From NSD1 to Sotos syndrome : a genetic and functional analysis

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Chapter 10

Comprehensive genetic analysis of relevant four genes in 49 patients with Marfan syndrome or Marfan related phenotypes

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Abstract

In order to evaluate the contribution of *FBN1, FBN2, TGFBR1,* and *TGFBR2* mutations to the Marfan syndrome (MFS) phenotype, the four genes were analyzed by direct sequencing in 49 patients with MFS or suspected MFS as a cohort study. A total of 27 *FBN1* mutations (22 novel) in 27 patients (55%, 27/49), one novel *TGFBR1* mutation in one (2%, 1/49) and two recurrent *TGFBR2* mutations in two (4%, 2/49) were identified. No *FBN2* mutation was found. Three patients with either *TGFBR1* or *TGFBR2* abnormality did not fulfill the Ghent criteria, but expressed some overlapping features of MFS and Loeys-Dietz syndrome (LDS). In the remaining 19 patients, either of the genes did not show any abnormalities. This study indicated that *FBN1* mutations were predominant in MFS but *TGFBRs* defects may account for approximate 5-10% of patients with the syndrome.

Introduction

Marfan syndrome (MFS, OMIM #154700) is an autosomal dominant connective tissue disorder primarily involving skeletal, ocular, and cardiovascular systems. The incidence is estimated to be one in 5,000 - 10,000 individuals (1,2). MFS was caused by mutations of the fibrillin-1 gene (*FBN1*) at 15q21.1 encoding the large, cysteine rich, extracellular matrix glycoprotein which is the major component of microfibrils (3). *FBN1* spans a 230-kb genomic region with 65 exons and at least 664 reported *FBN1* mutations spread throughout the gene and are mostly private in each affected family [UMD-FBN1 Mutation Database (http://www.umd.be:2030/)] (4). Recently we found that the transforming growth factor (TGF)-β receptor II gene (*TGFBR2*) was mutated in patients with Marfan syndrome not linked to *FBN1* aberrations (5). This gave the first genetic evidence of a direct link between abnormal TGF-β signaling and a human connective tissue disorder. Subsequently Loeys et al. reported a new dysmorphic syndrome with mutations in either *TGFBR2* or TGF-β receptor I (*TGFBR1*) (Loeys-Dietz syndrome, LDS, OMIM #609192) (6). Several symptoms are overlapped in MFS and LDS, including aortic root aneurysm, and other skeletal abnormalities. Furthermore *TGFBR2* mutations were also detected in patients with familial thoracic aortic aneurysms and dissections (TAAD) (7).

Congenital contractural arachnodactyly (CCA, OMIM#121050), a similar connective tissue disorder to MFS, is caused by mutations in the fibrillin 2 gene (*FBN2*) homologous to *FBN1* (8-10). It is often difficult to differentiate clinically between the two syndromes because of their phenotypic similarities in skeletal complications including arachnodactyly, dolichostenomelia, pectus deformities, and kyphoscoliosis. CCA usually presents with multiple joint contracture and crumpled ear helix and very occasionally with aortic root dilatation and eye involvement which may prevent clear distinction of the two syndromes (10-13).

Four genes, *FBN1*, *FBN2*, *TGFBR1*, and *TGFBR2*, were analyzed by direct sequencing in a total of 49 patients with MFS or suspected MFS as a cohort study. Contribution of mutations of each gene and corresponding phenotypes will be discussed.

Materials and Methods

Patients

The clinical features of the probands are given in Table 1. Among the 49 probands, 38 were reasonably evaluated according to the Ghent criteria, 14 of whom fulfilled the MFS criteria (14). After written informed consent, genomic DNA of blood leukocytes was isolated using DNA isolation systems [Quick Gene-800 (Fujifilm, Tokyo, Japan) and/or NA-3000 (Kurabo, Osaka, Japan)]. Hair and nails were obtained if necessary and their DNAs were extracted using Isohair (Nippon Gene, Tokyo, Japan), respectively. Experimental protocols were approved by the Committee for Ethical Issues at Yokohama City University School of Medicine.

