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DRUG-BASED THERAPIES FOR VASCULAR DISEASE IN MARFAN SYNDROME: FROM MOUSE MODELS TO HUMAN PATIENTS

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Abstract

Marfan syndrome is a congenital disorder of the connective tissue with a long history of clinical and basic science breakthroughs that have forged our understanding of vascular disease pathogenesis. The biomedical importance of Marfan syndrome was recently underscored by the discovery that the underlying genetic lesion impairs both tissue integrity and transforming growth factor- β regulation of cell behavior. This discovery has led to the successful implementation of the first pharmacological intervention in a connective tissue disorder otherwise incurable by either gene- or stem cell-based therapeutic strategies. More generally, information gathered from the study of Marfan syndrome pathogenesis has the potential to improve the clinical management of common acquired disorders of connective tissue degeneration.

Keywords

aortic aneurysm; connective tissue; fibrillin; losartan; Marfan syndrome; pharmacology; TGF β ; vascular therapy

Marfan syndrome (MFS) is a common congenital disorder of the connective tissue with cardinal manifestations involving the skeletal, cardiovascular, and ocular systems (1). The leading cause of morbidity and mortality in MFS is ascending aortic aneurysm that can precipitate acute dissection or rupture.

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For over a century, beginning in 1896 with Antoine Marfan's description of a young girl with unusual skeletal features, MFS has been the focus of intense clinical and basic science investigations that have led to a number of seminal discoveries and conceptual breakthroughs provided the evidence-based (2). In 1956, Victor McKusick compiled the first comprehensive description of the MFS phenotype. McKusick further identified MFS as the prototype of a group of "heritable disorders of connective tissue" that he predicted to be accounted for by structural or metabolic dysfunctions of extracellular matrix (ECM) proteins (3). Studies over the next three decades culminated in major clinical advances, such as the use of β -adrenergic blockade to reduce hemodynamic stress, the refinement of surgical interventions to replace the dilated aortic root, and the demonstration that mutations in the gene coding for the extracellular glycoprotein fibrillin-1 (*FBNI*) cause MFS (4–7). Aside from validating McKusick's earlier prediction, identification of the MFS gene led to the

development of molecular tools for pre-natal diagnosis and of several mouse models of the disease. A decade long characterization of these murine models of MFS established an unexpected correlation between mutations in fibrillin-1 and improper activation of transforming growth factor - β (TGF β) signaling, which has in turn provided the evidence-based rationale for a more effective therapeutic strategy to blunt aortic aneurysm progression in MFS patients (8). As such, the mouse findings have yielded important new insights into the clinical management of degenerative disorders of the connective tissue.

MARFAN SYNDROME OVERVIEW

MFS is inherited as an autosomal dominant trait with an estimated incidence of 1 per 5,000 individuals of which ~25% are sporadic cases due to de novo *FBNI* mutations. The disease shows high penetrance and marked inter- and intra-familial variability. Skeletal manifestations include disproportionate linear growth of digits, ribs and limbs, craniofacial abnormalities, scoliosis, osteopenia and joint hypermobility. Ocular disease in MFS includes dislocation of one or both lenses (ectopia lentis), strabismus, cataracts and/or glaucoma (9). Progressive enlargement of the ascending aorta and myxomatous valve changes are major cardiovascular manifestations that can cause chronic aortic regurgitation and aortic dissection. Additional abnormalities may involve the lungs, skin, integument, and muscle and adipose tissue.

MFS diagnosis is almost solely based on clinical findings and family history; molecular diagnosis is helpful in cases in which a familial *FBNI* mutation has been identified in affected family members. Guidelines established by the so-called Ghent nosology require a combination of manifestations in different organ systems that are defined as major and minor clinical criteria based on their frequency in the general population (10). These diagnostic criteria help differentiate classic MFS from a number of closely-related connective tissue disorders that share some similarities (*FBNI* mutations), but differ in their repertoire of manifestations, natural history, and response to treatment. Differential diagnosis is particularly important for the timely management of cardiovascular complications in pediatric patients; however, extensive phenotypic variability and age-dependent onset of informative manifestations constitute significant challenges to MFS diagnosis in these individuals.

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Vascular pathology in MFS is associated with changes in connective tissue architecture manifested by reduced mechanical compliance, unbalanced ECM remodeling, and progressive aneurysm development that ultimately increase the risk of aortic wall degradation (11). These histopathological alterations, traditionally and improperly referred to as cystic medial necrosis, are also associated with the normal aging process, bicuspid aortic valve disease, and with increased hemodynamic stress in hypertension (12–13). Until very recently, management of vascular disease in MFS has relied on routine echocardiograms to monitor aneurysm progression, administration of β -adrenergic blockers to reduce aortic wall stress, and appropriately-timed prophylactic surgery to prevent aortic complications (14–16). Although these interventions have extended life expectancy from approximately 45 to 72 years, alternative therapies are still needed to delay onset and progression of vascular disease, particularly in the most aggressive forms of MFS (17–18). Additionally, strategies to manage associated co-morbidities, such as musculoskeletal manifestations, are becoming increasingly important in the expanding population of aging MFS patients.

