MINIREVIEW

Recent progress in genetics of Marfan syndrome and Marfan-associated disorders

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Abstract Marfan syndrome (MFS, OMIM #154700) is a hereditary connective tissue disorder, clinically presenting with cardinal features of skeletal, ocular, and cardiovascular systems. In classical MFS, changes in connective tissue integrity can be explained by defects in fibrillin-1, a major component of extracellular microfibrils. However, some of the clinical manifestations of MFS cannot be explained by mechanical properties alone. Recent studies manipulating mouse *Fbn1* have provided new insights into the molecular pathogenesis of MFS. Dysregulation of transforming growth factor beta (TGFβ) signaling in lung, mitral valve and aortic tissues has been implicated in mouse models of MFS. *TGFBR2* and *TGFBR1* mutations were identified in a subset of patients with MFS (MFS2, OMIM #154705) and other MFS-related disorders, including Loeys–Dietz syndrome (LDS, #OMIM 609192) and familial thoracic aortic aneurysms and dissections (TAAD2, #OMIM 608987). These data indicate that genetic heterogeneity exists in MFS and its related conditions and that regulation of TGFβ signaling plays a significant role in these disorders.

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Introduction

Marfan syndrome (MFS, OMIM #154700) is a connective tissue disorder with autosomal dominant inheritance. MFS is clinically diagnosed according to the Ghent criteria, which describe pleiotropic manifestations affecting multiple organs (De Paepe et al. 1996). Typical MFS can affect the skeletal system (marfanoid habitus including arachnodactyly, dolichostenomelia, pectus deformity and scoliosis), the ocular system (ectopia lentis) and the cardiovascular system (aortic aneurysm/dissection and mitral regurgitation), as well as other systems, including lung, skin, integument, and dura mater. Significant phenotypic variability of MFS is commonly observed between affected members of different families and even among affected members within a single family. Neonatal MFS (nMFS) is the most severe type of MFS and is characterized by severe atrioventricular valve dysfunction, arachnodactyly, joint contracture, crumpled ears and pectus deformity. In addition to classic MFS, incomplete forms of MFS are seen, in which symptoms overlap with those of MFS but the phenotypes do not satisfy the Ghent criteria.

This review focuses on the recent advances in the genetics of MFS and its associated conditions, including Loeys–Dietz syndrome, non-syndromic thoracic aortic aneurysms and dissections, and Shprintzen–Goldberg craniosynostosis syndrome. Abnormal

transforming growth factor beta (TGFβ) signaling will be discussed as the core pathogenesis of MFS.

Genetics of Marfan syndrome and its related disorders

FBN1 mutation-related disorders

Marfan syndrome

Molecular diagnosis of MFS became possible after mutations had been identified in the *FBN1* gene (Dietz et al. 1991; Lee et al. 1991). *FBN1* is a 230 kb gene, containing 65 exons, which encodes the structural protein fibrillin-1 (Corson et al. 1993). More than 600 *FBN1* mutations are registered in the UMD-*FBN1* database for MFS and its associated disorders (http://www.umd.be:2030/) (Collod-Beroud et al. 2003). The mutation detection rate of *FBN1* in MFS varies among studies, ranging from 9% to 91% (Katzke et al. 2002; Loeys et al. 2004; Tynan et al. 1993). This variability could be explained, in part, by the different techniques used, but the most significant influencing factor is likely to be sample bias. The frequencies are quite different between patients fulfilling the Ghent criteria and those not fulfilling them (Biggin et al. 2004; Halliday et al. 2002; Loeys et al. 2001; Rommel et al. 2002, 2005; Tynan et al. 1993).

Extensive mutational analyses failed to show *FBN1* involvement in almost 10% or more of patients with MFS satisfying the Ghent criteria. Although one possible explanation could be due to so-called missing mutations in the promoter region or in other noncoding sequences, the existence of a second locus for MFS (*MFS2*) was hypothesized (Collod et al. 1994; Dietz et al. 1995; Gilchrist 1994). In 2004, patients with MFS2 were shown to have mutations in the *TGFBR2* gene, which encodes the transmembrane receptor type II of TGFβ (Mizuguchi et al. 2004). *TGFBR2* mutations were later linked to other clinically overlapping syndromes, described below (Kosaki et al. 2006; Loeys et al. 2005; Pannu et al. 2005a).

Other fibrillinopathies

FBN1 mutations were also found in incomplete forms of MFS as well as in several other MFS-related disorders such as nMFS, isolated ectopia lentis, Shprintzen–Goldberg craniosynostosis syndrome (SGS), familial thoracic aortic aneurysms and dissections (TAAD), and autosomal dominant Weill–Marchesani syndrome (Table 1) (Faivre et al. 2003; Francke et al. 1995; Kainulainen et al. 1994; Milewicz et al. 1996; Sood

et al. 1996). This resulted in the recognition of "fibrillinopathies" caused by *FBN1* aberrations (Charbonneau et al. 2004).

TGFBR mutation-related disorders

Marfan syndrome type 2

In 1993 Boileau et al. (1993) reported a large French family (MS1 family) with a Marfan-like phenotype that was not linked to the *FBN1* locus. The syndrome was subsequently designated Marfan syndrome type 2 (MFS2, OMIM #154705). In this review we are defining MFS2 genetically (not clinically) as the classic MFS phenotype (based on the Ghent criteria) caused by mutation in the *TGFBR2* locus.

