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ARTICLE

Classic, atypically severe and neonatal Marfan syndrome: twelve mutations and genotype–phenotype correlations in *FBN1* exons 24–40

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Mutations in the gene for fibrillin-1 (*FBN1*) cause Marfan syndrome, an autosomal dominant disorder of connective tissue with prominent manifestations in the skeletal, ocular, and cardiovascular system. There is a remarkable degree of clinical variability both within and between families with Marfan syndrome as well as in individuals with related disorders of connective tissue caused by *FBN1* mutations and collectively termed type-1 fibrillinopathies. The so-called neonatal region in *FBN1* exons 24–32 comprises one of the few generally accepted genotype–phenotype correlations described to date. In this work, we report 12 *FBN1* mutations identified by temperature-gradient gel electrophoresis screening of exons 24–40 in 127 individuals with Marfan syndrome or related disorders. The data reported here, together with other published reports, document a significant clustering of mutations in exons 24–32. Although all reported mutations associated with neonatal Marfan syndrome and the majority of point mutations associated with atypically severe presentations have been found in exons 24–32, mutations associated with classic Marfan syndrome occur in this region as well. It is not possible to predict whether a given mutation in exons 24–32 will be associated with classic, atypically severe, or neonatal Marfan syndrome. *European Journal of Human Genetics* (2001) **9**, 13–21.

Keywords: Marfan syndrome; fibrillin; temperature-gradient gel electrophoresis (TGGE); genotype–phenotype correlation

Introduction

The Marfan syndrome (MFS) is a relatively common heritable disorder of connective tissue characterised by manifestations such as aortic dilatation or dissection, scoliosis, pectus deformities, arachnodactyly, dolichostenomelia, and bilateral ectopia lentis. However, the number, age at onset, and constellation of such abnormalities vary widely among affected individuals, both within and between families.[1] The clinical criteria for MFS require a constellation of findings to make the diagnosis; a positive family history or the presence of a disease-causing mutation in *FBN1* may be used as a major criterion to support the diagnosis.[2]

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MFS is caused by mutations in the gene for fibrillin-1 (*FBN1*). *FBN1* contains 65 exons spanning 200 kb genomic DNA on chromosome 15q21.1[3,4] and codes for fibrillin-1, a 320 kD glycoprotein that is a main component of the extracellular microfibrils. Fibrillin-1 contains 47 motifs with homology to the human epidermal growth factor (EGF); 43 of these also contain a consensus sequence for calcium binding (cbEGF). The cbEGF modules are arranged in stretches of up to 12 tandem cbEGF modules that are interrupted by other modules including seven with homology to latent transforming growth factor-β1 binding protein (LTBP).[5]

To date, more than 137 *FBN1* mutations have been described,[6] the great majority of which have been found in only one affected family or individual. The mutations identified to date are distributed over all regions of the gene, with most of the reported mutations being missense mutations of the conserved cbEGF cysteine residues or residues of the calcium binding consensus sequence. Mutations of splice sites associated with exon skipping are also commonly found.[7]

In addition to mutations found in MFS patients, *FBN1* mutations have been found in a series of related connective tissue disorders, termed type-1 fibrillinopathies, including neonatal Marfan syndrome (nMFS), ectopia lentis, isolated skeletal abnormalities, and familial ascending aortic aneurysm (reviewed in Robinson and Godfrey[5]). The wide variability of disorders associated with *FBN1* mutations, as well as the clinical variability among patients with classic MFS, has made the investigation of genotype–phenotype correlations difficult.

We developed temperature-gradient gel electrophoresis assays for exons 24–40 of the fibrillin-1 gene and identified 12 mutations in patients with MFS including 10 novel mutations. A recurrent mutation (G1013R) in exon 24 was found in two unrelated patients with atypically severe clinical manifestations. Our results, together with a review of the literature, help further to refine knowledge about genotype–phenotype correlations in MFS.

