ORIGINAL INVESTIGATION

Genotype and Phenotype Analysis of 171 Patients Referred for Molecular Study of the Fibrillin-1 Gene *FBN1* Because of Suspected Marfan Syndrome

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Background: Marfan syndrome (MFS) is an underrecognized heritable connective tissue disorder resulting from mutations in the gene for fibrillin-1 (*FBN1*). Affected patients are at risk for aortic dissection and/or severe ocular and orthopedic problems. The diagnosis is primarily based on a set of well-defined clinical criteria (Ghent nosology). The age-related nature of some clinical manifestations and variable phenotypic expression may hinder the diagnosis, particularly in children. Molecular analysis may be helpful to identify at-risk individuals early and start prophylactic medical treatment. *FBN1* mutations have also been reported in patients with Marfan-related conditions, but it is unknown what proportion of all *FBN1* mutation carriers they represent.

Methods: We reviewed the clinical and molecular data of 171 consecutive patients referred for *FBN1* analysis because either MFS was diagnosed or they had signs suggestive of MFS. We compared the incidence of mutations in patients who fulfilled the clinical diagnostic criteria for MFS with those who did not.

Results: Diagnostic criteria for MFS were fulfilled in 94 patients, 62 (66%) of whom had an *FBN1* mutation. A significantly higher incidence of ectopia lentis was found in the patients with MFS with an *FBN1* mutation vs those without ($P = .04$). Among the 77 patients who did not meet the criteria, an *FBN1* mutation was found in 9 patients (12%). No correlation was found between the severity of the phenotype and the position and nature of the *FBN1* mutation.

Conclusions: This study showed a significant difference in the number of *FBN1* mutations between patients fulfilling and those not fulfilling the diagnostic criteria for MFS, which seems to be a good predictor of the presence of an *FBN1* mutation. A comprehensive clinical evaluation is mandatory before establishing a definitive diagnosis. An *FBN1* mutation analysis is helpful to identify individuals at high risk for MFS who need careful follow-up, particularly in families displaying phenotypic variability and in children.

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Marfan syndrome (MFS) (Mendelian Inheritance in Man [MIM] 154700) is a connective tissue disorder with autosomal dominant inheritance and a prevalence of 2 to 3 per 10 000 individuals.[1] Mutations in the fibrillin-1 gene (*FBN1*) (MIM 134797) on chromosome 15q21.1 cause MFS.[2] The cardinal features involve the ocular, skeletal, and cardiovascular systems. The syndrome shows complete penetrance but has a wide interfamilial and intrafamilial variability in phenotypic expression.[3] The most important complication is a progressive dilatation of the aortic root and ascending aorta, leading to aortic valve incompetence and aortic dissection. Early recognition of at-risk individuals, either by clinical or molecular investigations, is important in view of the available medical and surgical treatments that can significantly improve life expectancy.[4,5]

Mutation analysis of the *FBN1* gene can detect at-risk individuals at an early stage and offers the possibility for prenatal diagnosis. In fact, we are increasingly confronted with requests for *FBN1* screening to confirm a presumed diagnosis of MFS. However, despite advances in the molecular understanding of the disease, the diagnosis of MFS is still primarily clinical, which relies on the recognition of a number of clinical manifestations in different body systems. In 1986, the Berlin nosology outlined a set of diagnostic criteria,[6] which, after the introduction of mutation analysis of the *FBN1* gene, were shown to be prone to cause an overdiagnosis of MFS.[7] The Berlin nosology was therefore revised into a set of more stringent criteria in the Ghent nosology (**Table 1**).[8]

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PATIENTS, MATERIALS, AND METHODS

CLINICAL EVALUATION

This study included 171 consecutive patients in whom *FBN1* mutation analysis was performed. All patients were evaluated against the revised diagnostic criteria for MFS (Ghent nosology), either through personal examination by one of the investigators (B.L. and A.D.P.) or by another clinical geneticist familiar with MFS. A detailed clinical checklist was completed for each patient. The patients included 103 male subjects and 68 female subjects with a mean age of 23.3 years; 96 patients were adults (≥18 years), and 75 patients were children (<18 years).