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However the long held belief that MFS is solely caused by loss of tissue integrity, together with the notion that fibrillin-1 assembly is largely confined to embryogenesis, represented a theoretically insurmountable impediment to the implementation of rational gene- and/or cell-based therapies. This view has dramatically changed with recent findings that (i) fibrillin-1 may be involved in elastic fiber maturation, homeostasis, and remodeling during postnatal life and that (ii) the aortic architecture in fibrillin-1 mutant mice is normal at birth (19). Specifically, murine models of MFS discussed in this review include *Fbn1^{mgR/mgR}* mice, which under-express fibrillin-1 and die of ruptured aortic aneurysm at ~2 months of age; *Fbn1^{C1039G/+}* mice, which produce normal and structurally abnormal fibrillin-1 molecules and develop aortic aneurysm without dissection and rupture, and *Fbn1^{-/-}* mice, which do not produce fibrillin-1 and die soon after birth due to the structural collapse of an improperly matured aortic wall (20–22).

FIBRILLIN-1 BIOLOGY AND PATHOPHYSIOLOGY

Fibrillin-1 is a 350Kd cysteine-rich glycoprotein that polymerizes together with the structurally related fibrillin-2 into filamentous assemblies with an average diameter of 10 nm (23). Fibrillin microfibrils impart key physical properties to virtually every organ system through the temporal and hierarchical assembly of tissue-specific macro-aggregates through interactions with proteins such as elastin, proteoglycans, fibulins, and microfibril-associated glycoproteins (23–28). In addition to its structural function, fibrillin microfibrils also control cell behavior through the sequestration of latent TGFβ complexes in the ECM via the dual interaction of LTBP with fibrillins and latent pro-TGFβ complexes (Figure 1) (27). TGFβ-1, -2 and -3 (hereafter collectively referred to as TGFβ) are multifunctional signaling molecules that orchestrate a large variety of cellular activities in a context-specific manner. TGFβ signals through receptor-induced stimulation of Smad-2 and -3 (R-Smads) followed by the formation, nuclear translocation and binding of activated R-Smad:Smad4 complexes to specific DNA targets in association with transcriptional activators and/or repressors (Figure 2) (29–30). Inhibitory Smads, intracellular receptor trafficking, co-receptor activities, parallel activation of and cross talk with MAPK signaling pathways, and interactions with ECM components constitute the multilayered regulatory system that modulates TGFβ bioavailability and controls the intensity and duration of TGFβ signaling (31). Mouse models of MFS have strongly supported the notion that ECM sequestration imparts functional context to TGFβ activity by modulating the spatial and temporal release, as well as the concentration and presentation of the ligands (32).

Early work has shown that ruptured aortic aneurysm in *Fbn1^{mgR/mgR}* mice is preceded by a series of secondary cellular events that, in overlapping temporal succession, include medial calcification, excessive ECM accumulation and degradation, intimal hyperplasia and adventitial inflammation (22).

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Overall, these secondary events imply an unproductive tissue remodeling/repair response that promotes intense elastolysis and which in turn exacerbates the collapse of a structurally compromised aortic wall. Subsequent studies implicated promiscuous TGFβ signaling as the molecular driver of improper tissue formation and remodeling in other *Fbn1* mutant mice (8).

In addition to cardiovascular manifestations, MFS patients can present with pulmonary complications, including chronic obstructive lung disease and predisposition for pneumothorax (33–34). Traditionally, this lung phenotype has been equated to destructive emphysema resulting from impaired tissue integrity (1). Contrary to this prediction, however, widening of distal pre-alveolar saccules in newborn *Fbn1*-deficient mice occurs in the absence of inflammation or tissue destruction suggesting a developmental defect as the underlying cause (8). Upon closer inspection, the mouse phenotype was correlated with elevated TGF β activity (as evidenced by greater nuclear accumulation of R-Smads) and increased apoptosis of lung cells, suggesting a developmental origin of the defect. Importantly, systemic administration of TGF β -neutralizing antibodies during perinatal life rescued destructive emphysema in aged mutant mice, implying that early perturbations of tissue morphogenesis can predispose to late-onset degenerative processes. Similarly, increased cell proliferation and decreased apoptosis secondary to promiscuous TGF β activity were associated with architectural alterations in the mitral valves of *Fbn1*^{C1039G/+} mice (35). Once again, systemic TGF β antagonism during perinatal life corrected mitral valve abnormalities in *Fbn1*^{C1039G/+} mice, in addition to improving aortic wall architecture and enhancing regeneration of injured muscles (36). Collectively, these findings established a causal correlation between impaired microfibril biogenesis and improper latent TGF β activation. They also provided compelling evidence that pharmacological targeting of TGF β signaling could be a more effective strategy than β -adrenergic blockade in preventing or restraining aortic aneurysm in MFS.