Clinical summaries

Genomic DNA was collected from 124 unrelated patients with MFS according to the Berlin[8] or the revised Gent nosology,[2] as well as three patients with probable Shprintzen-Goldberg syndrome. Among the 124 patients with MFS, one had nMFS and has been previously reported,[9] and two (B15, D60) had atypically severe presentations as described below. Two (B73, D46) had atypically mild findings and have been previously reported,[10] and one further patient had bilateral ectopia lentis and joint hypermobility. The remaining patients had classic Marfan syndrome. Where possible, DNA was collected from affected and unaffected family members. The pertinent clinical data from the 12 patients described in this study are summarised in Table 1, and any unusual characteristics are noted below.

Table 1 Clinical features of the patients described in this study

Feature/finding	*B7*	*B15*	*B22*	*B27*	*B35*	*B47*	*D1*	*D13*	*D27*	*D36*	*D54*	*D60*
Age at last examination (years)	13	16	32	35	10	3	50	28	35	11	33	8
Inheritance	fam.	spor.	fam.	n/a	fam.	fam.	fam.	fam.	fam.	fam.	spor.	spor.
Gender	f	f	m	f	f	f	m	f	m	f	m	f
Skeletal system												
Height > 98P	+	–	+	–	+	+	+	–	+	+	+	+
Pectus deformity	car.	exc.	car.	asym.	car.	–	–	–	car.	–	exc.	–
Wrist and thumb sign	+	+	+	+	+	±	–	n/a	n/a	+	+	+
Scoliosis > 20°	–	+	+	+	+	–	–	+	–	–	+	+
Hyperextensible joints	–	–	–	–	+	+	+	n/a	n/a	+	–	+
Other	HP	HP, D	HP, D	DE	HP, D	HP	HP, D	–	HP, D	–	St	HP, D, En
Ocular system												
Ectopia lentis	–	+	+	–	–	n/a	+	+	+	+	+	+
Myopia (diopters)	+ (–2)	+	+ (–10)	+ (–2)	mild	n/a	+ (–13)	+ (–6)	+	n/a	–	+ (–6)
Cardiovascular system												
Dilation of the ascending aorta (mm)	+ (38)	+ (75)	+ (50)	+ (40)	+ (28)	– (20)	+ (45)	+ (48)	+	+	+	+
Aortic root replacement (age)	–	16	32	34	28	–	–	–	31	–	30	3
Mitral valve	MVP	MR	MVP	MVP	MVP	–	MR	MVP, MR	n/a	MVP	n/a	MVrep
Other	–	AR	–	AR	–	–	–	–	Pneu	–	–	–
Unusual features (see text)	–	+	–	+	–	–	–	–	–	–	–	+

+: feature present; –: feature absent; ±: clinically borderline finding; n/a: information not available; asym: asymmetric pectus deformity; exc: pectus excavatum; car: pectus carinatum; HP: high arched palate with dental crowding; D: dolichocephaly; MVP: mitral valve prolapse; MR: significant mitral regurgitation; AR: significant aortic regurgitation; St: striae atrophicae; En: enophthalmus; Pneu: recurrent spontaneous pneumothorax; MVrep: mitral valve replacement.

B15: Exon 24 (G3037A, G1013R)

Patient B15 was born as the second child of healthy parents after 36 weeks of pregnancy with a birth weight of 3 kg and length of 57 cm. She was initially diagnosed with MFS at the age of 8 months because of arachnodactyly, kyphoscoliosis, pectus excavatum, severe myopia, thin, almost parchment-like skin, and cardiovascular involvement.

Since infancy, the patient was extremely thin, with weight always beneath the third percentile and height above the 97th percentile. Rapid progression of scoliosis after the age of 6 years necessitated two scoliosis operations at the age of 8 and 10 years.

The patient had progressive and severe myopia from early childhood, and complete luxation of both lenses into the vitreous body at the age of 12 years. At the age of 16, dilatation of the aortic root to 75 mm was observed together with second-degree mitral insufficiency. Prophylactic aortic valve replacement with homograft replacement of the ascending aorta was performed, but the patient died suddenly one week following the operation due to suture dehiscence and internal haemorrhage. The patient's parents and two siblings are healthy and show no clinical signs of connective tissue disease.