Sequence analysis

FBN1, *FBN2*, *TGFBR1* and *TGFBR2*, were analyzed. *FBN1* and *TGFBR2* were sequenced in all patients. In whom both genes were normal, *TGFBR1* and subsequently *FBN2* were analyzed. Primer sequences for *FBN1* and *TGFBR2* were basically described elsewhere (5,15). Those for exon 7 of *FBN1*, exon 1 of *TGFBR2*, all exons of *TGFBR1* and *FBN2* were newly designed in this study (available on request). All coding exons of *FBN1* (65 exons), *FBN2* (65 exons), *TGFBR1* (9 exons), and *TGFBR2* (7 exons) and their flanking intronic regions were amplified by PCR. In one patient (MFS31), sufficient amount of DNA was not available, thus *FBN2* could not be analyzed. PCR products were purified with ExoSAP-IT (USB, Cleveland, OH) and sequenced by a standard protocol using BigDye terminator (Applied Biosystems, Foster City, CA) on an ABI PRISM 3100 Genetic analyzer (Applied Biosystems). The nucleotide substitution was confirmed using the SeqScape software ver. 2.0 (Applied Biosystems). If nucleotide changes were identified in patients whose parental samples were unavailable, at least 50 normal controls were additionally screened to confirm whether they were polymorphisms or not.

Results

A total of 27 mutations in *FBN1*, two in *TGFBR2*, and one in *TGFBR1* were detected in 30 patients in this study (Table 1). No mutation in either of four genes was found in the remaining 19 patients. Interestingly 4495A>T (S1499C) and 7978A>C (S2660R) in *FBN1* were identified in one family [MFS41a (mother) and MFS41b (daughter)], suggesting that both of the mutations resided on one allele as they were of maternal origin. Four *FBN1* mutations,

1285C>T (R429X), 1904A>G (Y635C), 4285T>A (C1429S), and 7399C>T (Q2467X), and two *TGFBR2* mutations, 1067G>C (R356P) and 1336G>A (D446N), were described previously UMD-FBN1 Mutation Database [http://www.umd.be:2030/] (16-20).

FBN1 mutations comprised of 17 missense mutations, 6 nonsense mutations, three frameshift mutations, and a splice site mutation. The splice site mutation, IVS46+5G>A, was found in two independent probands (MFS20 and MFS56). Eleven of 17 missense mutations were occurred at cb-EGF module (calcium-binding epidermal growth factor like module). A total of 13 missense mutations created (15%; 4/27) at or substituted (33%; 9/27) to a cysteine residue in fibrillin-1. Two recurrent missense mutations in *TGFBR2* in two and one novel missense mutation in *TGFBR1*, 1135A>G (M379V) in one were identified. The *TGFBR1* mutation occurred at a well-conserved amino acid of the kinase domain (Figure 1). No *FBN2* mutation was found. Furthermore polymorphisms of *FBN1, FBN2*, and *TGFBR2* found in this study were listed in Table 2. We regarded a nucleotide change as a polymorphism if it was identified in either of a patient's healthy parents or normal controls, or was registered as a SNP in the databases.

Clinical information based on the Ghent criteria was reasonably available from a total of 38 probands. Among 14 patients fulfilling the criteria, 9 possessed *FBN1* mutations (64%). Among 24 patients not fulfilling the criteria, 10 had *FBN1* abnormalities (42%), two *TGFBR2* mutations (8%), and one *TGFBR1* mutation (4%). Two patients with *TGFBR2* mutations (MFS34 and MFS55) presented with a similar face found in LDS and some skeletal abnormalities (Table 3). The 58-year-old patient with *TGFBR1* abnormality (MFS60b) does not show a typical LDS face but presented with DeBakey IIIb type ascending aortic dissection which was operated at her 51 years (Table 3 and Figure 2).