ANGIOTENSIN RECEPTOR BLOCKER THERAPY

The renin-angiotensin system is essential for cardiovascular homeostasis. Angiotensin II (AngII) binds two G-protein coupled receptor (GPCR) receptors (angiotensin II type 1 receptor (AT1R) and angiotensin II type 2 receptor (AT2R)) with opposing effects (37). AT1R activation by AngII, autoantibodies, or mechanical stress can stimulate cell growth and migration, as well as inflammation and vasoconstriction (38). Previous work has demonstrated highly localized expression of the AT1R in the aortic arch to regions of non-laminar flow (39). Additional *in vitro* studies have shown that cardiomyocytes under mechanical stress exhibit increased AT1R signaling in the absence of AngII (40). Activation of AT1R signaling is the result of a mechanically induced conformational change of the receptor and can be inhibited by angiotensin receptor blockers (ARBs) (40). AT2R activation can result in vasodilation, apoptosis, or inhibition of AT1R signaling (41). The differential outcomes of the Ang II receptors are probably best illustrated by the observation that AT1R antagonism can prevent development of abdominal aortic aneurysm whereas AT2R antagonism accelerates it (42). Experimentally induced renal and cardiac fibrosis in murine models has linked AngII activation to TGF β signaling through up-regulation of thrombospondin-1 (an activator of latent TGF β), in addition to documenting the therapeutic efficacy of AngII-converting enzyme (ACE) inhibitors or ARBs in fibrotic disorders (43–46).

Based on these correlative lines of evidence, the ARBs emerged as a promising therapeutic intervention for vascular therapy in MFS. The FDA-approved ARB losartan was therefore employed in a mouse pre-clinical trial to validate this hypothesis (5). *Fbn1*^{C1039G/+} mice were administered losartan, propranolol, or placebo beginning at embryonic day 14 or at 7 weeks of age for 10 or 6 months, respectively. Mice were sacrificed and aortic thickness and architecture were evaluated. Prenatal or postnatal administration of losartan, but not propranolol, prevented dilation of the aorta and destruction of aortic wall architecture. Losartan therapy rescued aortic architecture to a greater extent than administration of TGF β neutralizing antibody (5). Similar findings were more recently obtained in a losartan pre-clinical trial that employed *Fbn1*^{mgR/mgR} mice, thus reiterating drug efficacy in curbing

aneurysm progression in clinically distinct forms of MFS (our unpublished data). Importantly, the positive outcome of these mouse experiments provided proof-of-concept for the possible use ARBs in treating MFS patients. A retrospective study of eighteen pediatric patients with severe and rapidly progressive MFS who had been subject to ARB treatment for at least 1 year revealed a statistically appreciable decrease in the rate of aortic-root diameter growth (47).

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The positive results from both the mouse experiments and the retrospective study have resulted in a push toward better understanding the differences in the clinical efficacies of β -adrenergic, angiotensin receptor blockade and dual therapy. Currently there are multiple ongoing clinical randomized trials designed to assess the efficacy of the β -adrenergic blockers atenolol or nebivolol versus the ARB losartan in MFS individuals (Clinical Trial Numbers: NCT00429364, NCT00593710, NCT00683124) (48–49).

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For example, ARBs are contraindicated in pregnancy as inhibition of the fetal renin-angiotensin system can lead to fetopathy including renal failure, hypotension, oligohydramnios, and pulmonary hypoplasia (50–51). Pregnant women represent a subset of the MFS patients that are at an increased risk for vascular complications secondary to increases in blood volume and heart rate associated with pregnancy (52). MFS patients with aortic diameters >4cm have a 10% risk of vascular complications during pregnancy (53). Current therapies include β -blockers or elective surgical repair of the ascending aorta before conception to reduce the risk of complications (52). In addition, long-term losartan treatment might exacerbate other morbid manifestations seen in MFS patients, including increased stiffness of the ascending aorta. Pulse pressure, defined as the difference between systolic and diastolic pressures and is influenced by arterial stiffness, has been shown to positively correlate with ascending aortic diameter (54). Medical therapies which mitigate the arterial stiffness may further prevent vascular complications. Increased aortic stiffness has been observed in *Fbn1*^{C1039G/+} mice in addition to decreased NO synthase activity and treatment with losartan for 9 months was unable to mitigate the suppression of endothelial NO pathway (55–57). Losartan, unlike other ARBs, is metabolized to an active metabolite EXP3174 through CYP2C9 and has been previously demonstrated to have differential metabolism due to specific CYP2C9 polymorphisms (58). It is theoretically possible that, under current dosing guidelines, MFS patients may appear refractory to losartan therapy due to polymorphisms in CYP2C9. Future studies should investigate whether orally active ARBs, such as telmisartan, are more effective in these populations.