B27: Exon 27 (A3344G, D1115G)

Patient B27, a 35-year-old woman, was first diagnosed with MFS at the age of 13 years. As an adult, the patient was 168 cm tall and did not display a typical marfanoid habitus or dolichostenomelia, however, she did have thoracic scoliosis and an asymmetric pectus deformity (mainly carinatum), arachnodactyly with ulnar deviation of the fingers of the left hand, and a narrow midface with dental malocclusion. An MRI scan demonstrated the presence of sacral dural ectasia and possible intervertebral disc prolapse at L5/S1.

Echocardiography showed mitral valve prolapse with trivial regurgitation and a patent foramen ovale, in addition to relatively mild dilatation of the aortic root (4 cm) which subsequently remained stable. At age of 34, a previously undiagnosed aneurysm of the interventricular septum associated with progressive cardiac decompensation (thought to be due to a ventricular septal defect which had spontaneously closed in childhood) was surgically corrected, and prophylactic repair of the aortic root was performed at the same sitting.

D60: Exon 24 (G3037A, G1013R)

Patient D60, an 8-year-old girl, received a mitral valve graft with remodelling of the aortic valve and ascending aorta at the age of 3 years. Bilateral ectopia lentis was diagnosed at the age of 1 year, and bilateral lensectomy was performed at the age of 6 years. The patient's parents and her older brother are clinically unaffected.

Methods

Polymerase chain reaction (PCR)

PCR was performed from genomic DNA to amplify *FBN1* exons 24 to 40 and their flanking intronic sequences. Primer pairs were designed with the program package Melt87 in order to obtain primers suitable for use in TGGE.[11] One primer of each pair was modified with psoralen to enable UV cross-linking of both strands following PCR. Both primers for exon 24 were modified with psoralen, a procedure which has been shown to improve the melting characteristics of fragments not otherwise optimal for analysis with TGGE.[12] The primer sequences and PCR conditions are shown in Table 2.

Temperature-gradient gel electrophoresis (TGGE)

TGGE was performed as previously described[13] using a Diagen apparatus (Düsseldorf, Germany). For psoralen-cross-linking, the PCR products were irradiated with UV light (365 nm) before electrophoresis. The 8% polyacrylamide gel contained 8.0 M urea and MOPS (3-(N-morpholino)-propane-sulfonic acid)-EDTA buffer. TGGE conditions are described in Table 2.

Sequencing

Samples with abnormal TGGE band patterns were subjected to DNA sequencing using the Amersham T7 sequenase version 2.0 DNA sequencing kit. All mutations were characterised by sequencing both strands.

Results

Genomic DNA from 127 unrelated individuals with MFS or related clinical syndromes was analysed for mutations in *FBN1* exons 24–40 as part of a study of all 65 exons (in preparation). PCR was carried out with primers specific for each exon including intron/exon boundaries, and amplicons were analysed with TGGE. The analysis identified a total of 12 probable disease-causing mutations (Table 3). Examples of TGGE results are shown in Figure 1. All mutations identified created a characteristic band pattern with which TGGE was used to rule out the change in all other patients in the test group. Only one of the mutations reported here has been previously identified. This mutation, G1013R, was identified in two unrelated patients (B15 and D60) who both had atypically severe, early-onset manifestations. Patient D60 had early-onset aortic dilatation requiring operation at the age of 3, and patient B15 had unusually severe clinical manifestations and died of complications related to aortic root repair at the age of 16. In both cases the mutation was ruled out in the clinically unaffected parents.

