiency in kinase function. In support of this hypothesis, it was shown that transgenic expression of a mutant, kinase domain-deleted form of TβRII leads to increased TGFβ signaling, including stimulation of the intracellular signaling cascade and increased output of TGFβ-responsive genes.[33] This is clearly suggestive of a gain-of-function mechanism for mutant TGFβ receptors in LDS.

In theory, some aspects of the MFS phenotype might be caused by altered regulation of TGFβ signaling, whereas others might relate to a deficiency of the structural role of fibrillin-1. A comprehensive consideration of the composite LDS phenotype is very informative in this regard.[23,24] The stark observation is that nearly all manifestations of MFS can be seen in patients with LDS. LDS-affected patients do not develop lens dislocation and do not tend to have the same degree of long-bone overgrowth, generally showing stature that is similar to their unaffected family members. Under the assumption that all LDS manifestations reflect altered TGFβ signaling, these data suggest that most manifestations of MFS will be amenable to therapeutic strategies aimed at modulating the activity of TGFβ. Preliminary studies performed by Ben Brooke suggest that losartan can also productively modulate aortic-root growth in children with severe and rapidly progressive MFS (H.C.D. and B. Brooke, unpublished data). On the basis of our studies, the Pediatric Heart Network of the National Heart, Lung, and Blood Institute has launched a large multicenter clinical trial of losartan in people with MFS.

The relevance of this work may extend beyond patients with MFS and related connective-tissue disorders. For example, it is interesting to note that the mechanisms of two aortic aneurysm syndromes (MFS and LDS) converge on the TGFβ-signaling pathway. Recently, in a collaboration led by Paul Coucke and Anne DePaepe, it was demonstrated that loss of function of the facilitative glucose transporter glut10 causes arterial tortuosity syndrome (ATS), another systemic vasculopathy.[34] On the basis of overlap with LDS, it was reasoned that perhaps LDS also reflects too much TGFβ activity. We observed increased TGFβ signaling in the vessel wall of ATS-affected patients and near-absent expression of decorin, a negative regulator of TGFβ signaling that shows glucose-dependent expression.[34] Mark Halushka, in the lab, is now investigating whether altered TGFβ signaling underlies other syndromic and nonsyndromic presentations of aortic aneurysm. With similar reasoning, Ronni Cohn has now performed studies to show that excessive TGTβ signaling contributes to failed muscle regeneration and fibrosis in a mouse model of Duchenne

muscular dystrophy and that losartan can preserve skeletal muscle architecture and performance in dystrophin-deficient mice.[16] Our unpublished data suggest that the failed ability for muscle regeneration to keep pace with muscle destruction in selected forms of muscular dystrophy may also respond to TGFβ antagonism with losartan.

The view that the pathogenesis of MFS simply reflects excessive TGFβ activity is undoubtedly an oversimplification. It has proven, however, a tractable and productive reduction of available data. If there are lessons that can be generalized from our study of MFS, from the perspective of a clinician who happens to do research to improve the length and quality of life of patients, they would include (1) ask clinically relevant questions; (2) keep your eye on the prize (for MFS, the aorta), but explore the use of other systems that might provide a more immediate and robust readout for the elucidation of mechanisms or testing of therapies (e.g., lung septation); (3) look for net effects, even if all intermediate steps are not understood; (4) ameliorate, if not "cure"; (5) focus on "translatable" strategies; (6) make aggressive use of animal models; (7) perform clinical trials, when feasible and appropriate; (8) engage the participation of individuals from diverse disciplines, to explore the expanded relevance of new insights; and, most importantly, (9) recruit and promote trainees who are much smarter than you are. For those contemplating combined M.D.-Ph.D. training, I must stress that every important hypothesis in our work derived directly from a clinical encounter.

I would like to acknowledge the William S. Smilow Center for Marfan Syndrome Research, the National Marfan Foundation, the National Institutes of Health, and the Howard Hughes Medical Institute for providing substantial funding, often discretional, which allowed us to explore all possibilities, not just the derivative "next step." I again stress my deep gratitude to my patients, who have given of themselves, both literally and figuratively, to make this work possible and who provide enduring inspiration and motivation. Finally, I thank my wife (and medical geneticist), Ada Hamosh, and my daughter, Nina Dietz, for tolerating both my absent minded–professor routine and the fact that anxiety is my connective tissue. Once again, I thank you all for this great honor.