We accepted the diagnosis of MFS in an isolated patient if 2 major manifestations were present and at least 1 other body system was involved (Table 1). Only 50 (all older than 16 years) of the 171 patients had an evaluation of the lumbar spine by computed tomography or magnetic resonance imaging. We therefore relied mainly on the involvement of the skeletal, ocular, and cardiovascular systems to classify them. In familial cases, the diagnosis of MFS was established if at least 1 major criterion and the involvement of a second body system were present and 1 other family member fulfilled the diagnostic criteria independently. The patients who did not fulfill the diagnostic criteria for MFS were classified in one of the Marfan-related phenotypes.[8,12,15-17] From each patient we obtained a blood sample, a skin biopsy specimen for fibroblast culture, or both.

MOLECULAR ANALYSIS OF THE *FBN1* GENE

Genomic DNA (gDNA) was extracted from peripheral blood leukocytes by the QIAamp DNA blood mini kit (Qiagen Inc, Valencia, Calif) or from skin fibroblasts by the Easy-DNA kit (Invitrogen Corp, Carlsbad, Calif). Total RNA was prepared from skin fibroblasts with TRIzol Reagent 100 mL (Life Technologies Inc, Rockville, Md), and first strand complementary DNA (cDNA) was synthesized by Moloney murine leukemia virus reverse transcriptase (MMLV-RT) (Life Technologies).

Initially, we disposed only of primers for genomic DNA screening, provided by the international Marfan consortium (coordinated by H. Dietz, MD, PhD, Baltimore, Md). Mutation screening of the *FBN1* genomic sequences was performed by amplification of 65 fragments, each presenting 1 exon with flanking intron sequences with an average size of 260 base pairs (bp).

In a later stage of the study, we obtained primer sequences for cDNA screening from D. Milewicz, MD, and E. Putman, PhD (Houston, Tex). The complete cDNA analysis comprised amplification of 24 overlapping fragments with an average size of 450 bp. The *FBN1* cDNA reference sequence with GenBank accession No. NM_000138 was used (National Center for Biotechnical Information, Bethesda, Md; available at: http://www.ncbi.nlm.nih.gov).

The *FBN1* mutation analysis was performed in 113 patients on gDNA only, in 23 on cDNA only, and in 33 on both. In 2 patients with severe neonatal presentation of MFS, only the middle region of the *FBN1* gene (exon 23-32) could be analyzed because no more DNA was available.

For mutation screening of polymerase chain reaction fragments, 2 different approaches were used: conformation sensitive gel electrophoresis[18,19] and single-strand conformational polymorphism.[20] These techniques have been used successfully by us for collagen mutation screening[21] and by others for *BRCA1*[22] and *TSC2*[23] mutation analysis, for which the mutation detection has been estimated to be between 60% and 80%.

Interpretation of the diagnostic criteria is not always straightforward because of phenotypic variability in MFS, incomplete expression in young children, and clinical overlap with Marfan-related conditions. One concern is that a too strict interpretation of the Ghent nosology can cause an underdiagnosis of MFS.

FBN1 mutations have occasionally been reported in Marfan-related conditions such as familial ectopia lentis,[9-11] Marfan-like habitus,[11,12] or aortic dissection.[13,14] This led to the conclusion that *FBN1* mutations may underlie a range of "fibrillinopathies" including severe as well as mild conditions, all of which share to some extent clinical manifestations of MFS. What proportion of *FBN1* mutation carriers they represent is not known.

We have reviewed the clinical and molecular data obtained during the last 4 years in 171 consecutive patients referred for *FBN1* analysis with a clinical diagnosis or signs suggestive of MFS. Our purpose was to assess the incidence of *FBN1* mutations in the patients fulfilling and not fulfilling the criteria for MFS and to evaluate the contribution of molecular studies of the *FBN1* gene to the diagnosis of MFS. Finally, we looked for correlations between the clinical phenotype and the nature of the *FBN1* mutation.

RESULTS

CLINICAL CLASSIFICATION OF PATIENT GROUPS

Ninety-four patients (56 adults and 38 children) presented with an unequivocal diagnosis of MFS. Among those, 7 patients had very severe expression of the syndrome from the neonatal period and were therefore diagnosed as having so-called neonatal MFS, which is characterized by severe congestive heart failure due to mitral valve or tricuspid insufficiency, joint contractures, crumpled ears, and loose skin. The remaining 87 patients all presented with classic MFS according to the Ghent nosology. In all but 1 (patient 59) the clinical diagnosis could be made even if the information on the dura was not taken into account. The distribution of the major manifestations in the 87 patients was as follows: ectopia lentis in 43 patients (49%), significant aortic dilatation or dissection in 73 patients (84%), and major skeletal involvement in 47 patients (54%). Thirty of the 87 patients had a computed tomo-