Table 1. List of the clinical and molecular data

MFS ID	Age (year)	Sex	Height (cm)	Skele-tal	Ocu-lar	Cardio-vascular	Pulmo-nary	Skin	Dura	Fami-lial Hx	Ghent criteria	Gene	Exon	Nucleotide change	Amino acid change	Nature	Novelty
37	10	Female	153	m	M	-	-	-	-	-	Not fulfilling	*FBN1*	2	211T > C	W71R	De novo	Novel
59	39	Female		m		-	m	-	-	+	Not fulfilling	*FBN1*	4	400T > G	C134G		Novel
19												*FBN1*	7	772C > T	Q258X		Novel
10a												*FBN1*	8	937delT	C313A Fs	Familial	Novel
52	35	Male	192	m	-	M	m	m	-	-	Not fulfilling	*FBN1*	10	1285C > T	R429X		Matyas et al. (20)
42												*FBN1*	15	1904A > G	Y635C		UMD-*FBN1* database
7	13	Male	124	m	-	M	-	-		-	Not fulfilling	*FBN1*	16	2097T > A	C699X	De novo	Novel
39a	38	Female	185	M	M		-	m		M	MFS	*FBN1*	24	2942G > C	C981S	Familial	Novel
40												*FBN1*	24	3043G > A	A1015T		Novel
26	2	Male	101	M	-	M	-	-		-	Not fulfilling	*FBN1*	25	3125G > A	G1042D		Novel
25	16	Female	174	M	M	M	-	-		-	MFS	*FBN1*	33	4099T > C	C1367R		Novel
43a												*FBN1*	34	4283-4284insG	R1428R Fs	Familial	Novel
2	10	Female	148	M	M	M	-	-		-	MFS	*FBN1*	34	4285T > A	C1429S	De novo	Collod-Beroud et al. (16)
5												*FBN1*	35	4405delC	R1469A Fs		Novel
41a		Female		M	M					M	MFS	*FBN1*	36	4495A > T	S1499C	Familial	Novel

Table 1. ***(continued)***

MFS ID	Age (year)	Sex	Height (cm)	Skele-tal	Ocu-lar	Cardio-vascular	Pulmo-nary	Skin	Dura	Fami-lial Hx	Ghent criteria	Gene	Exon	Nucleotide change	Amino acid change	Nature	Novelity
41b		Female		M	M					M	MFS	*FBN1*	36	4495A > T	S1499C	Familial	Novel
38	35	Female	169	m	M	-	-	-	-	-	Not fulfilling	*FBN1*	38	4781G > T	G1594V		Novel
44	34	Female	176	m	M	m				M	MFS	*FBN1*	40	4988G > T	C1663F	Familial	Novel
49	9	Male	143	m	-	-	-	-	-	-	Not fulfilling	*FBN1*	43	5368C > T	R1790X		Novel
57a	40	Female	175	m	-	M	-	m	-	M	MFS	*FBN1*	43	5404A > T	K1802X	Familial	Novel
57b	13	Female	183	M	-	M	-	-	-	M	MFS	*FBN1*	43	5404A > T	K1802X	Familial	Novel
33a	4	Male	111	m	M	-	-	-		M	MFS	*FBN1*	44	5539T > C	C1847R	Familial	Novel
33b	31	Male	198	m	M	M	-	-		M	MFS	*FBN1*	44	5539T > C	C1847R	Familial	Novel
20												*FBN1*	46	IVS46 + 5G > A			Nijbroek et al. (21)
56		Male	184	M	M	M	-	m		-	MFS	*FBN1*	46	IVSS46 + 5G > A		De novo	Nijbroek et al. (21)
50	49	Male	178	-	-	M	-	-	-	-	Not fulfilling	*FBN1*	50	6236C > G	S2079C		Novel
1a	6	Female	129	-	-	M	-	-		M	Not fulfilling	*FBN1*	58	7241G > A	R2414Q	Familial	Novel
1b	32	Female	171	m	-	M	m	-		M	Not fulfilling	*FBN1*	58	7241G > A	R2414Q	Familial	Novel
54	13	Male	150	M		m	-			-	Not fulfilling	*FBN1*	59	7342T > C	C2448R		Novel
9	3	Male	107	m	-	-	M	-	-	M	MFS	*FBN1*	59	7399C > T	Q2467X	Familial	Halliday et al. (18)
3a		Female	174	m	-	M	-			M	MFS	*FBN1*	59	7409G > A	C2470Y	Familial	Novel