Marfan-like symptoms observed in this family consisted of severe cardiovascular findings, including sudden death of affected members at young age, probably due to a thoracic aortic dissection, and typical MFS skeletal features, but no significant ocular findings were seen. However, one affected family member (IV-83 from the large French family, MS1) was recently reported to suffer from ectopia lentis, which is clinically compatible with classic MFS according to the Ghent criteria but surprising in light of the absence of the condition in other family members (Mizuguchi et al. 2004). Clinical re-evaluation of this individual as well as other affected members is warranted.

Genetic analysis of the French family enabled a successful mapping of the second locus for MFS (*MFS2*) to 3p24.2-p25 (Collod et al. 1994). A Japanese boy with MFS was later shown to have de novo complex chromosomal rearrangements involving 3p24.1, which is close to the *MFS2* locus (Mizuguchi et al. 2004). Detailed genomic analysis revealed that the 3p24.1 breakpoint disrupted the *TGFBR2* gene. Subsequent *TGFBR2* sequence analysis in the MS1 family identified in all affected members a nucleotide substitution c.1524G > A (p.Q508Q) of *TGFBR2*, which is

Table 1 Marfan syndrome-related disorders and mutated genes

Disorder	Gene
Marfan syndrome	*FBN1, TGFBR1, TGFBR2*
Neonatal Marfan syndrome	*FBN1*
Familial thoracic aortic aneurysms and dissections	*FBN1, TGFBR1, TGFBR2*
Isolated ectopia lentis	*FBN1*
Shprintzen–Goldberg craniosynostosis syndrome	*FBN1, TGFBR2*
Autosomal dominant Weill–Marchesani syndrome	*FBN1*
Loyes-Dietz syndrome	*TGFBR1, TGFBR2*

hypothesized to result in protein truncation due to abnormal splicing. Further analysis of 19 unrelated probands unlinked to *FBN1* identified c.923T > C (p.L308P), c.1346C > T (p.S449F) and c.1609C > T (p.R537C; recurrent in two independent families) (Table 2 and Fig. 1). These missense mutations affect an evolutionarily conserved amino acid in the serine/threonine kinase domain of TGFβ receptor type II. This finding confirmed that the MFS phenotype can be caused not only by mutations in *FBN1* but also by mutations in *TGFBR2*.

Loeys–Dietz syndrome

Loeys et al. (2005) reported a new aortic aneurysm syndrome presenting with cardiovascular and skeletal manifestations consistent with those seen in MFS, along with other features not present in MFS. Loeys–Dietz syndrome (LDS, OMIM #609192) is characterized by hypertelorism, bifid uvula, cleft palate, generalized arterial tortuosity, and ascending aortic aneurysm and dissection. Hypothesizing that abnormal TGFβ signaling might cause vascular and craniofacial phenotypes, Loeys et al. (2005) investigated *TGFBR2* as a candidate gene for LDS. Heterozygous *TGFBR2* mutations were found in six of ten LDS patients: five missense mutations in the serine/threonine kinase domain [c.1006T > A (p.Y336N), c.1063G > C (p.A355P), c.1069G > T (p.G357W), c.1582C > T (p.R528C), c.1583G > A (p.R528H)], as seen in MFS2, and a single mutation in a splice-acceptor site (IVS1-2A > G) (Table 2, Fig. 1). The remaining four patients were shown to have *TGFBR1* mutations: three missense mutations in the serine/threonine kinase domain [c.953T > G (p.M318R), c.1199A > G (p.D400G) and c.1460G > C (p.R487P)] and a missense mutation in the glycine/serine-rich (GS) domain [c.599C > T (p.T200I)] (Table 2, Fig. 1). Owing to the clinical overlap of MFS and Shprintzen–Goldberg craniosynostosis syndrome (SGS) with LDS, Loeys et al. (2005) also screened seven MFS patients (unlinked to *FBN1*) and five SGS patients for mutations in *TGFBR1* and *TGFBR2*, but no abnormalities were seen at these loci.

Two other *TGFBR2* mutations [c.773T > G (p.V258G) and c.1067G > C (p.R356P)] and another *TGFBR1* mutation [c.722C > T (p.S241L)] have since been reported in other patients meeting the clinical description of LDS by other groups (Table 2, Fig. 1) (Ki et al. 2005; Matyas et al. 2006). Finally, Loeys et al. (2006) collected 30 more probands of LDS and found *TGFBR2* mutations in 21 and *TGFBR1* mutations in nine (Table 2, Fig. 1) (Loeys et al. 2006). Furthermore, LDS type II (LDS2) without craniofacial features was also proposed. Eight *TGFBR2* and four *TGFBR1* mutations were found in LDS2 patients (Tables 2 and 3, Fig. 1) (Loeys et al. 2006).