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Table 1. Diagnostic Criteria According to the Ghent Nosology[8]*

Criterion	Major	Minor
Skeletal system		
Manifestations	Pectus carinatum or pectus excavatum requiring surgery, arm span to height ratio >1.05 or reduced US/LS <0.86 (adults), positive wrist and thumb sign, scoliosis >20° or spondylolisthesis, limited elbow extension (<170°), ples planus, protrusio acetabuli (radiography)	Facial appearance, joint hypermobility, pectus excavatum of moderate severity, highly arched palate
Involvement	4 of 7 major present	2 of 7 major present or 1 of 7 major and 2 of 4 minor present
Ocular system		
Manifestations	Ectopia lentis	Myopia, flat cornea, iris or ciliary muscle hypoplasia
Involvement	Ectopia lentis present	2 of 3 minor present
Cardiovascular system		
Manifestations	Aortic ascendens dilatation with or without aortic regurgitation and involving the sinuses of Valsalva, aorta ascendens dissection	Mitral valve prolapse, annulus mitralis calcification (age of onset, <40 y), pulmonary artery dilatation, aorta descendens or aorta abdominalis dilatation or dissection (age of onset, <50 y)
Involvement	1 of 2 major present	1 of 4 minor present
Pulmonary system		
Manifestations	None indicated	Pneumothorax, apical blebs (chest radiography)
Involvement	None indicated	1 of 2 minor present
Skin		
Manifestations	None indicated	Striae atrophicae (not associated with weight changes or pregnancy), recurrent or incisional herniae
Involvement	None indicated	1 of 2 minor present
Dura		
Manifestions	Lumbosacral dural ectasia by CT or MRI	None indicated
Involvement	Dural ectasia present	None indicated
Family		
Involvement	First degree family member independently fulfilling diagnostic criteria, mutation in *FBN1* known to cause MFS	None indicated

*US/LS indicates the ratio of the upper segment (in meters) to the lower segment (in meters); CT, computed tomography; MRI, magnetic resonance imaging; and MFS, Marfan syndrome.

graphic or magnetic resonance scan of the dura, 18 (60%) of whom had dural ectasia.

Overall, 55 (59%) of the 94 patients had a positive family history, whereas 39 were sporadic, including the 7 neonates. The remaining 77 patients (40 adults and 37 children) did not fulfill the diagnostic criteria according to the Ghent nosology. Among them, 12 children presented a phenotype highly suggestive of MFS, ie, a characteristic appearance and/or positive family history. Eight of these patients had 1 major and 1 minor diagnostic criterion for MFS, 3 had ectopia lentis (among whom 1 also had minor skeletal features), and 1 had major skeletal involvement and a positive family history. We assumed that they had not fulfilled the diagnostic criteria because of their young age and represented cases of "emerging" MFS.

The 25 other children and the 40 adults did not fulfill the diagnostic criteria because they presented only 1 major criterion or only 1 or more minor criteria. Even if they develop an additional major criterion in the future (such as aortic dilatation or dural ectasia), they would still not be diagnosed as having MFS according to the Ghent nosology. The patients were classified into 1 of the Marfan-related categories on the basis of their phenotypic characteristics:

- Nineteen patients (16 adults and 3 children) had aortic aneurysm and/or dissection as the only or predominant feature (MIM 132900). In the adult group, 11 patients had only aortic disease, 4 had both aortic disease and minor skeletal involvement, and 1 had cutaneous striae. One child presented only aortic dilatation, and in the 2 other children, aortic aneurysm was found together with either aortic valve stenosis or aortic valve insufficiency.
- Six patients (5 adults and 1 child) were diagnosed as having predominant ectopia lentis (MIM 129600). One adult presented isolated ectopia lentis, 1 had both ectopia lentis and minor skeletal involvement, 1 had mild aortic dilatation (below 2 SDs), 1 adult only had associated striae, and 1 adult had striae and minor skeletal involvement. Based on the family history, the child most likely represented an example of autosomal recessive ectopia lentis (MIM 225200).
- Seven patients (4 adults and 3 children) had the MASS phenotype (MIM 157700), defined by the presence of at least 2 of the following symptoms: myopia, mitral valve prolapse, mild aortic dilatation below 2 SDs, cutaneous striae, and minor skeletal involvement.
- Eight patients (6 adults and 2 children) presented mitral valve prolapse syndrome, based on the presence of mitral valve prolapse together with some skeletal manifestations.
- Twenty-two patients (9 adults and 13 children) presented a Marfan-like habitus and had a major or minor criterion in the skeletal system. No patient had major cardiac or ocular involvement, 6 had striae, 2 had mild myopia, and 1 had pneumothorax.
- Three children presented Shprintzen-Goldberg syndrome (MIM 182212).

