Editorial

Potential Phenotype–Genotype Correlation in Marfan Syndrome
When Less is More?

Harry C. Dietz, MD

A recent emphasis on individualized medicine reflects a desire to transition currently prevailing evidence-based practices (What works best for the average patient?) to more informed and tailored approaches (What will work best for this patient or subgroup?). It has been both expected and realized that genotypic information will contribute prominently to this endeavor. In this issue of *Circulation Cardiovascular Genetics*, Franken et al[1] report that the angiotensin receptor blocker (ARB) losartan is particularly effective at suppressing aortic root aneurysm progression in patients with Marfan syndrome (MFS) that are heterozygous for mutations in the fibrillin-1 gene (*FBN1*) that create a premature termination codon, when compared with other mutation classes expected to express abnormal protein from the mutant allele. Given the intuitive importance of this finding, it is worth exploring its potential biological foundations, while also considering alternative explanations.

Proposed Pathogenesis of MFS

MFS is an autosomal-dominant systemic disorder of connective tissue with prominent involvement of the ocular, skeletal, and cardiovascular systems, including a strong predisposition for eye lens dislocation (ectopia lentis) and myopia, long-bone overgrowth and other skeletal deformity, and progressive aortic root dilatation and tear (dissection), respectively. The condition is caused by heterozygous mutations in *FBN1* that encodes the extracellular matrix protein fibrillin-1. Fibrillin-1 monomers aggregate to form complex extracellular structures called microfibrils and an extracellular deficiency of microfibrils is thought to be the sentinel event in MFS pathogenesis. Missense mutations are the most common type causing MFS, with particularly high frequency of substitutions that either destroy or create cysteine residues in repetitive epidermal growth factor–like or latent transforming growth factor-β (TGF-β)–binding protein–like domains that each have 6 or 8 obligate cysteines with predictable spacing, respectively, that are required for proper intradomain folding. The *FBN1* mutational repertoire also includes all other types of mutations, including insertions/deletions (indels) or altered splicing events that alter the coding sequence but maintain the open reading frame and events that create a premature termination codon, including nonsense and frameshift mutations and out-of-frame perturbations of splicing. Finally, a small subset of patients with MFS show large deletions that remove most or all of the disrupted allele. Premature termination codon mutations in FBN1 that disrupt the open reading frame before the distal portion of the penultimate are expected and observed to induce instability and efficient clearance of the mutant mRNA through nonsense-mediated mRNA decay, effectively precluding the production of much (or any) abnormal fibrillin-1 protein. Cell lines and tissues from patients and mouse models with MFS show diminished matrix deposition of fibrillin-1, with levels <50% threshold predicted by heterozygosity. Early pathogenic models suggested that this may relate to interference with use of the normal protein derived from the wild-type (WT) allele by mutant protein derived from the mutant allele, a so-called dominant-negative (DN) effect. However, a similar degree of deficiency of extracellular fibrillin-1 is seen in cell lines and tissues heterozygous for mutant alleles that have no or limited capacity to express mutant protein (true and functional haploinsufficiency, respectively). These and other data suggest that efficient fibrillin-1 deposition into the matrix is reliant on a critical threshold of normal fibrillin-1 accumulation at the cell surface, and that loss of contribution from one allele to this pool by any mechanism is sufficient to significantly impair efficient use of protein derived from the WT allele in MFS.[2,3] There is also evidence, however, that matrix incorporation of mutant fibrillin-1 (unique to DN-type mutations) can promote proteolytic clearance of microfibrils over time, perhaps further exacerbating the functional deficit and contributing to phenotypic progression.[4,5] Thus, although theoretical mechanistic distinctions between mutations with and without DN potential (DN mutations and haploinsufficiencyinducing (HI) mutations, respectively) are blurred, they are not necessarily irrelevant.