Familial thoracic aortic aneurysms and dissections

Non-syndromic thoracic aortic aneurysms and dissections (TAAD) have complex and heterogeneous etiology, with some families inheriting TAAD in an autosomal dominant fashion, with decreased penetrance and variable expression. To date, at least three TAAD loci have been mapped by linkage studies of large single families (Pannu et al. 2005b): *TAAD1* at 5q13–q14 (Guo et al. 2001), the familial aortic aneurysm 1 locus (*FAA1*) at 11q23–q24 (Vaughan et al. 2001), and *TAAD2* at 3p24–p25 (Hasham et al. 2003).

The realization that TAAD2 (OMIM, #608987) and MFS2 are clinically overlapping diseases that both map to 3p24–p25 led Pannu et al. (2005a) to look for *TGFBR2* mutations in 80 unrelated TAAD families, including the large family with disease linkage to 3p24–p25. Two *TGFBR2* mutations [c.1378C > T (p.R460C) and c.1379G > A (p.R460H)], both affecting the same amino acid residue in the serine/threonine kinase domain, were identified in four families, or approximately 5% of the TAAD cases (Pannu et al. 2005a) (Table 2, Fig. 1). Each mutation occurred in the unique haplotype block, indicating an independent mutation event.

The mutational hotspot at the p.R460 residue in TAAD2 suggested a positive phenotype–genotype correlation, and this observation was supported by the discovery of another family with p.R460H that was initially diagnosed as having TAAD2 and, later, as having a distinctive condition with cardiovascular findings consistent with TAAD2, together with arterious tortuosity and aneurysm (Law et al. 2005, 2006). Three additional missense *TGFBR2* mutations [c.1159G > A (p.V387G), c.1181G > A (p.C394Y), c.1657T > A (p.S553T)] and, more importantly, one *TGFBR1* mutation [c.1460G > A (p.R487Q)] were found in four TAAD patients (Matyas et al. 2006).

Shprintzen–Goldberg craniosynostosis syndrome

Shprintzen–Goldberg craniosynostosis syndrome (SGS, OMIM, #182212) is characterized by craniosynostosis and other craniofacial features, marfanoid skeletal abnormalities, and developmental delay (Robinson et al. 2005). Furlong syndrome (FS) is a similar marfanoid disorder with craniosynostosis, which differs from SGS by the absence of mental retardation (Furlong et al. 1987).

Table 2 Summary of *TGFBR2* and *TGFBR1* mutations

Gene	Disorder	Exon	Mutations	Domain	Splicing abnormality	Nature	Ghent criteria	References
TGFBR2	MFS	–	Chromosomal rearrangements	–	–	De novo	Fulfilled	Mizuguchi et al. (2004)
		4	c.923T > C (p.L308P)	Kinase	–	De novo	Fulfilled	Mizuguchi et al. (2004)
		4	c.1067G > C (p.R356P)	Kinase	–	De novo	Not fulfilled[a]	Sakai et al. (2006)
		4	c.1106G > T (p.G369V) and c.1159G > C (p.V387L)[b]	Kinase	–	Familial	Not fulfilled	Matyas et al. (2006)
		4	c.1151A > G (p.N384S)	Kinase	–	Familial	Not fulfilled	Singh et al. (2006)
		4	c.1188T > G (p.C396W) and c.-334T > A[c]	Kinase	–	De novo	Fulfilled	Singh et al. (2006)
		5	c.1273A > G (p.M425V)	Kinase	–	Familial	Fulfilled	Disabella et al. (2006)
		5	c.1336G > A (p.D446N)	Kinase	–	De novo	Not fulfilled	Disabella et al. (2006)
		5	c.1336G > A (p.D446N)	Kinase	–		Not fulfilled[a]	Sakai et al. (2006)
		5	c.1322C > T (p.S441F)	Kinase	–	De novo	Not fulfilled	Singh et al. (2006)
		5	c.1346C > T (p.S449F)	Kinase	–	Familial	Not fulfilled	Mizuguchi et al. (2004)
		5	c.1378C > T (p.R460C)	Kinase	–	Familial	Fulfilled	Singh et al. (2006)
		5	c.1379G > A (p.R460H)	Kinase	–	Familial	Fulfilled	Disabella et al. (2006)
		6	c.1489C > T (p.R497X)	Kinase	–	De novo?	Not fulfilled	Singh et al. (2006)
		6	c.1524G > A (p.Q508Q)	Kinase	+	Familial	Fulfilled	Mizuguchi et al. (2004)
		7	c.1561T > C (p.W521R)	Kinase	–	De novo	Fulfilled	Matyas et al. (2006)
		7	c.1609C > T (p.R537C)	Kinase	–	Familial	Fulfilled	Mizuguchi et al. (2004)
	LDS	–	IVS1-2A > G	–	+	De novo?	–	Loeys et al. (2005)
		4	c.773T > G (p.V258G)	Kinase	–	Familial	Fulfilled	Matyas et al. (2006)
		4	p.A329T	Kinase	–	?	–	Loeys et al. (2006)
		4	c.1006T > A (p.Y336N)	Kinase	–	Familial	–	Loeys et al. (2005)
		4	c.1063G > C (p.A355P)	Kinase	–	Familial	–	Loeys et al. (2005)
		4	c.1067G > C (p.R356P)	Kinase	–	De novo	–	Ki et al. (2005)
		4	c.1069G > T (p.G357W)	Kinase	–	De novo	–	Loeys et al. (2005)
		4	p.N384S	Kinase	–	?	–	Loeys et al. (2006)
		5	p.P427L	Kinase	–	?	–	Loeys et al. (2006)
		5	p.P427S	Kinase	–	?	–	Loeys et al. (2006)
		5	p.Y448H	Kinase	–	?	–	Loeys et al. (2006)
		5	p.S449F	Kinase	–	?	–	Loeys et al. (2006)
		5	p.M457K	Kinase	–	?	–	Loeys et al. (2006)
		5	p.R460H	Kinase	–	?	–	Loeys et al. (2006)
		5	p.C461Y	Kinase	–	?	–	Loeys et al. (2006)
		–	IVS5-1G > A		+	?	–	Loeys et al. (2006)
		6	p.R495X	Kinase	–	?	–	Loeys et al. (2006)
		7	p.E519K	Kinase	–	?	–	Loeys et al. (2006)
		7	p.C520Y	Kinase	–	?	–	Loeys et al. (2006)
		7	p.D524N	Kinase	–	?	–	Loeys et al. (2006)
		7	p.A527V	Kinase	–	?	–	Loeys et al. (2006)
		7	c.1582C > T (p.R528C)	Kinase	–	De novo	–	Loeys et al. (2005, 2006)
		7	c.1583G > A (p.R528H)	Kinase	–	De novo	–	Loeys et al. (2005, 2006)
		7	p.L529F	Kinase	–	?	–	Loeys et al. (2006)
		7	p.C533R	Kinase	–	?	–	Loeys et al. (2006)
		7	p.C533F	Kinase	–	?	–	Loeys et al. (2006)
		7	p.R537C	Kinase	–	?	–	Loeys et al. (2006)
		7	p.R537G	Kinase	–	?	–	Loeys et al. (2006)
	TAAD	4	c.1159G > A (p.V387M)	Kinase	–	Familial	–	Matyas et al. (2006)
		4	c.1181G > A (p.C394Y)	Kinase	–	?	–	Matyas et al. (2006)
		5	c.1378C > T (p.R460C)	Kinase	–	Familial	–	Pannu et al. (2005a)
		5	c.1379G > A (p.R460H)	Kinase	–	Familial	–	Pannu et al. (2005a) and Law et al. (2006)