Web Resource

The URL for data presented herein is as follows:

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim/ (for LDS)
Pediatric Heart Network, http://www.pediatricheartnetwork.org/marfan.asp

References

1. Hollister DW, Godfrey M, Sakai LY, Pyeritz RE (1990) Immunohistologic abnormalities of the microfibrillar-fiber system in the Marfan syndrome. N Engl J Med 323:152–159
2. Milewicz DM, Pyeritz RE, Crawford ES, Byers PH (1992) Marfan syndrome: defective synthesis, secretion, and extracellular matrix formation of fibrillin by cultured dermal fibroblasts. J Clin Invest 89:79–86
3. Kainulainen K, Pulkkinen L, Savolainen A, Kaitila I, Peltonen L (1990) Location on chromosome 15 of the gene defect causing Marfan syndrome. N Engl J Med 323:935–939
4. Dietz HC, Pyeritz RE, Hall BD, Cadle RG, Hamosh A, Schwartz J, Meyers DA, Francomano CA (1991) The Marfan syndrome locus: confirmation of assignment to chromosome 15 and identification of tightly linked markers at 15q15-q21.3. Genomics 9:355–361
5. Dietz HC, Cutting GR, Pyeritz RE, Maslen CL, Sakai LY, Corson GM, Puffenberger EG, Hamosh A, Nanthakumar EJ, Curristin SM, et al (1991) Marfan syndrome caused by a recurrent de novo missense mutation in the fibrillin gene. Nature 352: 337–339
6. Pereira L, Andrikopoulos K, Tian J, Lee SY, Keene DR, Ono R, Reinhardt DP, Sakai LY, Biery NJ, Bunton T, et al (1997) Targetting of the gene encoding fibrillin-1 recapitulates the vascular aspect of Marfan syndrome. Nat Genet 17:218–222
7. Pereira L, Lee SY, Gayraud B, Andrikopoulos K, Shapiro SD, Bunton T, Biery NJ, Dietz HC, Sakai LY, Ramirez F (1999) Pathogenetic sequence for aneurysm revealed in mice underexpressing fibrillin-1. Proc Natl Acad Sci USA 96:3819–3823
8. Bunton TE, Biery NJ, Myers L, Gayraud B, Ramirez F, Dietz HC (2001) Phenotypic alteration of vascular smooth muscle cells precedes elastolysis in a mouse model of Marfan syndrome. Circ Res 88:37–43
9. Neptune ER, Frischmeyer PA, Arking DE, Myers L, Bunton TE, Gayraud B, Ramirez F, Sakai LY, Dietz HC (2003) Dysregulation of TGF-β activation contributes to pathogenesis in Marfan syndrome. Nat Genet 33:407–411
10. Moren A, Olofsson A, Stenman G, Sahlin P, Kanzaki T, Claesson-Welsh L, ten Dijke P, Miyazono K, Heldin CH (1994) Identification and characterization of LTBP-2, a novel latent transforming growth factor-β-binding protein. J Biol Chem 269:32469–32478
11. Dallas SL, Miyazono K, Skerry TM, Mundy GR, Bonewald LF (1995) Dual role for the latent transforming growth factor-β binding protein in storage of latent TGF-β in the extracellular matrix and as a structural matrix protein. J Cell Biol 131:539–549
12. Yin W, Smiley E, Germiller J, Mecham RP, Florer JB, Wenstrup RJ, Bonadio J (1995) Isolation of a novel latent transforming growth factor-β binding protein gene (LTBP-3). J Biol Chem 270:10147–10160
13. Dallas SL, Keene DR, Bruder SP, Saharinen J, Sakai LY, Mundy GR, Bonewald LF (2000) Role of the latent transforming growth factor beta binding protein 1 in fibrillin-containing microfibrils in bone cells in vitro and in vivo. J Bone Miner Res 15:68–81
14. Isogai Z, Ono RN, Ushiro S, Keene DR, Chen Y, Mazzieri R, Charbonneau NL, Reinhardt DP, Rifkin DB, Sakai LY (2003) Latent transforming growth factor β-binding protein 1 interacts with fibrillin and is a microfibril-associated protein. J Biol Chem 278:2750–2757
15. Ng CM, Cheng A, Myers LA, Martinez-Murillo F, Jie C, Bedja D, Gabrielson KL, Hausladen JM, Mecham RP, Judge DP, et al (2004) TGF-β-dependent pathogenesis of mitral valve prolapse in a mouse model of Marfan syndrome. J Clin Invest 114:1586–1592
16. Cohn RD, van Erp C, Habashi JP, Soleimani AA, Klein EC,

Lisi MT, Gamradt M, ap Rhys CM, Holm TM, Loeys BL, et al (2007) Angiotensin II type 1 receptor blockade attenuates TGF-β-induced failure of muscle regeneration in multiple myopathic states. Nat Med 13:204–210 (erratum 13:511)