Microfibrils serve diverse functions, including a direct contribution to the biomechanical properties of tissues, coordination of elastic fiber biogenesis and homeostasis, and regulation of the bioavailability and activity of growth factors that influence the performance of neighboring cells. The relative contribution of each of these functions to physiological and hence pathological tissue performance probably shows both spatial and temporal heterogeneity. For example,

ectopia lentis is thought to manifest simple structural failure of supporting structures called ciliary zonules that are largely composed of bundles of microfibrils. Other phenotypic manifestations of MFS are less easily reconciled with pathogenic mechanisms that singularly invoke tissue weakness, such as bone overgrowth, craniofacial dysmorphism, and skeletal muscle myopathy. The original hypothesis that TGF-β contributes to the pathogenesis of MFS stems from the observation that fibrillin-1 has homology to and physically interacts with components of the large latent complex of TGF-β in the extracellular environment.[6,7] Additional studies demonstrated increased TGF-β activity in multiple tissues that are altered in MFS in both people and mouse models, as evidenced by increased TGF-β–dependent phosphorylation (ie, activation) of signaling effectors, such as SMAD2 or SMAD3 (phosphorylation of SMAD2/3) or extracellular signal-regulated kinase (phosphorylation of extracellular signal-regulated kinase) and increased expression of prototypical TGF-β–driven gene products. Attenuation of TGF-β signaling in vivo, either directly using specific neutralizing antibodies or indirectly using ARBs (eg, losartan), associated with dramatic improvement in multiple disease manifestations in MFS mice, including developmental emphysema, myxomatous degeneration of the mitral valve, skeletal muscle myopathy, and aortic root aneurysm.[8] The conclusion that dysregulated TGF-β signaling contributes to the multisystem manifestations of MFS was solidified on recognition that mutations in the genes encoding TGF-β pathway factors are sufficient to cause diseases that associate virtually all the features of MFS (with the exception of eye lens dislocation) with other diagnosis-specific findings. Loeys–Dietz syndrome can be caused by mutations in genes encoding TGF-β ligands (*TGFB2*),[9,10] receptor subunits (*TGFBR1* or *TGFBR2*)[11,12] or intracellular signaling effectors (*SMAD3*),[13] whereas Shprintzen–Goldberg syndrome is caused by mutations in *SKI* that encodes a direct negative regulator of the TGF-β transcriptional response.[14,15] That heterozygous loss-of-function mutations in either positive or negative effectors of TGF-β signaling cause substantially overlapping phenotypes remains puzzling, however, there is consensus that the aortic root wall shows a clear tissue signature for increased TGF-β signaling in both people and mouse models with these conditions. It is also notable that manipulations in mouse models that attenuate disease progression associate with normalization of this signature, whereas the opposite is seen for those that worsen disease. Although genetic or pharmacological abrogation of TGF-β signaling does not routinely induce aortic aneurysm in otherwise unprovoked WT mice, such manipulation can initiate aortic wall pathology specifically in the C57BL/6J inbred background on angiotensin II infusion[16] or in WT mice on complete conditional silencing of TGF-β receptor subunit expression in vascular smooth muscle cells if induced in the early perinatal period, but not thereafter when people with MFS routinely show the onset or progression of aneurysm.[17] In both the circumstances, overtly inflammatory vessel wall pathology and aortic dissection evolve within days after provocation, findings not characteristic of human inherited aneurysm predispositions. In a mouse model of MFS that is homozygous for a hypomorphic *Fbn1* allele, Cook et al[18] showed that TGF-β antagonism with a panspecific

neutralizing antibody accelerated aortic disease if initiated in the perinatal period, but mitigated aneurysm growth and dissection if initiated even slightly later in postnatal life. These findings highlight a somewhat mysterious dimorphic role of TGF-β in the vessel wall at the earliest stages of postnatal development that might relate, at least in part, to a physiological surge of TGF-β signaling during early postnatal life and, perhaps a proposed divergent role for microfibrils in the regulation of TGF-β signaling; serving both to concentrate TGF-β ligands at sites of intended function and to suppress TGF-β activation.[19] For the purposes of this discussion, however, the salient point is that a deficiency of extracellular fibrillin-1 is thought sufficient to initiate failure of both the structural and regulatory functions of microfibrils.