Table 2 continued

Gene	Disorder	Exon	Mutations	Domain	Splicing abnormality	Nature	Ghent criteria	References
		7	c.1657T > A (p.S553T)	–	–	De novo?	–	Matyas et al. (2006)
	SGS (LDS?)	–	IVS5-2A > G	–	+	De novo	–	Kosaki et al. (2006)
TGFBR1	MFS	4	c.759G > A(p.M253I)	Kinase	–	Familial	Fulfilled	Singh et al. (2006)
		4	c.799A > C (p.N267H)	Kinase	–	Familial	Not fulfilled	Matyas et al. (2006)
		5	c.934G > A (p.G312S)	Kinase	–	Familial	Fulfilled	Singh et al. (2006)
		7	c.1135A > G (p.M379V)	Kinase	–	?	Not fulfilled	Sakai et al. (2006)
	LDS	4	c.599C > T (p.T200I)	GS	–	De novo	–	Loeys et al. (2005)
		4	p.K232E	Kinase	–	?	–	Loeys et al. (2006)
		4	p.F234L	Kinase	–	?	–	Loeys et al. (2006)
		4	c.722C > T (p.S241L)	Kinase	–	De novo	Fulfilled	Loeys et al. (2006) and Matyas et al. (2006)
		5	c.953T > G (p.M318R)	Kinase	–	De novo	–	Loeys et al. (2005)
		6	p.A350E	Kinase	–	?		Loeys et al. (2006)
		6	p.G353V	Kinase	–	?		Loeys et al. (2006)
		6	p.G374E	Kinase	–	?		Loeys et al. (2006)
		7	c.1199A > G (p.D400G)	Kinase	–	De novo	–	Loeys et al. (2005)
		9	p.N478S	Kinase	–	?		Loeys et al. (2006)
		9	c.1460G > C (p.R487P)	Kinase	–	Familial	–	Loeys et al. (2005, 2006)
		9	p.R487Q	Kinase	–	?		Loeys et al. (2006)
		9	p.R487W	Kinase	–	?		Loeys et al. (2006)
	TAAD	9	c.1460G > A (p.R487Q)	Kinase	–	De novo	–	Matyas et al. (2006)
	FS	4	c.722C > T (p.S241L)	Kinase	–	De novo	Fulfilled	Ades et al. (2006)

MFS Marfan syndrome, *LDS* Loeys-Dietz syndrome, *TAAD* Familial thoracic aortic aneurysms and dissections, *SGS* Shprintzen-Goldberg craniosynostosis syndrome, *FS* Furlong syndrome

[a] LDS facial features were recognized

[b] Two nucleotide changes in one allele

[c] Compound heterozygote

Although a question was raised on the involvement of *FBN1* abnormality in SGS (Robinson et al. 2005), at least two *FBN1* mutations were identified (Kosaki et al. 2006; Sood et al. 1996).