Table 1. ***(continued)***

MFS ID	Age (year)	Sex	Height (cm)	Skele-tal	Ocu-lar	Cardio-vascular	Pulmo-nary	Skin	Dura	Fami-lial Hx	Ghent criteria	Gene	Exon	Nucleotide change	Amino acid change	Nature	Novelity
3b	36	Female	170	m	-					M		*FBN1*	59	7409G > A	C2470Y	Familial	Novel
3c	9	Male	121	M	-	M	-	-		M	MFS	*FBN1*	59	7409G > A	C2470Y	Familial	Novel
3d	7	Female	134	m	-	M	-	-		M	MFS	*FBN1*	59	7409G > A	C2470Y	Familial	Novel
41a		Female		M	M					M	MFS	*FBN1*	63	7978A > C	S2660R	Familial	Novel
41b		Female		M	M					M	MFS	*FBN1*	63	7978A > C	S2660R	Familial	Novel
34	16	Female	159	m	-	-	-	-	-	-	Not fulfilling	*TGFBR2*	4	1067G > C	R356P	De novo	Ki et al. (19)
55	1	Male	96	m	-	M	-	-		-	Not fulfilling	*TGFBR2*	5	1336G > A	D446N		Disabella et al. (17)
60b	58	Female	162	m	-	m	-	-		M	Not fulfilling	*TGFBR1*	7	1135A > G	M379V		Novel

M, major criteria is satisfied; m, minor criteria is satisfied; -, no symptom

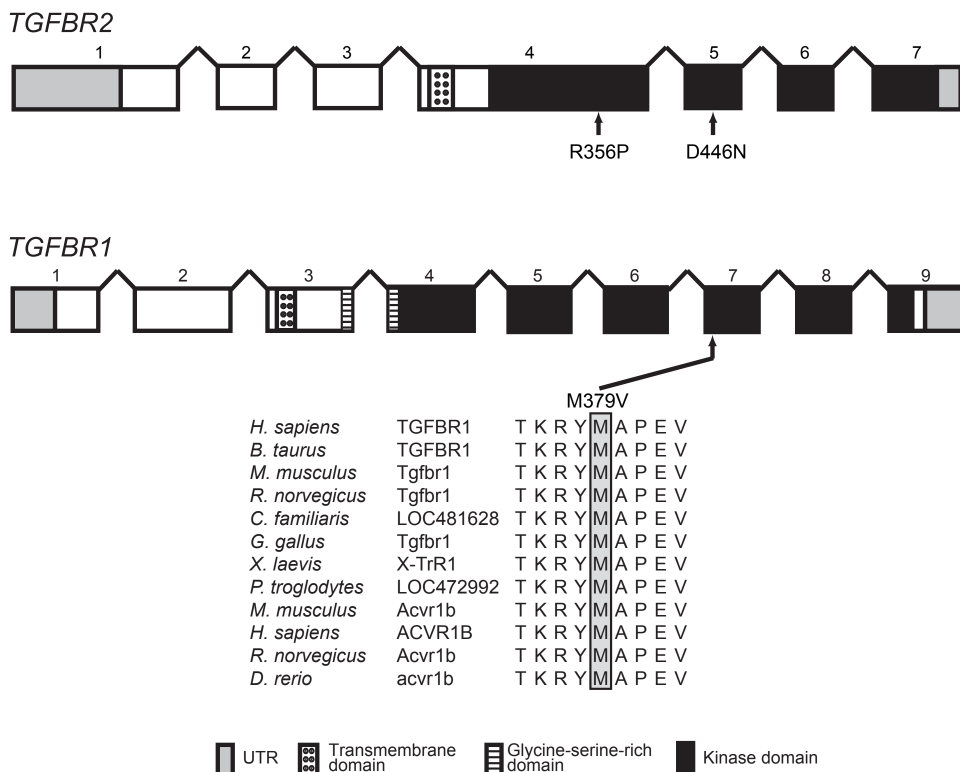

Figure 1. Genomic structure of *TGFBR2* and *TGFBR1* and mutations found in this study

TGFBR2 and *TGFBR1* consist of 7 and 9 exons, respectively. Square indicates exon. A transmembrane domain, a glycine-serine-rich domain, a kinase domain, and UTR are shown as a dotted box, a striped box, a black box, and a grey box, respectively. Two missense mutations in *TGFBR2*, 1067G>C (R356P) and 1336G>A (D446N), are found in MSF34 and MFS55, respectively. One *TGFBR1* mutation in MFS60b was also identified. Two *TGFBR2* mutations were previously described (17,19). Multiple sequence alignment using the web-based software, CLUSTALW (http://clustalw.genome.ad.jp/), clearly demonstrated the *TGFBR1* mutation occurred at an evolutionally conserved amino acid of a kinase domain.