Phenotype–Genotype Correlations in MFS

Attempts to elicit phenotype–genotype correlations in MFS have met with limited success. Contributing factors may include the extreme allelic heterogeneity in MFS, the fact that most phenotypes show continuous (rather than discrete) variation, and the potential influence of both the genetic and environmental modifiers of disease. The few associations that have passed repeated scrutiny include enrichment for ectopia lentis with DN-type mutations, increased skeletal severity with HI mutations, and the enhanced potential for particularly severe presentations of MFS (including cardiovascular manifestations) in patients with in-frame mutations in a central region of the fibrillin-1 protein, somewhat loosely defined by those encoded within exons 24–32[20–22]. Overall, DN-type mutations are more likely to be associated with pediatric presentations with greater severity, although there are exceptions to this rule. Such distinctions are enhanced when specifically comparing cysteine substitutions with mutations that create a premature termination codon. Contrary to a premise put forth by Franken et al,[1] HI-type mutations (including entire *FBN1* allele deletions) are not more likely to be associated with phenotypic homogeneity, but rather have proven capable of association with phenotypes ranging from particularly mild (even subdiagnostic) presentations without cardiovascular involvement in adulthood to classic MFS, although particularly severe pediatric disease has not been described.[21,23,24] Taken together, these data suggest tremendous overlap between phenotypes induced by DN- and HI-type *FBN1* mutations, with a context-dependent potential for greater severity in the former.

Treatment Strategies for MFS

Commencing in the early 1990s, multiple studies explored the therapeutic benefit of β-blockers in suppressing the rate of aortic root dilatation in both children and adults with MFS[25] with the prediction that a reduction in heart rate, blood pressure, or dP/dt in the proximal aorta would prove beneficial. Although uniformly small, largely nonrandomized and often retrospective in nature, most studies found a significant protective effect in patients receiving β-blockers versus no medical therapy. Limitations in definitively interpreting these data include a wide range of age, phenotypic severity, and agent or dose of medication used. Atenolol was the most common β-blocker tested, with an average dose ranging from <1 to 1.9

mg/kg per day. The 1 study that failed to document protection from β-blockers used the lowest average dose of atenolol (0.92 mg/kg per day). A meta-analysis of these data found strong evidence for a reduced rate of aortic root growth in patients with MFS receiving β-blockers when compared with no medical therapy with a standardized mean difference of −1.3 mm/y in the β-blocker group (*P*<0.005).[25] Taken together, these data support the contention that β-blocker therapy is protective against aortic root enlargement in MFS, with at least the suggestion of a dosage-dependent effect. This conclusion is further supported by observations in a mouse model of MFS with a heterozygous cysteine substitution in an epidermal growth factor–like domain of fibrillin-1 (C1039G/+), the most common class of mutation causing MFS in people.[26] MFS mice receiving propranolol had a relative but significant reduction in aortic root growth rate when compared with placebo-treated animals. Unlike human trials, this type of study can precisely control for fibrillin-1 genotype, genetic background, dose of medication, compliance, and environmental exposures.

The conclusion that excessive TGF-β signaling contributes to the multisystem complications of MFS in mouse models prompted a trial of ARBs, a class of medications that both lowers hemodynamic stress and had previously shown the ability to attenuate TGF-β signaling in rodent models of chronic kidney disease and cardiomyopathy.[27,28] C1039G/+ MFS mice showed normalization of aortic root growth rate and aortic wall architecture, with significant improvement in both the parameters when compared with either placebo- or propranolol-treated affected littermates.[26] On the basis of this work, Brooke et al[29] launched an observational study comparing the performance of children with severe MFS (mean aortic root *z* score of 7.21) receiving losartan or irbesartan (n=17 at a sustained dose of 1.4 mg/kg per day or n=1 at 2.0 mg/kg per d, respectively) on top of their previous dose of atenolol (mean of 1.8 mg/kg per d) to that on previous medical therapy (antenolol with or without angiotensin-converting enzyme inhibitor or calcium channel blocker). Mean aortic root growth rate decreased from 3.54 to 0.46 mm/y and mean rate of change in aortic root *z* score decreased from +0.97 to −0.50 per year. In a similarly designed study, Chiu et al[30] compared the performance of 28 patients with MFS on losartan (maximum tolerated dose ≤50 mg in children and 100 mg in young adults) plus β-blocker (maximum 1 mg/kg per day) to their previous performance on β-blocker alone (maximum 2.0 mg/ kg per d). They observed a decline in aortic root growth rate in the losartan group from 0.89 to 0.10 mm/y with an attendant significant decline in rate of change of aortic root *z* score and aortic annulus and sinotubular junction dimension. Pees et al[31] studied losartan monotherapy (mean of 1.2 mg/kg per day) in children and adolescents who had or had not previously received β-blocker therapy. They observed a significant decline in body surface area–normalized aortic root size (−3.0 mm/ m²) and aortic root *z* score (−0.28) at the end of 35 months of follow-up, with evidence for a greater response in those with a younger age and a longer duration of treatment. There was an attendant significant decline in body surface area –normalized aortic annulus, sinotubular junction, and ascending aortic size. Finally, Groenink et al[32] performed a randomized controlled trial (Cozaar in Marfan Patients Reduces Aortic Enlargement