Furthermore, the initial study of five SGS patients failed to reveal mutations in either *TGFBR1* or *TGFBR2* (Loeys et al. 2005), but Kosaki et al. (2006) recently reported a *TGFBR2* mutation [IVS5-2A > G] (Table 2, Fig. 1) in an SGS patient with craniofacial and skeletal abnormalities, mild developmental delay, and cardiovascular features, including aortic regurgitation, annuloaortic ectasia and sigmoid configuration of the brachiocephalic, left common carotid and left subclavian arteries. Robinson et al. (2006), however, suggested that this patient might be more appropriately diagnosed as having LDS, due to the presence of bifid uvula and arterial manifestations.

An identical *TGFBR1* mutation [c.722C > T (p.S241L)] was reported in two unrelated patients described as having probable FS (Table 2, Fig. 1) (Ades et al. 2006). One of these patients had learning difficulties, and the other had normal intelligence (Ades et al. 2006). Systemic arterial tortuosity was not evaluated in either of them, but one showed bifid uvula,

consistent with LDS. The same mutation was also reported in an LDS patient with hypertelorism, tortuous arteries and bifid uvula (Matyas et al. 2006).

Germline *TGFBR* mutations and connective tissue disorders

The various mutations, as well as gene disruption by chromosomal structural abnormality, suggest that loss-of-function mutations of *TGFBR2* are responsible for a wide spectrum of connective tissue disorders, but no simple genotype–phenotype correlation has been observed. It is intriguing that domain-specific germline mutations of *TGFB1* cause Camurati–Engelmann disease (CED, OMIM #131300) (Kinoshita et al. 2000) associated with marfanoid habitus, despite the absence of connective tissue fragility. *TGFB1* mutations in CED were shown to cause increased TGFβ signaling (Janssens et al. 2003). These findings suggest that abnormal TGFβ signaling could be responsible for the skeletal features of MFS. This hypothesis is corroborated by earlier studies describing the roles of TGFβ signaling in skeletal development (Alvarez and Serra 2004; Serra et al. 1999).

Fig. 1 Mutation spectrum of *TGFBR2* and *TGFBR1* identified in Marfan syndrome and its related disorders. *Numbered boxes* indicate exons of each gene. *Open and filled circles* represent missense and splicing abnormality mutations, respectively. Each mutation was found in a different family. Functional domains are shown as *patterned boxes*. *MFS* Marfan syndrome, *LDS* Loeys-Dietz syndrome, *TAAD* Familial thoracic aortic aneurysms and dissections, *SGS* Shprintzen-Goldberg craniosynostosis syndrome, *FS* Furlong syndrome
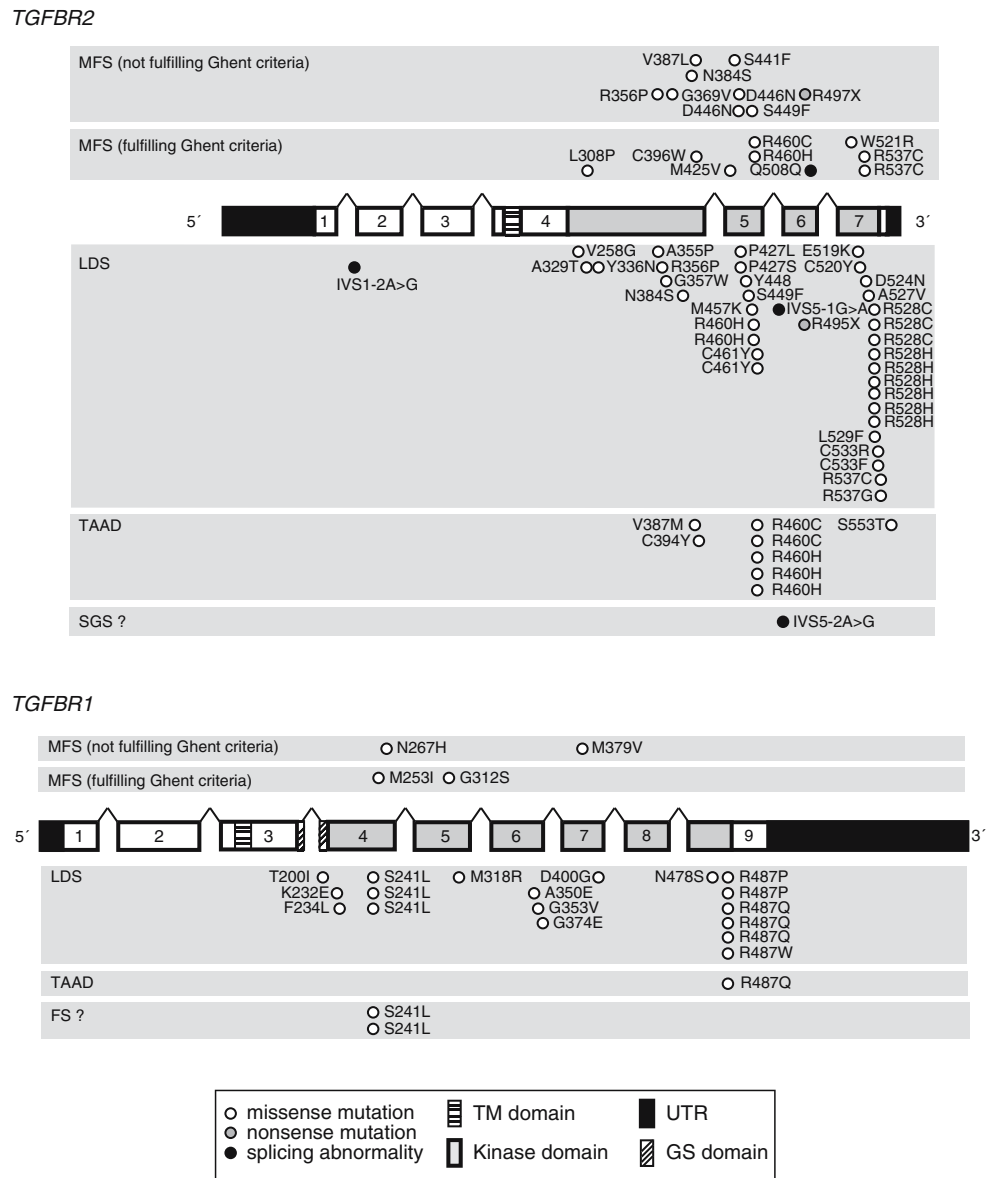
TGFBR2 and *TGFBR1* mutations found in MFS and its related disorders are generally associated with severe vascular consequences that differ somewhat from those found in patients with *FBN1* mutations. The vascular consequences associated with *TGFBR2* and *TGFBR1* mutations include aortic dissections in young age and aneurysms at sites distant from the aortic root. Thus, careful screening of arterial tortuosity and aneurysms from head to leg is highly recommended in this patient population.