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Nineteen patients had a computed tomographic or magnetic resonance scan of the dura, and dural ectasia was absent in all cases.

MOLECULAR DATA

A total of 71 *FBN1* mutations were found in the 171 patients in whom *FBN1* mutation analysis was performed. Four mutations were found in 7 patients with the neonatal Marfan phenotype, and 58 mutations were identified in the remaining 87 patients with classic MFS (36 mutations in adults and 22 in children) (**Table 2**). In the 3 families with MFS in whom no mutation was found, results of a linkage analysis showed cosegregation of the Marfan phenotype with the *FBN1* gene. However, the size of the families was too small to obtain a significant log of the odds score.

In the 77 patients who did not fulfill the MFS criteria, a total of 9 mutations (12%) were identified. Importantly, 6 mutations (patients 1-6) were found among 12 children with highly suggestive signs of MFS. In addition, 1 mutation was found in a 43-year-old man (patient 65) with severe mitral valve prolapse, and 2 mutations were detected in patients with predominant ectopia lentis (patients 66 and 67) (Table 2).

Overall, the detection rate of *FBN1* mutations in the group with MFS was 66% (62 of 94 patients). This percentage might be higher because 2 patients with neonatal MFS were screened for the middle region of the gene only. In the group of young children with emerging MFS, the incidence of mutations was 50% (6 of 12 patients). The incidence of *FBN1* mutations in the group with Marfan-related conditions was 5% (3 of 65 patients).

The type of *FBN1* mutation identified was heterogeneous and comprised 42 missense mutations, 9 nonsense mutations, and 20 deletions/insertions causing in-frame or out-of-frame mutations. Seventy percent (50 of 71 patients) of the mutations resided within one of the calcium binding epidermal growth factor–like motifs. Nineteen mutations influenced a crucial cysteine residue or another highly conserved amino acid involved in calcium binding. The causal nature of the other missense mutations was assumed on the basis of the familial segregation of the mutation, its absence in 50 controls, and/or the occurrence of the same mutation in an unrelated patient with MFS.

GENOTYPE-PHENOTYPE CORRELATION

We could not identify distinguishing features in patients with MFS with or without an *FBN1* mutation except for the presence of ectopia lentis, which was significantly higher in the mutation group (33 of 58 patients vs 10 of 29; $\chi^2_1=4.4$, $P=.04$). No significant difference was observed between both groups with respect to the familial presentation, the number of affected relatives,[24] and the distribution of major manifestations (aortic dilatation or dissection or major skeletal involvement) (Table 2 and **Table 3**).

Nine of the 71 *FBN1* mutations reported here are recurrent (R545C, C1835Y, IVS 46+5G>A, G1013R, R1541X, C781R, R2282W, I2585T, and R122C), which means that they have either been described by others previously and/or were found by us in unrelated patients. Overall comparison of the phenotypic manifestations in patients carrying the same mutation showed comparable findings.[24-30] The atypical joint effusion described in association with the R122C mutation in one family[10] may be coincidental because it was not found in patients 1 and 11 or other literature reports.[29]

Three mutations identified in patients with neonatal MFS (patients 69-71) resided within the middle region of the *FBN1* gene (exons 23-32) and 1 was in exon 4 (patient 68). Mutation A1337P (patient 71) is the first missense mutation in exon 32 associated with neonatal MFS because only exon-skipping mutations in this exon have been found in patients with neonatal MFS.[31] The 6 other mutations in the middle region were found in patients with classic MFS (patients 27-32) and the mutation in exon 27 was characterized in a patient with mitral valve prolapse (patient 65).

Variability in phenotypic severity was seen in association with mutations at the same codon leading to different amino acid substitutions. For example, the substitution of cysteine 1055 by tryptophan (patient 70) resulted in neonatal MFS as did the substitution of cysteine 1055 by glycine,[32] whereas the substitution of this residu by tyrosine gave rise to classic MFS (patient 29). On the other hand, extensive phenotypic variability within the same family was also regularly observed. In contrast to the findings of Collod-Beroud et al,[33] we found that cysteine mutations in the middle region of the gene (C1055Y and C1339Y) can be associated with ectopia lentis in classic MFS.