A novel missense mutation (T1020A) and a novel mutation of the +1 position of the splice donor site in intron 24 (IVS24 + 1 G to T) were also identified by TGGE analysis of exon 24 fragments. The affected patients had classic manifestations of MFS. Since all three mutations found in exon 24 created a characteristic band pattern (Figure 1a), TGGE was used to

Table 2 Primers and PCR and TGGE conditions. After an initial denaturing step of 5min at 95°C, PCR was run for 40 cycles at the annealing temperatures indicated in the table. Since psoralen-mediated crosslinking is optimal when psoralen is attached via a dApdA to the 5' end of the primer, 'extra' adenosines were added if necessary (indicated by '+1bp' or '+2bp' in the product size column). Pso: 5' modification with psoralen. The sense primers for exons 27 and 28 were previously described[17]

Exon	*Sense primer*	*Antisense primer*	*Position*	*Product size*	*Annealing temp.*	*TGGE conditions*
24	5'-Ps-(AA)C-CGT-GTG-GCT-CTA-TTT-AAC-CTC-3'	5'-Ps-(AA)T-TGG-CCA-TGG AAA-ACG-TAA-C-3'	-–40 to +26	296+4bp	58°C	30–60°C, 4h
25	5'Ps-(AA)T-TTG-CCC-CAC-ATT-TTC-TTA-TTC-TT-3'	5'-TCC-CCT-CTC-CTG-GCC-CTT-A-3'	–28 to +36	190+2bp	49°C	30–60°C, 2h 30min
26	5'-ATT-AAG-GCT-GTC-CTG-AGA-CTC-3'	5'-Ps-(AA)G-AAT-CCT-TCT-CTT-TCT-GTG-TTG-3'	–50 to +43	222+2bp	49°C	30–60°C, 2h 30min
27	5'-Ps-(AA)G-GCC-CCC-ACC-TTT-AAC-AT-3'	5'-GAA-AGT-CTT-TGC-TCC-TTA-C-3'	–37 to +19	182+2bp	52°C	35–65°C, 1h 30min
28	5'-TGC-CAA-AGT-TGG-AAG-CTT-ATG-3'	5'-Ps-(AA)T-AAC-ATA-ACA-TAA-CAT-AAA-ATA-AAG-3'	–54 to +45	225+2bp	52°C	20–45°C, 2h
29	5'-Ps-(AA)C-AGA-CAT-CCA-AAC-CAT-ATC-A-3'	5'-CTG-AGA-GAT-TCA-ACA-TGA-GCT-AGA-3'	–49 to +34	206+2bp	52°C	35–65°C, 2h
30	5'-CAA-CCT-GTG-GTT-GTT-GGT-TT-3'	5'-Ps-AAC-AGT-GCT-TAT-GAC-TAA-CAA-GA-3'	–32 to +54	212bp	54°C	30–60°C, 2h
31	5'-Ps-(A)AC-TCA-ATG-ATA-TCA-AAT-AGC-TAC-A-3'	5'-ACT-ACT-TAA-TAT-TTT-ATT-GTT-CTA-C-3'	–51 to +39	216+1bp	55°C	30–60°C, 2h
32	5'-Ps-AAG-ACA-TTT-GTG-CTG-AGC-CT-3'	5'TGT-AAT-CTA-TGC-AGT-CCT-TGA-TAA-3'	–48 to +50	221bp	52°C	30–60°C, 2h
33	5'-Ps-(A)AT-TGG-TTT-TAA-ATA CCA-CCC-T-3'	5'-AGT-TGT-TTC-CAG-CGT-GAA-3'	–55 to +23	201+1bp	54°C	30–60°C, 3h
34	5'-Ps-(AA)C-GAG-GAA-GAG-TAA-CGT-GTG-TTT-3'	5'-AGT-GGC-TTC-CCC-ATC-AGT-T-3'	–35 to +21	182+2bp	53°C	30–60°C, 3h
35	5'-Ps-AAG-TTT-TTG-CTT-TTT-CTC-CCT-C-3'	5'-GGA-GCT-GAT-TTT-GAT-GCC-A-3'	–30 to +32	185bp	52°C	35–65°C, 2h 30min
36	5'-AAC-TCC-ACT-ACT-CAC-TGT-TCG-3'	5'-Ps-AAT-ACA-CAG-TAT-GCT-TGC-TTC-T-3'	–28 to +45	196bp	Duplex Exon 32	Duplex Exon 32
37	5'-Ps-AAA-GAT-TCT-GCC-TGA-TGC-T-3'	5'-AAC-TGG-CTG-GAG-TTG-AAA-T-3'	–43 to +47	255bp	Duplex Exon 33	Duplex Exon 33
38	5'-Ps-(A)AG-ATT-CAA-AAC-AAC-TCA-ATT-TG-3'	5'-CAA-GTT-GTG-TGT-GCT-TTA-AGA-3'	–39 to +39	147+1bp	Duplex Exon 34	Duplex Exon 34
39	5'-Ps-(A)AT-TTA-CAA-TGC-TAA-AGG-AAT-G-3'	5'-AAG-ACC-TTA-TCA-TCC-TAC-CAG-3'	–42 to +31	199+1bp	54°C	30–60°C, 3h
40	5'-Pso-AAG-TTT-TCA-TAT-TCA-CAT-ACC-AC-3'	5'-CAT-GCA-TTA-CTG-AGA-AAA-GC-3'	–41 to +30	194bp	55°C	30–60°C, 2h 30min