Whether an MFS2 phenotype exists as a separate disorder from LDS is debatable. *TGFBR1* and *TGFBR2* aberrations are highly prevalent in LDS, which is characterized by severe cardiovascular features and family histories of aortic dissection with sudden death. Following the first report of *TGFBR2*

mutations in MFS (Mizuguchi et al. 2004), LDS, TAAD2, SGS, and FS were recognized as *TGFBR* mutation-related disorders. Although the original MFS2 patients with *TGFBR2* mutations (Mizuguchi et al. 2004) could not be reasonably re-examined for the presence of LDS features such as bifid uvula, hypertelorism, craniosynostosis, and arterial tortuosity, at least three reports have since described *TGFBR1* or *TGFBR2* mutations in classic MFS patients in whom LDS had been ruled out (Disabella et al. 2006; Matyas et al. 2006; Singh et al. 2006). Four missense mutations [c.1273A > G (p.M425V), c.1378C > T (p.R460C), c.1379G > A (p.R460H), and c.1561T > C (p.W521R)] and compound heterozygous mutations [c.1337G > A (p.D446N) and c.-334T > A] in *TGFBR2* were identified in five patients with MFS that satisfied the Ghent

Table 3 Frequency of *TGFBR2/TGFBR1* mutations in MFS-related disorders

Diagnosis	FBN1 involvement	Gene tested	Mutations (%)		References
MFS	Negative	TGFBR2	25	(5/20)	Mizuguchi et al. (2004)
	Negative	TGFBR1	0	(0/7)	Loeys et al. (2005)
		TGFBR2	0	(0/7)	
	Negative	TGFBR2	100?	(3/3)[a]	Disabella et al. (2006)
	Negative or unknown	TGFBR2	3	(1/30)[b]	Ki et al. (2005)
	Negative	TGFBR1	5	(2/41)	Singh et al. (2006)
		TGFBR2	12	(5/41)	
	Negative	TGFBR1	5	(1/22)	Sakai et al. (2006)
		TGFBR2	9	(2/22)	
LDS	Unknown	TGFBR1	40	(4/10)	Loeys et al. (2005)
		TGFBR2	60	(6/10)	
	Unknown	TGFBR1	30	(9/30)	Loeys et al. (2006)
		TGFBR2	70	(21/30)	
LDS2	Unknown	TGFBR1	10	(4/40)	Loeys et al. (2006)
		TGFBR2	20	(8/40)	
TAAD	Unknown	TGFBR2	0	(0/10)	Mizuguchi et al. (2004)
	Unknown	TGFBR2	5	(4/80)	Pannu et al. (2005a)
SGS	Unknown	TGFBR1	0	(0/5)	Loeys et al. (2005)
		TGFBR2	0	(0/5)	
	Negative	TGFBR1	0	(0/1)	Kosaki et al. (2006)
		TGFBR2	100	(1/1)	
MD-CS/MR (FS)	Negative	TGFBR1	100	(2/2)[c]	Ades et al. (2006)
		TGFBR2	0	(0/2)	
MFS-related phenotypes	Negative	TGFBR1	4	(3/70)	Matyas et al. (2006)
		TGFBR2	9	(6/70)	

MFS Marfan syndrome, *LDS* Loeys-Dietz syndrome, *LDS2* Loeys-Dietz syndrome type II, *TAAD* Familial thoracic aortic aneurysms and dissections, *SGS* Shprintzen-Goldberg craniosynostosis syndrome, *MD-CS/MR* Marfan–craniosynostosis/mental retardation, *FS* Furlong syndrome

[a] Total number of patients screened was not described

[b] The patient was initially diagnosed as having an MFS variant, which was later refined as LDS

[c] The two patients with a *TGFBR1* mutation were categorized to Furlong syndrome

criteria. Seven more patients with MFS that did not satisfy the Ghent criteria were also reported to have mutations in *TGFBR2* (Disabella et al. 2006; Matyas et al. 2006; Sakai et al. 2006; Singh et al. 2006). In addition, two *TGFBR1* missense mutations [c.759G > A (p.M253I) and c.934G > A (p.G312S)] were found in two MFS patients and two *TGFBR1* mutations [c.799A > C (p.N267H) and c.1135A > G (p.M379V)] were identified in two patients not fulfilling MFS. Thus, *TGFBR2* and *TGFBR1* aberrations were observed in typical cases of classic MFS with no signs of LDS (Singh et al. 2006).