Figure 2. Photographs of the 58-year-old patient with a *TGFBR1* mutation
Abnormal face implying Loeys-Diets syndrome or arachnodactyly was not identified.

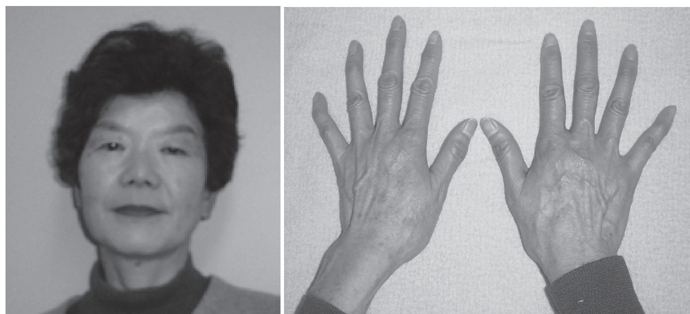

Table 2. Polymorphisms found in this study

Gene	Exon	Polymorphism	SNP ID	Allele frequency in normal control
FBN1	1	79G > A (A27T)	rs25397	
FBN1	4	396T > C (D132D)		1/106
FBN1	11	1415G > A (C472Y)	rs4775765	
FBN1	15	1875T > C (N625N)	rs8033037	
FBN1	22	IVS21-12T > C		Found in a healthy parent
FBN1	27	3442C > G (P1148A)	rs7175654/rs140598	
FBN1	27	IVS27 + 3A > G		Found in a healthy parent
FBN1	42	IVS42 + 14 G > A	rs140650	
FBN1	55	6855T > C (D2285D)	rs363836	
FBN1	56	6888G > A (Q2296Q)	rs363830	
FBN2	6	728T > C (I243T)	NM_001999	
FBN2	12	1643a > c (D548A)		1/106
FBN2	23	2893G > A (V965I)	rs154001	
FBN2	27	3518C > G (T1173S)		1/46
FBN2	29	3762C > T (D1274D)	rs2279582	
FBN2	46	5823T > C (H1941H)	NM_001999	3/40
FBN2	54	6833C > T (T2278M)	rs2307109	
FBN2	54	IVS54 + 28G > A		2/102
FBN2	55	6931A > G (M2311V)	rs32209	
FBN2	57	7200T > C (S2400S)	rs190450	
FBN2	61	7739C > T (S2580L)	NM_001999	3/40
TGFBR2	4	1167C > T (N389N)	rs2228048	

Table 3. Loeys-Dietz Syndrome features in patients with *TGFBR2* or *TGFBR1* abnormality

Symptom	MFS34	MFS55	MFS60b	Frequency (%)[1]
Hypertelorism	+	+	+	93
Cleft palate/bifid uvula	-	+	-	100
Aortic root aneurysm		-	-	100
Arterial tortuosity		-	-	100
Aneurysm of other vessels		-	+	92
Craniosynostosis		-	-	36
Malar hypoplasia	+	+	-	85
Blue sclerae	-	+	-	62
Ectopia lentis	-	-	-	0
Arachnodactyly	+	-	+	57
Dolichostenomelia	+	-	+	29
Pectus deformity	+	-	-	64
Scoliosis	+	+	-	71
Talipes varus	-	+	-	29
Camptodactyly	-	+	+	43
Joint laxity	+	+	+	86
Patent ductus arteriosus	-	-	-	54
Atrial septal defect	-	-	-	31
Chiari type I		-	-	20
Developmental delay	-	-	-	21
Hydrocephalus	-	-	-	15
Others		VSD, bicuspid aortic valve, aortic root dilatation, bilateral strabismus, umbilical hernia		

[1] Reported by Loeys et al. (6)