FUTURE RESEARCH DIRECTIONS AND THERAPEUTIC MODALITIES

The current model of MFS pathogenesis postulates that mutations affecting the structure or expression of fibrillin-1 preclude or decrease sequestration of latent TGF β complexes in the ECM; thus, rendering them more prone to or more accessible for activation. This view

implies that physiological levels of latent TGF β activators are sufficient to drive disease progression in MFS. An alternative and not mutually exclusive view is that a structurally abnormal ECM may also promote additional cellular responses that contribute to MFS pathogenesis by heightening latent TGF β activation through the action of integrins, proteases and/or other molecules.

In accordance with the above considerations, abnormally high p38 MAPK signaling has been reported to be an early determinant of greater R-Smad signaling in the aortas of *Fbn1*^{-/-} mice, which mimic the neonatal lethal form of MFS (Figure 2) (19). This finding is consistent with the notion that TGF β can also signal through JNK, p38, and ERK1/2 MAPKs with different effects on cell performance (59–63). Furthermore, MAPKs can be activated by a large number of environmental stimuli, including those mediated by AT1R, and MAPKs can regulate the expression of extracellular modulators of TGF β signaling and influence R-Smad activity as well. Moreover, there is experimental evidence that AngII can also induce the activity of ERK1/2 and JNK signaling (64). It is therefore conceivable to argue that, depending on the nature of the *FBN1* mutation and on modifiers of stress responses and/or TGF β activators, aortic aneurysm in MFS may progress by sequential activation of one or more MAPKs with discrete consequences for TGF β signaling and cell performance. These complex molecular interactions may therefore account for the significant clinical variability of vascular disease in MFS.

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Aortic extracts from *Fbn1*^{mgR/mgR} mice have been reported to induce macrophage chemotaxis leading to a profound inflammatory response and activation of ECM-degrading and latent TGF β -activating metalloproteinases (MMPs) (65). Inability to sequester TGF β through *FBN1* mutations in MFS coupled with increased MMP activation could exacerbate unopposed TGF β signaling. Such a model of vascular pathogenesis would require the focused effort of losartan action on TGF β signaling and other pharmacological means to mitigate the inflammatory response. Consistent with this proposed model, systemic MMP inhibition by doxycycline has been shown to improve aortic wall architecture and delay ruptured aneurysm in *Fbn1*^{C1039G/+} and *FBN1*^{mgR/mgR} mice, respectively (66). Along the same lines, the preliminary finding that aortic architecture is improved to a greater extent using losartan as opposed to TGF β neutralizing antibody implicates the participation of factors other than improper TGF β signaling in driving aneurysm progression in MFS.

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In this respect, it is worth noting that vascular manifestations similar to those of MFS are observed with mutations that affect smooth muscle cell contraction, such as mutations in myosin heavy chain 11 (MYH11) and alpha-2 actin (ACTA2), or the plasma membrane receptor LRP1, an integrator of PDGF and TGF β signals in the aortic wall (67–69). Accordingly, it is possible that mutations in fibrillin-1 may negatively impact both ECM structural integrity and modulation of TGF β bioavailability with multiple and complex consequences for aortic compliance and cell behavior. Ongoing investigations are therefore focusing on the mechanism of latent TGF β activation and other potential contributors to aneurysm onset and progression with the intent of identifying new biological targets to improve vascular disease therapy in MFS.

CONCLUSION

As in the past, MFS research has yielded new insights into the pathophysiology of human diseases. The new paradigm that ECM sequestration is a critical regulator of local TGF β signaling has provided the conceptual framework of using traditional pharmacological means of therapy to counteract degeneration in MFS and conceivably, in age-related conditions affecting the integrity of connective tissues. Moreover, inappropriate TGF β signaling in MFS has conceptualized the origin of clinical variability in this disease by identifying a number of candidate modifiers that are part of the regulatory network of TGF β signaling. More generally, MFS represents a unique example of how characterization of monogenic disorders can inform and guide the clinical management of diseases in which gene or stem cell-based therapies are impracticable or impossible.

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Figure 1.
Fibrillin microfibrils modulate TGFβ incorporation into the extracellular matrix through binding to LTBP

Figure 2. Simplified review of TGFβ signaling pathways. Molecules are color coded according to their action. Yellow: transcription factors. Orange: kinases. Red: inhibitory protein. Gray: GTPase