Table 3 Summary of the results of this study. Sequence changes were confirmed by restriction analysis where possible

Exon	*Patient*	*Mutation*	*Predicted amino acid change*	*Restriction enzyme*	*Co-segregation*
24	B15	G3037A	G1013R	Msp I	+
24	B47	A3058G	T1020A	n/a	n/a
24	D54	IVS24 +1 G to T	Skipping of exon 24	Rsa I	n/a
24	D60	G3037A	G1013R	Msp I	+
27	B27	A3344G	D1115G	Hph I	n/a
30	B35	A3713G	D1238G	n/a	n/a
31	D1	A3898G	K1300E	n/a	n/a
32	B7	G3973T	E1325X	Mse I	+
34	D36	A4217G	D1406G	Sty I	+
34	D13	G4223T	C1408F	Bmy I	+
37	D27	C4615T	R1539X	Taq I	n/a
40	B22	G4955A	C1652Y	n/a	+

n/a: no known restriction site was changed by the mutation; under co-segregation +: mutation was shown to co-segregate with the disease phenotype in all tested family members; n/a: family samples not available.

screen 50 normal controls; no abnormal band patterns were detected.

The other mutations were found in patients with classic MFS, including two nonsense mutations, two mutations predicted to change cysteine residues of cbEGF modules, and four mutations affecting various residues of the calcium-binding consensus sequence of different cbEGF modules (Table 3). Cosegregation of the mutation with the disease phenotype was shown in all cases for which family samples were available (Table 3).