COMMENT

This article presents the results of *FBN1* mutation analysis in a group of 171 patients with a clinical diagnosis or signs suggestive of MFS. Our data show a significant difference in the number of *FBN1* mutations between patients fulfilling and not fulfilling the diagnostic criteria for MFS (66% vs 12%, respectively) ($\chi^2_1=53.4$, $P<.001$). All 71 mutations were identified in patients with neonatal or classic MFS except for 9 patients, among whom 6 children had emerging MFS. This suggests that at least in adults the fulfillment of the diagnostic criteria according to the Ghent nosology is a good predictor for the presence of an *FBN1* mutation. Moreover, in most patients with MFS, clinical diagnosis could be established without knowing the dural involvement.

When comparing the patients with MFS with a mutation with those without, the incidence of ectopia lentis was the only significantly discriminating factor, whereas there were no major differences regarding family history or distribution of other clinical manifestations. Two (patients 66 and 67) of the 3 patients with a Marfan-related condition in which an *FBN1* mutation was found also had ectopia lentis. As such, the presence of ectopia lentis justifies an *FBN1* mutation analysis.

We found a high incidence (6 of 12) of mutations in children with a phenotype highly suggestive of MFS who were too young to fulfill the diagnostic criteria. This emphasizes that the expression of MFS in young

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Table 2. List of the 71 *FBN1* Mutations With Clinical and Molecular Data*

Patient No./ Age, y	Skeletal System	Ocular System	Cardiovascular System	Pulmonary System	Skin	Dura	Family History	Diagnosis	g/c	Exon	*FBN1* Motif	Mutation: Missense/ Nonsense	Mutation: Frameshift
1/15	m	M	m	0	0	2	0	Highly suggestive MFS	g+	4	EGF-like #02	R122C	
2/8	m	0	M	0	0	2	0	Highly suggestive MFS	g+	14	cbEGF-like #05	G592D	
3/4	0	M	0	0	0	2	0	Highly suggestive MFS	g+	46	cbEGF-like #28	R1915S	
4/13	M	0	m	0	0	2	0	Highly suggestive MFS	g+	47	cbEGF-like #29	C1971Y	
5/16	M	0	m	0	0	2	0	Highly suggestive MFS	c+	62	cbEGF-like #41	I2585T	
6/15	M	0	0	0	0	2	M	Highly suggestive MFS	g+	63	cbEGF-like #42	G2618R	
7/19	M	0	M	0	m	M	0	Classic MFS	g–/c+	1	NH_2 terminal		124delG
8/52	m	0	M	0	m	2	M	Classic MFS	g–/c+	2	NH_2 terminal		del ex2
9/22	m	M	M	0	0	2	M	Classic MFS	g+	3	EGF-like #01		IVS 2–3C>G
10/28	M	0	M	0	m	2	0	Classic MFS	g+	3	EGF-like #01	C89F	
11/34	m	M	0	0	m	M	M	Classic MFS	g+	4	EGF-like #02	R122C	
12/31	M	M	M	0	0	0	0	Classic MFS	g+	6	Hybrid motif #01	R240C	
13/49	m	0	M	0	m	0	M	Classic MFS	c–/g+	8	cbEGF-like #02		959_960ins A→320X
14/14	m	M	M	0	m	M	M	Classic MFS	g+	9	8-Cys #01	W366C	
15/46	m	0	M	0	0	2	M	Classic MFS	g+	9	8-Cys #01		1147G>A
16/3	m	M	M	0	0	2	M	Classic MFS	g+	13	cbEGF-like #04	G560S	
17/19	m	M	M	0	m	2	M	Classic MFS	g+	13	cbEGF-like #04	R545C	
18/36	M	M	0	0	0	2	M	Classic MFS	g+	13	cbEGF-like #05	C570Y	
19/41	m	M	0	0	m	M	M	Classic MFS	g+	14	cbEGF-like #05	C598W	
20/5	M	M	M	0	0	2	0	Classic MFS	c+	15	cbEGF-like #06		1843_1845 delAAC
21/4	M	M	M	0	0	2	0	Classic MFS	g–/c+	17	8-cys #02		IVS 16–1G>C →del ex17
22/7	m	M	M	0	0	0	0	Classic MFS	g+	19	cbEGF-like #08	C781R	
23/9	M	M	M	0	m	2	0	Classic MFS	c–/g+	19	cbEGF-like #08	C776Y	
24/24	m	M	M	0	0	2	0	Classic MFS	c+	19	cbEGF-like #08	C781R	
25/16	M	M	M	0	0	0	M	Classic MFS	c+	21	Hybrid motif #02		2677 G>A (ex 21: del 4bp)
26/39	M	0	M	m	m	M	M	Classic MFS	g+	21	Hybrid motif #02		2586_2587 insT
27/32	0	M	M	m	0	M	M	Classic MFS	g+	23	cbEGF-like #10	E913G	
28/18	M	0	M	0	0	2	M	Classic MFS	g+	24	8-Cys #03	G985R	
29/16	m	M	M	0	m	0	0	Classic MFS	c+	25	cbEGF-like #11	C1055Y	
30/17	m	M	M	0	0	2	0	Classic MFS	g+	26	cbEGF-like #12	Y1101C	
31/3	m	0	M	0	0	2	M	Classic MFS	g+	29	cbEGF-like #15		3623delG
32/33	m	M	M	0	m	2	0	Classic MFS	c+	32	cbEGF-like #18	C1339Y	
33/24	m	M	M	0	m	2	M	Classic MFS	g+	34	cbEGF-like #20	C1429S	
34/14	M	M	M	0	m	2	0	Classic MFS	c+	36	cbEGF-like #22		IVS 35–8G> A→ins 6 nt IVS 35
35/41	M	M	0	0	0	2	M	Classic MFS	g+	37	8-Cys #04	R1541X	
36/25	M	M	M	0	m	M	M	Classic MFS	c–/g+	37	8-Cys #04	R1539X	
37/26	m	m	M	0	m	0	M	Classic MFS	g+	37	8-Cys #04		4699_4721 dup23nt
38/15	M	0	M	m	m	2	M	Classic MFS	c+	38	8-Cys #04	E1584X	
39/29	m	M	M	0	m	M	M	Classic MFS	c–/g+	38	8-Cys #04	R1596X	
40/26	M	0	M	0	m	2	0	Classic MFS	g+	39	cbEGF-like #23	R1644X	
41/38	m	0	M	0	m	2	M	Classic MFS	c–/g+	43	cbEGF-like #25	C1791Y	
42/30	M	M	M	0	m	M	M	Classic MFS	c+	43	cbEGF-like #25	R1790P	
43/41	m	m	M	0	m	2	M	Classic MFS	g+	44	cbEGF-like #26		5468_5484 dup15nt