Discussion

The overall detection rate of *FBN1* mutations in this study was 55% (27 out of 49 cases). The rate goes up to 64 % in patients fulfilling the criteria and down to 42% in those not fulfilling MFS, being similar to the previous study by Halliday et al. (77% versus 60%) (18). According to the UBM-*FBN1* database, *FBN1* mutations can be divided into two classes, a protein truncation type (38.6% of the 562 mutations) and a missense type (60.3%) (4). The latter mostly occurs in cb-EGF-like domains (78%). In this study, the former type (6 nonsense, three frameshift mutations and a splice site mutation) represent 37%, and the latter (17 missense mutations) 63%, all of which were found at a well conserved amino acid through human, cow, pig, dog, rat, mouse, and chicken Fbn1 homologues (data not shown). Among missense mutations found, 11 are located in cb-EGF-like domains (65%), fitting to the database. Eight missense mutations in cb-EGF-like domains either introducing (2) or substituting (6) cysteine residues may result in abnormal disulfide bonding and misfolding as previously described (4). It is also noteworthy that seven out of 8 families presenting with major eye involvement showed either missense mutations involving cysteine residues or a splice site mutation as previously suggested (22) (Table 1).

Two recurrent missense mutations of *TGFBR2*, R356P and D446N, have been identified in two (MFS34 and MFS55) out of 22 patients (9%) with MFS or suspected MFS unlinked to *FBN1* aberrations. The former was previously found in a Korean LDS patient (19) and the latter also in a patient with severe cardiovascular disease and skeletal involvement but not compatible with either MFS or LDS (17). Previously we identified three *TGFBR2* mutations in four of 19 *FBN1*-unrelated MFS patients (21%) (5). Three *TGFBR2* mutations were reported in two full MFS probands and one MFS-suspected patient unassociated with any *FBN1* mutations (17). Instead, no *TGFBR2* mutations were found in 30 classic MFS patients, although some of them may have *FBN1* aberration (19). It is obvious that clinical spectrum of *TGFBR2* mutations includes MFS (see also a supportive paper by Rommel et al.(22), but incidence of *TGFBR2* mutations would be approximate 5% of MFS or suspected MFS, much less than that of *FBN1* mutations (30-90%) (Figure 1).

The new syndrome, LDS, with either *TGFBR2* or *TGFBR1* mutations is characterized by hypertelorism, bifid uvula, cleft palate, generalized arterial tortuosity, ascending aortic aneurysm, and dissection (6). None of LDS patients did meet the Ghent criteria for MFS, but

the two syndromes share clinical phenotypes including aortic aneurysms and other skeletal abnormalities (6,23). All *TGFBR2* missense mutations identified in LDS were also located at well conserved amino acids of the kinase domain (6,23). The recent report of a pure type of familial TAAD associated with *TGFBR2* mutations strongly indicated that *TGFBR2* mutations result in severe cardiovascular consequences (7). It is remarkable that affected family members of the TAAD also had descending aortic disease and aneurysms of other arteries. The two patients (MFS34 and MFS55) with *TGFBR2* mutations in this report did not meet the MFS criteria and presented with some facial characteristics of LDS and a few skeletal abnormalities. However MFS55 did not show any aneurysm of aortic root and other vessels, and information of cardiovascular phenotypes was not available in MFS34, thus it remains inconclusive whether these patients are classified to LDS (Table 2) or MFS. Similarly MFS60b with a *TGFBR1* mutation did not satisfy the Ghent criteria or fit to a LDS phenotype, but her aortic phenotype was severe. No *FBN2* mutation was found in this series, supporting that it is unnecessary to investigate *FBN2* in MFS.

In conclusion, we have found 27 *FBN1* mutations in 27, two *TGFBR2* mutations in two, and one *TGFBR1* mutation in one out of 49 patients with MFS or MFS related disorders. *TGFBRs* screening should be considered in MFS or suspected MFS unlinked to *FBN1* abnormalities, although their abnormalities are occasional, and more information of clinical phenotypes caused by *TGFBRs* mutations is absolutely necessary. No mutation in either of four genes was found in the 19 patients. It is possible that there is an additional causative gene(s) for MFS-related phenotypes. Accumulation of such 19 patients with so far unknown genetic origin will be useful as a resource for finding new MFS-related genes.

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