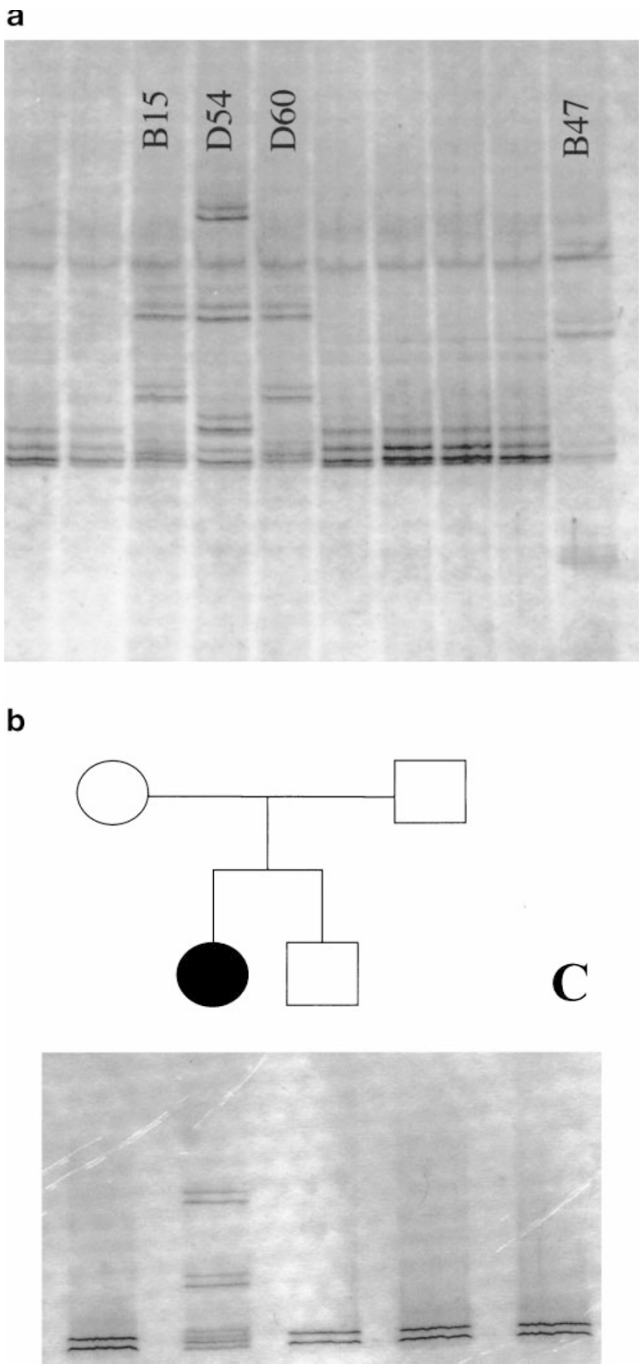

Figure 1 **a** TGGE analysis of exon 24 PCR fragments. Each of the three mutations identified in this exon created a unique band pattern upon TGGE analysis. The mutation G3037A (G1013R) was found in two unrelated patients (B15 and D60). The mutation IVS24 + 1 G to T was found in patient D54, and the mutation A3058G was found in patient B47. **b** TGGE analysis of exon 24 PCR fragments for patient D60 and her clinically unaffected brother and parents. C: healthy control.

A novel neutral polymorphism was identified in exon 26 (C3294T, N1098N) with an allele frequency of 1.5% in 124 MFS probands. The previously described $(TTTTA)_n$ repeat polymorphism in intron 28[14] was identified by TGGE and DNA sequencing and shown to have an allele frequency in 133 MFS probands of 87% (6 repeats), 13% (5 repeats) and 0.4% (8 repeats) with a heterozygote frequency of 25%. A novel sequence variant was identified in exon 27 by TGGE and DNA sequencing in a 7-year-old female proband screened because of mild aortic root dilatation (27 mm, or 1 mm above the 95% confidence interval) in the context of a bicuspid aortic valve. The sequence change, T3368A, is predicted to lead to the substitution of a glutamic acid for lysine at position 1123 (L1123Q) and was confirmed by restriction with *Xcm*I. The change affects a non-conserved residue one position upstream of the second cysteine residue of the cbEGF module encoded by exon 28. L1123Q was not found in any of the 124 MFS probands nor in 50 normal controls, but it was present in the proband's unaffected 10-year-old brother, so that it most likely represents a rare, neutral polymorphism. Although the mild aortic root dilatation in this proband is most likely due to the bicuspid aortic valve, a modifying effect of the sequence variant L1123Q cannot be excluded.

In addition to the 12 mutations identified by TGGE screening, we here provide further information on another unrelated patient who was shown to carry mutation G1013R (G3037A) by heteroduplex screening in a patient who was not a member of the initial screening group. The mutation, previously reported in abstract form,[15] was identified in a 10-month-old girl (patient N24) with severe clinical involvement. The patient underwent placement of a ventriculoperitoneal shunt for hydrocephalus of unknown origin (there was no clinical or computer tomographic signs of craniosynostosis). At the age of 10 months, she was found to have mitral valve prolapse and regurgitation, pulmonary stenosis, and a dilated ascending aorta with an aortic root dimension of 2.1 cm. Other abnormalities included arachnodactyly and anterior chest deformity. However, features characteristic of nMFS such as congenital joint contractures, crumpled ears, cutis laxa, and down-slanting palpebral fissures were not noted. Fibrillin immunostaining of dermal fibroblast cultures[16] revealed almost no stainable fibrils; however, fibrillin immunostaining of skin sections was within normal limits (not shown).