(continued)

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Table 2. List of the 71 *FBN1* Mutations With Clinical and Molecular Data* (cont)

Patient No./ Age, y	Skeletal System	Ocular System	Cardiovascular System	Pulmonary System	Skin	Dura	Family History	Diagnosis	g/c	Exon	*FBN1* Motif	Mutation: Missense/ Nonsense	Mutation: Frameshift
44/29	m	M	M	m	0	M	M	Classic MFS	c+	44	cbEGF-like #26	C1835Y	
45/49	m	0	M	0	0	2	M	Classic MFS	g+	44	cbEGF-like #26		5499_5500 insT
46/10	M	M	M	0	0	2	0	Classic MFS	g+	46	cbEGF-like #28		IVS 46+5G>A
47/7	M	M	M	0	0	2	0	Classic MFS	g+	46	cbEGF-like #28	I1909T	
48/10	M	0	M	0	0	2	M	Classic MFS	g+	47	cbEGF-like #29		5898delA
49/1	m	M	M	0	0	2	0	Classic MFS	g+	48	cbEGF-like #30	C1977Y	
50/26	M	M	M	0	0	M	M	Classic MFS	c–/g+	49	cbEGF-like #31	Q2054X	
51/40	M	M	M	0	m	2	0	Classic MFS	g–/c+	52	cbEGF-like #32		del ex52
52/40	M	M	M	0	0	M	M	Classic MFS	g–/c+	52	cbEGF-like #32		IVS 52+2TG →del ex52
53/12	m	M	M	0	m	2	M	Classic MFS	g+	53	cbEGF-like #33	G2195X	
54/15	M	0	M	0	0	2	M	Classic MFS	g+	54	cbEGF-like #34	N2223H	
55/27	M	M	M	0	m	2	0	Classic MFS	g+	55	cbEGF-like #35	R2282W	
56/4	m	0	M	0	0	2	M	Classic MFS	g+	55	cbEGF-like #35	R2282W	
57/14	M	0	M	0	0	2	M	Classic MFS	c+	58	cbEGF-like #37	C2406Y	
58/4	M	0	M	0	0	2	M	Classic MFS	g+	59	cbEGF-like #38	Y2466X	
59/50	m	0	M	0	0	M	0	Classic MFS	g+	61	cbEGF-like #40		7576/ 7577delA
60/21	M	m	M	0	m	2	0	Classic MFS	c+	62	cbEGF-like #41	I2585T	
61/35	m	0	M	0	0	2	M	Classic MFS	g+	62	cbEGF-like #41	C2581F	
62/30	0	0	M	0	m	0	M	Classic MFS	g+	63	cbEGF-like #42	G2668C	
63/23	m	0	M	0	m	2	M	Classic MFS	g+	63	cbEGF-like #42	N2624K	
64/10	m	M	M	0	0	0	0	Classic MFS	g+	64	cbEGF-like #43	E2717X	
65/43	m†	0	m	0	0	0	0	MVP	g+	27	cbEGF-like #13	V1128I	
66/23	0	M	m‡	0	0	2	0	Ectopia lentis	c–/g+	37	8-Cys #04	R1530C	
67/45	m	M	0	0	0	0	0	Ectopia lentis	g+	52	cbEGF-like #32	P2154R	
68/neonatal	. . .	. . .	. . .	. . .	. . .	. . .	. . .	nMFS	g+	4	EGF-like #02		390delT
69/neonatal	. . .	. . .	. . .	. . .	. . .	. . .	. . .	nMFS	g+	24	8-Cys #03	G1013R	
70/neonatal	. . .	. . .	. . .	. . .	. . .	. . .	. . .	nMFS	g+	25	cbEGF-like #11	C1055W	
71/neonatal	. . .	. . .	. . .	. . .	. . .	. . .	. . .	nMFS	g+	32	cbEGF-like #18	A1337P	