17. Habashi JP, Judge DP, Holm TM, Cohn RD, Loeys BL, Cooper TK, Myers L, Klein EC, Liu G, Calvi C, et al (2006) Losartan, an AT1 antagonist, prevents aortic aneurysm in a mouse model of Marfan syndrome. Science 312:117–121
18. Shihab FS, Bennett WM, Tanner AM, Andoh TF (1997) Angiotensin II blockade decreases TGF-beta1 and matrix proteins in cyclosporine nephropathy. Kidney Int 52:660–673
19. Khalil A, Tullus K, Bakhiet M, Burman LG, Jaremko G, Brauner A (2000) Angiotensin II type 1 receptor antagonist (losartan) down-regulates transforming growth factor-β in experimental acute pyelonephritis. J Urol 164:186–191
20. Chua CC, Hamdy RC, Chua BH (1997) Regulation of thrombospondin-1 production by angiotensin II in rat heart endothelial cells. Biochim Biophys Acta 1357:209–214
21. Naito T, Masaki T, Nikolic-Paterson DJ, Tanji C, Yorioka N, Kohno N (2004) Angiotensin II induces thrombospondin-1 production in human mesangial cells via p38 MAPK and JNK: a mechanism for activation of latent TGF-β_1. Am J Physiol Renal Physiol 286:F278–F287
22. Zhou Y, Poczatek MH, Berecek KH, Murphy-Ullrich JE (2006) Thrombospondin 1 mediates angiotensin II induction of TGF-β activation by cardiac and renal cells under both high and low glucose conditions. Biochem Biophys Res Commun 339: 633–641
23. Loeys BL, Chen J, Neptune ER, Judge DP, Podowski M, Holm T, Meyers J, Leitch CC, Katsanis N, Sharifi N, et al (2005) A syndrome of altered cardiovascular, craniofacial, neurocognitive and skeletal development caused by mutations in *TGFBR1* or *TGFBR2*. Nat Genet 37:275–281
24. Loeys BL, Schwarze U, Holm T, Callewaert BL, Thomas GH, Pannu H, De Backer JF, Oswald GL, Symoens S, Manouvrier S, et al (2006) Aneurysm syndromes caused by mutations in the TGF-β receptor. N Engl J Med 355:788–798
25. Mizuguchi T, Collod-Beroud G, Akiyama T, Abifadel M, Harada N, Morisaki T, Allard D, Varret M, Claustres M, Morisaki H, et al (2004) Heterozygous *TGFBR2* mutations in Marfan syndrome. Nat Genet 36:855–860
26. Ki CS, Jin DK, Chang SH, Kim JE, Kim JW, Park BK, Choi JH, Park IS, Yoo HW (2005) Identification of a novel *TGFBR2* gene mutation in a Korean patient with Loeys-Dietz aortic aneurysm syndrome; no mutation in *TGFBR2* gene in 30 patients with classic Marfan's syndrome. Clin Genet 68:561–563
27. Pannu H, Fadulu VT, Chang J, Lafont A, Hasham SN, Sparks E, Giampietro PF, Zaleski C, Estrera AL, Safi HJ, et al (2005) Mutations in transforming growth factor-β receptor type II cause familial thoracic aortic aneurysms and dissections. Circulation 112:513–520
28. Ades LC, Sullivan K, Biggin A, Haan EA, Brett M, Holman KJ, Dixon J, Robertson S, Holmes AD, Rogers J, et al (2006) *FBN1, TGFBR1,* and the Marfan-craniosynostosis/mental retardation disorders revisited. Am J Med Genet A 140:1047–1058
29. Law CJ, Bunyan D, Castle B, Day L, Simpson I, Westwood G, Keeton B (2006) Clinical features in a family with an R460H mutation in transforming growth factor β receptor 2 gene. J Med Genet 43:908–916
30. Matyas G, Arnold E, Carrel T, Baumgartner D, Boileau C, Berger W, Steinmann B (2006) Identification and in silico analyses of novel *TGFBR1* and *TGFBR2* mutations in Marfan syndrome-related disorders. Hum Mutat 27:760–769
31. Singh KK, Rommel K, Mishra A, Karck M, Haverich A, Schmidtke J, Arslan-Kirchner M (2006) *TGFBR1* and *TGFBR2* mutations in patients with features of Marfan syndrome and Loeys-Dietz syndrome. Hum Mutat 27:770–777
32. Frischmeyer PA, Dietz HC (1999) Nonsense-mediated mRNA decay in health and disease. Hum Mol Genet 8:1893–1900
33. Denton CP, Zheng B, Evans LA, Shi-wen X, Ong VH, Fisher I, Lazaridis K, Abraham DJ, Black CM, de Crombrugghe B (2003) Fibroblast-specific expression of a kinase-deficient type II transforming growth factor β (TGFβ) receptor leads to paradoxical activation of TGFβ signaling pathways with fibrosis in transgenic mice. J Biol Chem 278:25109–25119
34. Coucke PJ, Willaert A, Wessels MW, Callewaert B, Zoppi N, De Backer J, Fox JE, Mancini GM, Kambouris M, Gardella R, et al (2006) Mutations in the facilitative glucose transporter GLUT10 alter angiogenesis and cause arterial tortuosity syndrome. Nat Genet 38:452–457