Discussion

G1013R: A relatively common recurrent mutation in patients with unusually severe manifestations of Marfan syndrome

The mutation G1013R (G3037A) was first reported by Nijbroek *et al*[17] in a patient with atypically severe manifestations including panvalvular dysfunction, gross aortic dilatation, and congestive heart failure, as well as ectopia lentis and

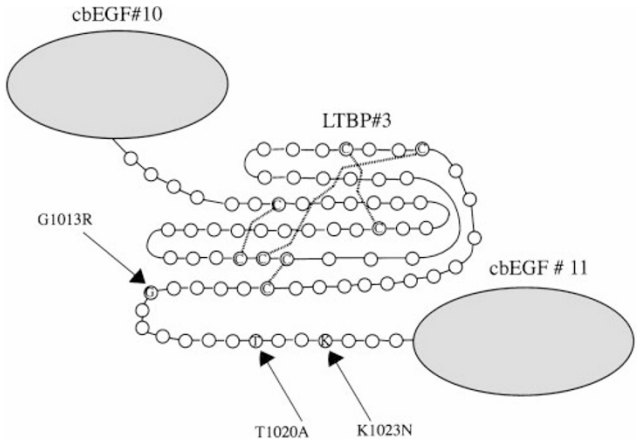

Figure 2 Mutations found to date in the interdomain linkage region between LTBP module no. 3 (encoded by exon 24) and cbEGF module no. 12 (encoded by exon 25): G1013R (present work and Nijbroek *et al*[17]), T1020A (present work), and K1023N.[21]

severe skeletal manifestations. We identified G1013R (G3037A) in two further unrelated patients in our screening group with atypically severe manifestations (B15, D60) as well as in an additional patient with atypically severe manifestations (N24). Therefore, G1013R has been identified in four unrelated patients, all of whom had atypically severe clinical involvement. G1013R affects a highly conserved residue located five residues after the eighth and final cysteine residue of LTBP molecule no. 3 in the interdomain linkage region between the LTBP motif and cbEGF motif no. 11, encoded by exon 25. The interdomain linkage region is highly conserved in fibrillin-1 and -2 and may play a role in interdomain flexibility.[18,19] Glycine, which has more conformational freedom than any other amino acid, may be particularly crucial at this position (Figure 2). Interestingly, an analogous mutation (G1056D) has been reported in the gene for fibrillin-2 in a patient with the phenotypically related disorder congenital contractural arachnodactyly.[20]

We identified another mutation in the interdomain linkage region in a family with variable manifestations of classic MFS. This mutation, T1020A, is predicted to lead to the substitution of alanine, a hydrophobic amino acid, by threonine, a polar amino acid. One may speculate that T1020A could cause a similar, but less pronounced, disturbance of interdomain flexibility. Together with the mutation K1023N, reported in a patient with nMFS,[21] a total of three unique mutations have been reported in the interdomain region between LTBP no. 3 and cbEGF no. 11, suggesting that this region plays a critical role in the function of fibrillin-1 (Figure 2).