*m indicates minor involvement; M, major involvement; 0, not involved; 2, unknown; MFS, Marfan syndrome; g, genomic DNA analysis; and c, complementary DNA analysis; plus sign, mutation identified; minus sign, mutation not identified; EGF, epidermal growth factor; cb, calcium binding; MVP, mitral valve prolapse; nMFS, neonatal MFS; and ellipses, not applicable.

†Only enlarged arm span to height ratio and highly arched palate.

‡Mild aortic dilatation (<2 SDs).

children may be incomplete, and a proportion of them represent cases of emerging MFS. It also illustrates the importance of careful follow-up before establishing or excluding a diagnosis of MFS.

In our cohort, 3 *FBN1* mutations were found among the 65 patients who did not fulfill the MFS criteria: 2 patients (patients 66 and 67) had predominant ectopia lentis and 1 (patient 65) had mitral valve prolapse and mild skeletal features. Overall, the current data from the literature, taken together with the low number of mutations (3 of 65 patients) in this present study, suggest that the incidence of *FBN1* mutations in the Marfan-related phenotypes may be low. In some instances, these phenotypes represent examples of milder fibrillinopathies. In view of the marked intrafamilial variability found within families with MFS, the distinction between MFS and other fibrillinopathies may be arbitrary. Moreover, multiple clinical evaluations over time may be necessary before classifying a patient with one of the Marfan-related conditions. For example, Kainulainen et al[9] reported 2 patients with so-called predominant ectopia lentis and mild skeletal features who later developed cardiac manifestations of MFS.[29]

A wide range of *FBN1* mutations was observed in this present study. No correlation was found between the severity of the Marfan phenotype and the position or nature of the *FBN1* mutation. Some authors suggested a relationship between the severity of ocular or cardiac involvement and the presence of either a cysteine substitution[34] or an *FBN1* mutation in the middle region of the *FBN1* gene,[33] but our results do not support these observations. In the absence of any significant genotype-phenotype correlation, the question remains which factors determine the severity of the Marfan phenotype in a patient. The present understanding of the molecular pathogenesis is such that for most mutations (most of which are missense mutations) a fair amount of the mutant transcript is expressed and exerts a dominant negative effect over the normal gene product during the assembly of normal and abnormal fibrillin monomers into microfibrils.[35,36] However, severe Marfan phenotypes were also seen in association with nonsense mutations or with frameshift mutations that are believed to lead to a nonfunctional *FBN1* allele (haploinsufficiency), such as the mutation R1539X (patient 36) and 5898delA (patient 48). In these 2 patients, the level of mutant transcript mea-

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Table 3. List of Patients With Classic MFS Without *FBN1* Mutations With Clinical and Molecular Data*