[COMPARE]) of losartan (100 mg/d) plus previous medical therapy (β-blockers or calcium channel blockers) versus previous medical therapy alone in 233 adults with MFS. Among patients with a native aortic root at randomization, the addition of losartan was associated with a significantly slower aortic root growth rate (0.77 versus 1.35 mm/3 y, as assessed by MRI). Patients with previous aortic root surgery who received losartan showed significantly slower growth of the more distal ascending aorta (0.5 versus 1.01 mm/3 y). In a large randomized pediatric trial, Lacro et al[33] showed equivalence of atenolol and losartan when monitoring aortic root growth rate and clinical outcomes in children and young adults with MFS of moderate severity (average aortic root *z* scores at enrollment of 4.0). The most notable difference in study design, when compared with previous studies, was the comparison between monotherapy with each agent and the high dose of atenolol achieved (mean of ≈3.0 mg/d and ≤4.0 mg/kg per d) using a medication titration strategy that had not previously been used in MFS (20% reduction in average daily heart rate). Both treatment arms associated with a decline in aortic root *z* score over time (−0.139±0.013 and −0.107±0.013 z/y, respectively) with both the slopes significantly different from 0.00 (*P*<0.001 for both the arms) and evidence for a greater response in younger patients. The decline in aortic root *z* score seen in both the groups compares favorably with the gain (+0.24 z/y) observed in a retrospective analysis of 65 children with MFS of moderate severity (mean *z* score of 3.25±1.52) who had been treated with atenolol using more typical treatment protocols (1–2 mg/ kg per d).[29] In addition, the rates of change in absolute aortic root dimension observed with atenolol and losartan in this trial (+0.069±0.004 and +0.075±0.004 cm/y, respectively) compare favorably with the rates observed previously in untreated pediatric patients with MFS (0.16±0.38, 0.18±0.90, 0.11±0.06, and 0.21±1.6 cm/y).[25,34–37] Although these data suggest that both atenolol and losartan achieved protective effects in this trial, the lack of a placebo group, as dictated by the prevailing view that β-blockers afford some protection, precluded a study design to specifically test this hypothesis.

Perspective

In this issue of *Circulation Cardiovascular Genetics*, Franken et al[1] stratify patients in the COMPARE trial with native aortic roots at enrollment by the apparent type of underlying *FBN1* mutation. Overall, 117 patients were considered; 79 (67.5%) and 38 (32.5%) with DN- or HI-type mutations, respectively. Overall, they again found a slower rate of aortic root growth in patients receiving previous therapy (largely β-blockers) plus losartan (n=58; 0.8±1.4 mm/3 y) compared with those receiving previous therapy alone (n=59; 1.3±1.5 mm/3 y); *P*=0.009. However, although a significant benefit of losartan therapy was seen in the HI group (0.5±0.8 mm/3 y with losartan; n=17 versus 1.8±1.5 mm/3 y without losartan; n=21; *P*=0.001), this was not observed in the DN group (0.8±1.3 mm/3 y with losartan; n=41 versus 1.2±1.7 mm/3 y without losartan; n=38; *P*=0.197). The authors conclude that losartan was uniquely effective in the HI group. One obvious question that was not addressed was whether sufficient sample sizes and hence power to reach robust conclusions was maintained in subgroup analyses. To my mind, however, equally

important considerations include the biological plausibility of this finding and the factor(s) that are driving the apparent discrepancy between groups. One hypothesis would be that DN- and HI-type mutations initiate disease pathogenesis by discretely different mechanisms. As discussed above, both the cell culture and the animal model experiments suggest that this is not the case, with both types of mutations impairing the secretion or matrix use of fibrillin-1 derived from the WT allele, culminating in a level of microfibrillar abundance and function that falls below a critical threshold necessary for proper tissue homeostasis. In this light, it is notable that mouse model experiments have documented remarkable efficacy of losartan in mitigating aortic pathology in lines harboring either missense (DN-type) or hypomorphic (HI-type) mutations in *Fbn1*, with or without the potential to express mutant protein, respectively.[18,26]