However, it should be noted that arterial tortuosity, a cardinal feature of LDS, was not systematically evaluated in any of the four studies (Disabella et al. 2006; Matyas et al. 2006; Sakai et al. 2006; Singh et al. 2006). Moreover, two research groups were unable to identify *TGFBR2* mutations in 29 MFS patients (*FBN1* was normal in 24 and unknown in five) (Ki et al. 2005) and seven patients (*FBN1* was normal) with MFS compatible with the Ghent criteria (Loeys et al. 2005). Thus, the question of whether an MFS2 phenotype and LDS should be classified as the same disorder remains unresolved.

At least six *TGFBR2* mutations (R356P, N384S, R460H, R460C, S449F, and R537C) and two *TGFBR1* mutations (S241L and R487Q) were recognized in two or three conditions (i.e., R460H found in MFS, LDS, and TAAD) (Ades et al. 2006; Disabella et al. 2006; Ki et al. 2005; Loeys et al. 2006; Matyas et al. 2006; Mizuguchi et al. 2004; Pannu et al. 2005a; Sakai et al. 2006; Singh et al. 2006) (Fig. 1, Table 2), implying that *TGFBR* mutations may cause various clinical consequences or that appropriate diagnosis is rather difficult for these disorders.

Pathogenesis of Marfan syndrome

Fibrillin-1 involvement in connective tissue disorders

Extracellular matrix (ECM) is formed by a number of macromolecules that are secreted and deposited into the space surrounding cells, where they are essential

for tissue development and homeostasis. Fibrillin-1 is an ECM protein that is assembled into microfibrillar networks, where it interacts with other ECM proteins. Fibrillin-rich microfibrils form peripheral components of elastic fibers, which play a role as an architectural foundation and provide elasticity to tissues (Kielty et al. 2002).

Fibrillin-1 is a multi-domain protein that contains three characteristic modules: an epidermal growth factor (EGF)-like motif, a latent TGFβ binding protein (LTBP) motif, and a fusion of the two (Fib motif). The majority of the EGF-like modules in the *FBN1* gene have a conserved calcium-binding sequence (cbEGF-like module).

Mutations associated with MFS are distributed over the entire *FBN1* gene. No genotype–phenotype correlation has been clearly established, except for neonatal MFS (nMFS). Most mutations causing nMFS seem to be clustered in exons 24–32, although other phenotypes are also associated with mutations in these exons.

The fibrillin-1 protein contains 47 EGF-like modules, characterized by six cysteine residues that form disulfide bonds with one another. Most MFS missense mutations occur in one of the 43 cbEGF-like modules (Boileau et al. 2005; Robinson and Godfrey 2000). These mutations are thought to influence the protein structure and calcium binding affinity of cbEGF-like modules, with various deleterious effects (Boileau et al. 2005; Downing et al. 1996).

In classical MFS the incidence of ectopia lentis was significantly higher in patients harboring cysteine substitutions in the cbEGF-like modules than in patients with premature termination codon (PTC) mutations (Arbustini et al. 2005; Biggin et al. 2004; Loeys et al. 2004; Rommel et al. 2005; Schrijver et al. 2002). Furthermore, isolated or predominant ectopia lentis is frequently associated with cysteine substitutions (Ades et al. 2004; Comeglio et al. 2002), suggesting that cysteine residues may have a critical function in suspensory ligaments of the eyes, as previously described (Rommel et al. 2005).

The dominant–negative mechanism of mutant fibrillin-1 in MFS pathology is an attractive hypothesis in the light of the polymerizing nature of fibrillin-1 molecules into microfibrils. Various studies have examined the correlation between the expression level of mutant mRNA produced by PTC mutations and the degree of clinical severity (Ades et al. 2004; Dietz et al. 1993; Schrijver et al. 2002). Nonsense-mediated mRNA decay could limit aberrant protein production from mutated alleles in heterozygous patients, but remnant mutant fibrillin-1 proteins may act in a dominant–negative fashion. However, the hypothesized associa-

tion between expression level and clinical severity is controversial. It is also possible that different expression levels of normal *FBN1* alleles leads to different phenotypes in MFS family members sharing the same heterozygous PTC mutation (Hutchinson et al. 2003).