Patient No./ Age, y	Skeletal System	Ocular System	Cardiovascular System	Pulmonary System	Skin	Dura	Family History	Diagnosis	g/c
72/34	m	M	M	0	m	M	M	Classic MFS	g–
73/8	M	0	m	0	0	2	M	Classic MFS	g–
74/24	M	m	M	0	m	2	M	Classic MFS	g–
75/32	M	0	M	0	m	M	0	Classic MFS	c–
76/17	M	0	M	0	m	2	M	Classic MFS	c–
77/8	m	M	M	0	0	2	0	Classic MFS	g–
78/27	M	0	m	0	m	0	M	Classic MFS	g–
79/14	m	M	m	0	0	2	M	Classic MFS	g–
80/12	M	0	M	m	0	2	0	Classic MFS	c–/g–
81/45	M	M	M	0	m	2	M	Classic MFS	g–
82/28	M	M	M	0	m	M	0	Classic MFS	c–/g–
83/19	m	M	M	m	0	2	0	Classic MFS	c–/g–
84/30	M	0	M	0	m	2	M	Classic MFS	g–
85/15	m	M	M	0	0	2	0	Classic MFS	g–/c–
86/44	M	0	M	0	m	0	M	Classic MFS	c–/g–
87/5	M	2	M	0	m	2	0	Classic MFS	c–/g–
88/17	M	0	0	0	m	M	0	Classic MFS	g–
89/45	M	0	M	0	0	2	M	Classic MFS	g–
90/31	M	0	m	0	m	2	M	Classic MFS	g–
91/37	m	0	M	0	0	M	M	Classic MFS	g–/c–
92/34	M	0	M	0	m	2	0	Classic MFS	g–
93/64	m	M	M	0	0	2	M	Classic MFS	g–
94/24	m	M	m	m	m	0	M	Classic MFS	g–
95/21	M	m	M	0	m	2	0	Classic MFS	g–
96/27	M	m	M	0	m	2	0	Classic MFS	g–
97/25	M	0	M	m	0	2	M	Classic MFS	g–
98/19	M	0	m	0	0	2	M	Classic MFS	g–
99/17	M	0	M	0	m	0	M	Classic MFS	g–
100/32	m	M	M	0	m	2	M	Classic MFS	g–

*MFS indicates Marfan syndrome; m, minor involvement; M, major involvement; 0, not involved; 2, unknown; g, genomic DNA analysis; and c, complementary DNA analysis.

sured by us was undetectable. This may reflect the fact that the amount of mutant transcript measured in fibroblasts is not representative of the amount of transcript in the target tissues (eg, aorta and zonula ciliaris). Also, the extensive phenotypic variability seen in some families suggests that other (epi)genetic or environmental factors may modulate the phenotypic outcome.

Our overall detection rate for *FBN1* mutations in the group with MFS is about 65%. This still leaves us with a detection failure rate of 35%. Whether this is due solely to technical reasons or to the presence of another MFS locus remains an open question. From our limited linkage data, we have no additional evidence for the latter hypothesis. We are aware that other methods such as denaturing high-performance liquid chromatography (DHPLC) and direct sequencing may be more sensitive for mutation screening. For example, for mutation screening of *BRCA1*, DHPLC is superior to single-strand conformational polymorphism.[37] For *FBN1* mutation screening there is limited experience with DHPLC, and the current literature data suggest a high sensitivity (76%-100%)[26,38] but with a high rate of false positives (52%), which induces a higher cost and workload.[38]

CONCLUSIONS

The diagnosis of MFS in affected adults can usually be made by the established clinical diagnostic criteria. In affected children, the clinical manifestations may be incomplete, and in those cases confirmation by molecular diagnosis can help support the decision making about regular follow-up and preventive cardiovascular treatment. Moreover, in families showing wide variability in clinical expression, identification of a disease causing *FBN1* mutation may be necessary to confirm or exclude the diagnosis and identify persons at risk. Despite the fact that the presence of an *FBN1* mutation does not predict the severity of the Marfan phenotype, it offers the possibility for and responds to an increasing demand for a prenatal or preimplantation genetic diagnosis. The application of more refined mutation screening techniques will be necessary to resolve the question about locus heterogeneity in MFS and to address issues about genotype-phenotype correlation. Follow-up over time will be necessary to determine whether the presence of an *FBN1* mutation influences the clinical prognosis, particularly in patients who do not meet the clinical diagnostic criteria for MFS.

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