An important finding that is not comprehensively addressed in the study by Franken et al[1] is the suggestion of increased aneurysm severity in the HI group, when compared with the DN group, with a trend for increased aortic root diameter normalized for age (1.49 versus 1.35 mm/y; P=0.09). Furthermore, the percentage of individuals with stable aortic dimensions throughout the trial was substantially increased in DN compared with HI patients receiving previous therapy alone (60.5% versus 19%, respectively); such a profound difference was not observed in the losartan groups (51% versus 58.8%, respectively). Indeed, a 2-way analysis of variance revealed no difference in the effect size of losartan between the DN and HI groups. In this light, it seems that the baseline severity of disease, as opposed to an isolated difference in the efficacy of losartan, is contributing to apparent differences in performance between the DN and HI groups.

How might an increased baseline severity in the HI group be explained? As previously discussed, this is not an expected finding given the overall enrichment for pediatric presentations of severe disease associated with DN-type mutations. One important distinguishing factor in this study is the adult age of participants (mean, 35.3 years; range 18–71 years) who did not require previous aortic root surgery. This would intuitively impose a selection bias for milder cases that would be particularly relevant to DN patients. Potential contributing factors include the location and nature of the underlying DN mutation that dictate its intrinsic pathogenic potential and the influence of genetic or environmental modifiers. It also seems possible that, independent of such factors, patients in the DN group achieved a greater effect of their previous therapy with β-blockers, which would effectively limit the potential to observe an additive benefit of losartan. This could relate to an earlier age of diagnosis and hence initiation of medical therapy—shown to positively influence response by the studies of Pees et al[31] and Lacro et al[33]—or to more aggressive dosing schedules based on earlier onset and more rapid progression of aortic disease. This would be in keeping with a general trend that is apparent when all therapeutic trials for MFS are considered; the magnitude of effect size varies inversely with age and directly with the severity of the study population overall and especially with the severity of the comparison group. In this light, it would be interesting for Franken et al[1] to determine whether the same apparent difference between the DN

and HI groups is observed when considering the performance of the more distal ascending aorta among patients who had previously required aortic root surgery. Another consideration is whether there is a practical maximal therapeutic effect that can be achieved with any therapy in MFS. A larger aortic root dimension at baseline would dictate higher wall stress, a situation compounded by an aortic segment that has minimized or even saturated its elastic capacity with biomechanical properties largely governed by rigid collagen, and by a rapid taper from an abnormally large to a more restrictive dimension at the sinotubular junction or more distal ascending aorta. In this light, is a growth rate distribution (of a tissue that remains, by necessity, under pressure) that centers on a fraction of a millimeter per year the best that we can hope for?

Substantial evidence suggests a strong contribution of extracellular signal-regulated kinase in the pathogenesis of aortic aneurysm in MFS and related conditions that may relate, at least in part, to activation by noncanonical TGF-β signaling cascades.[38,39] Both ARBs and β-adrenergic receptor blockade reduce hemodynamic stress and both can attenuate extracellular signal-regulated kinase activation in the cardiovascular system.[38,40] These data suggest that the protective effects of these 2 classes of medications may rely on partially but not necessarily wholly overlapping mechanisms. Comparable performance between the atenolol and losartan in the Lacro trial, despite the fact that only atenolol was titrated to hemodynamic effect, with a significant reduction in heart rate and mean arterial pressure when compared with the losartan group, is in keeping with this hypothesis. If validated, the use of the combination of β-blockers and ARBs may prove warranted. Although select studies have explored the therapeutic potential of the high end of β-blocker dosing that may not be achievable based on side-effects outside of the context of a patient-blinded trial, the dose of ARBs has been relatively modest when compared with reported tolerability thresholds and practices used for other TGF-β–related phenotypes. For example, studies have demonstrated superiority of ultrahigh dose ARB therapy in the management of chronic renal disease, when compared with typical antihypertensive regimens and independent of any apparent hemodynamic influence.[41] Trials of combined and monotherapy with ultrahigh dosing of losartan or newer generation ARBs seem warranted in MFS. Such initiatives may ultimately be informed by the nature of the underlying mutation, consideration of other potential genetic influences (eg, those influencing drug metabolism, affinity for targets, and downstream consequences), and the application of biomarkers of drug response and efficacy.

Disclosures

None.

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Harry C. Dietz

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