Support for the haploinsufficiency hypothesis as a mechanism in MFS pathogenesis comes from the finding of an *FBN1* deletion in a patient with a number of marfanoid characteristics (Hutchinson et al. 2003; Judge et al. 2004) as well as in mouse models (Judge et al. 2004). In transgenic mice carrying human mutant *FBN1* (p.C1663R), no obvious cardiovascular and skeletal signs have been recognized, despite the co-assembly of the mutant protein into mouse microfibrillar networks. Other knock-in mice (in which the p.C1039G mutation $Fbn1^{C1039G/+}$ targeted the endogenous $Fbn1$) showed decreased microfibrillar deposition, skeletal abnormalities and changes in the architecture of the aortic wall. Notably, the aortic wall phenotype was rescued by the wildtype *FBN1* transgene.

TGFβ signaling and connective tissue disorders

TGFβ is a secreted polypeptide that plays diverse roles in cell proliferation and differentiation, apoptosis, and extracellular matrix formation (Cohen 2003; Derynck et al. 2001; Ignotz and Massague 1986; Massague et al. 2000). TGFβ1 is abundant in the ECM. An inactive form of mature TGFβ1 stays in a complex with latency-associated polypeptide (LAP), which is an N-terminal peptide cleaved from proTGFβ1, and latent TGFβ binding protein (LTBP). Mature TGFβ1 and LAP are noncovalently associated in a small latency complex (SLC). The SLC binds to LTBP via disulfide bonds between LAP and LTBP, forming a large latency complex (LLC) (Annes et al. 2003). LTBP plays a role in folding and secreting TGFβ1, targeting it appropriately to the ECM, and modulating TGFβ activity (Charbonneau et al. 2004; Kaartinen and Warburton 2003; Ramirez et al. 2004; Rifkin 2005).

A recent study revealed that LTBP-1 (one of the LTBPs) and fibrillin interact in vitro and suggested that fibrillin-1 may stabilize the latent TGFβ complexes in the ECM (Isogai et al. 2003). Support for this hypothesis is seen in mouse models of MFS. Three strains of transgenic mice, each harboring a different type of *Fbn1* mutation, displayed several MFS features with variable severity (Judge et al. 2004; Pereira et al. 1997, 1999). Increased TGFβ activity was observed in at least four organs (lung, mitral valve, aortic and dural tissues), possibly as a result of excess free LLC due to inadequate stabilization within the ECM, as previously hypothesized (Habashi et al. 2006; Jones et al. 2005; Neptune

et al. 2003; Ng et al. 2004; Rifkin 2005). Administration of an anti-TGFβ neutralizing antibody rescued the lung, mitral valve, and aortic tissue phenotypes (Habashi et al. 2006; Neptune et al. 2003; Ng et al. 2004). Furthermore, aortic aneurysm was prevented by the administration of losartan, an angiotensin II type 1 receptor blocker that alleviates increased TGFβ activity (Habashi et al. 2006). Taken together, these findings support an association of abnormal TGFβ signaling with MFS pathogenesis. Interaction between the affected structural ECM components and aberrant TGFβ signaling may coordinately determine MFS phenotypes.

TGFβ transduces its signals via two distinct types of transmembrane receptors, type I (TβRI) and type II (TβRII), encoded by *TGFBR1* and *TGFBR2*, respectively (ten Dijke et al. 1996; Wrana et al. 1994). Both types of receptors consist of an extracellular domain, a transmembrane domain and a serine/threonine kinase domain. A glycine/serine-rich domain (GS domain) is specific for TβRI. The ligand-bound TβRII phosphorylates the GS domain, which then acts in signal transduction (Wieser et al. 1995).

It is likely that abnormal TGFβ signaling is involved in the human MFS phenotype. Identification of *TGFBR2* mutations in MFS2 supports the hypothesis and provided the first direct link between a human connective tissue disorder and abnormal TGFβ signaling. Loss-of-function *TGFBR2* mutations are hypothesized to cause MFS2, LDS and TAAD2. In our previous study mammalian cells were transfected with MFS2-related mutant *TGFBR2* constructs, and a luciferase assay clearly showed decreased TGFβ signaling activity (Mizuguchi et al. 2004). If the highly conserved p.R460 residue is essential for the structural integrity of the catalytic loop of TβRII, the p.R460 missense mutations found in TAAD2 could dramatically perturb TGFβ signaling (Pannu et al. 2005a).

By contrast, aortic tissues from LDS individuals showed increased TGFβ signaling activity. The heterozygous state (with one normal allele and the other mutant) of *TGFBR2* abnormality in affected individuals might not simply reflect a loss-of-function nature of the mutation, probably because of the complex regulation of the TGFβ signaling pathway (Loeys et al. 2005). Paradoxically, increased TGFβ signaling was also shown in the kinase-deficient TβRII transgenic mice (Denton et al. 2003).

Conclusion

Recent genetic studies of MFS, both in mice and humans, revealed that TGFβ signaling is involved in the pathogenesis of MFS and related disorders. *FBN1* abnormalities appear to be the major genetic cause of MFS, but not the only cause.

MFS-related disorders share many features with MFS. Clinical data should be carefully evaluated, with the recognition that incomplete data might lead to different diagnoses. Further studies are needed in order to establish frames of nosology for MFS and MFS-related disorders, by collection and analysis of extensive genetic and clinical data from many affected patients. An appropriate diagnostic system(s) is needed to differentiate MFS and MFS-related disorders.

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