Atypically severe Marfan syndrome

We were prompted by the above observations to review the literature for all mutations associated with atypically severe phenotypes. Putnam and co-workers[22] were the first to delineate a severe form of MFS distinct from nMFS but characterised by cardiovascular complications requiring surgery in childhood as well as by abnormal face and ears, with or without congenital contractures. Since there is no generally accepted clinical grading system for MFS, it seems reasonable to propose a preliminary definition of 'atypically severe' Marfan syndrome based on the age of occurrence of severe cardiovascular manifestations (because cardiovascular lesions such as aortic dissection represent the main cause of death in MFS). In MFS, aortic dissection develops in an age-dependent manner.[23] For the purposes of this work, we classified 'atypically severe Marfan syndrome' as clinical MFS with atypically severe manifestations including aortic dissection or the requirement for cardiovascular surgery at the age of 16 years or younger. Patients younger than 16 but

Table 4 Clinical manifestations of patients with atypically severe phenotypes associated with published *FBN1* missense mutations

Exon	*Mutation*	*Cardiovascular involvement*	*Other clinical manifestations*	*Reference*
4	C129Y	Progressive AD as a child, MVP, MR	JC, PE, EL, RD, Pneu	17
24	G1013R	PVD, AD and CHF. ARR before age 15	JC/JL, CrE, EL, SSD	17
24	G1013R	AD, ARR at age 16, MR, AR	PD; A, Sc, My, EL	this report, B15
24	G1013R	ARR at age 3, MVrep	EL, A	this report, D60
24	G1013R	AD, MVP, MR, PS at age 10 months	PD, A	this report, N24 and 15
26	E1073K	AD (1 year), MVP	Sc, A, EL	22 and 39
27	C1117G	MVP, MR, MVrep (age 6 years), AD	EL, PD, Sc, A	22
27	R1137P	MVrep, surgery for ascending and descending aortic dissections, death due to CHF at age 17	PE, My, EL, A, Sc, Hy, JL	40
31	del1301–1303, insH	MVrep, ARR in early childhood	SSD	17
31	C1320S	ARR at age 11, MVP, MR	PD, JC, A, EL, My	28
32	C1326R	AD at age one year, ARR at age 7, MVP, MR	PD, A, JL, EL, Pneu	28
40	C1663R	AD, ARR in early childhood	Classic and severe manifestations; surgical intervention for scoliosis	41

A: arachnodactyly; JC: joint contractures; JL: joint laxity; PD: pectus deformity; Sc: scoliosis; SSD: severe skeletal deformity; CrE: crumpled ears; Hy: muscular hypotonia; EL: ectopia lentis; My: high-grade myopia; RD: retinal detachment; PVD: panvalvular dysfunction; MVP: mitral valve prolapse; MR: mitral regurgitation; MVrep: mitral valve replacement or repair; AD: aortic root dilatation; ARR: aortic root replacement; CHF: congestive heart failure; PS: pulmonary stenosis; Pneu: spontaneous pneumothorax; IH: surgical repair of inguinal hernias in childhood; PE: pectus excavatum.

displaying abnormalities predicted to necessitate cardiovascular surgery, such as an aortic root diameter of 5 cm or more, were also included (Table 4).

A high degree of phenotypic variability is characteristic of MFS, and patients classified based on an early age of onset of severe cardiovascular manifestations may represent a heterogeneous group in other respects. However, it is striking that 12 of the 14 published missense mutations reported in patients with atypically severe MFS as defined above were found to cluster in exons 24–32 (Figure 3a). The clustering of mutations associated with atypically severe phenotypes as well as nMFS[9] in exons 24–32 strongly suggests a critical functional role for this region. The mutation G1013R has now been identified in four unrelated patients with atypically severe MFS and may be regarded as a kind of minor hot spot for this phenotype.

Exon-skipping mutations have also been shown to be often associated with severe phenotypes including nMFS.[24] This may be related to a requirement for precise lateral alignment of fibrillin monomers for polymerisation. However, not all exon-skipping mutations have been associated with severe phenotypes. Here, we present a novel mutation affecting the invariant splice donor site of intron 24 predicted to cause skipping of exon 24 in a 33-year-old patient with classic MFS, who had undergone prophylactic aortic root replacement at age 30. The mutation is predicted to delete LTBP motif no. 3 such that a tandem stretch of 13 cbEGF motifs is created (encoded by exon 23 and 25 to 36).

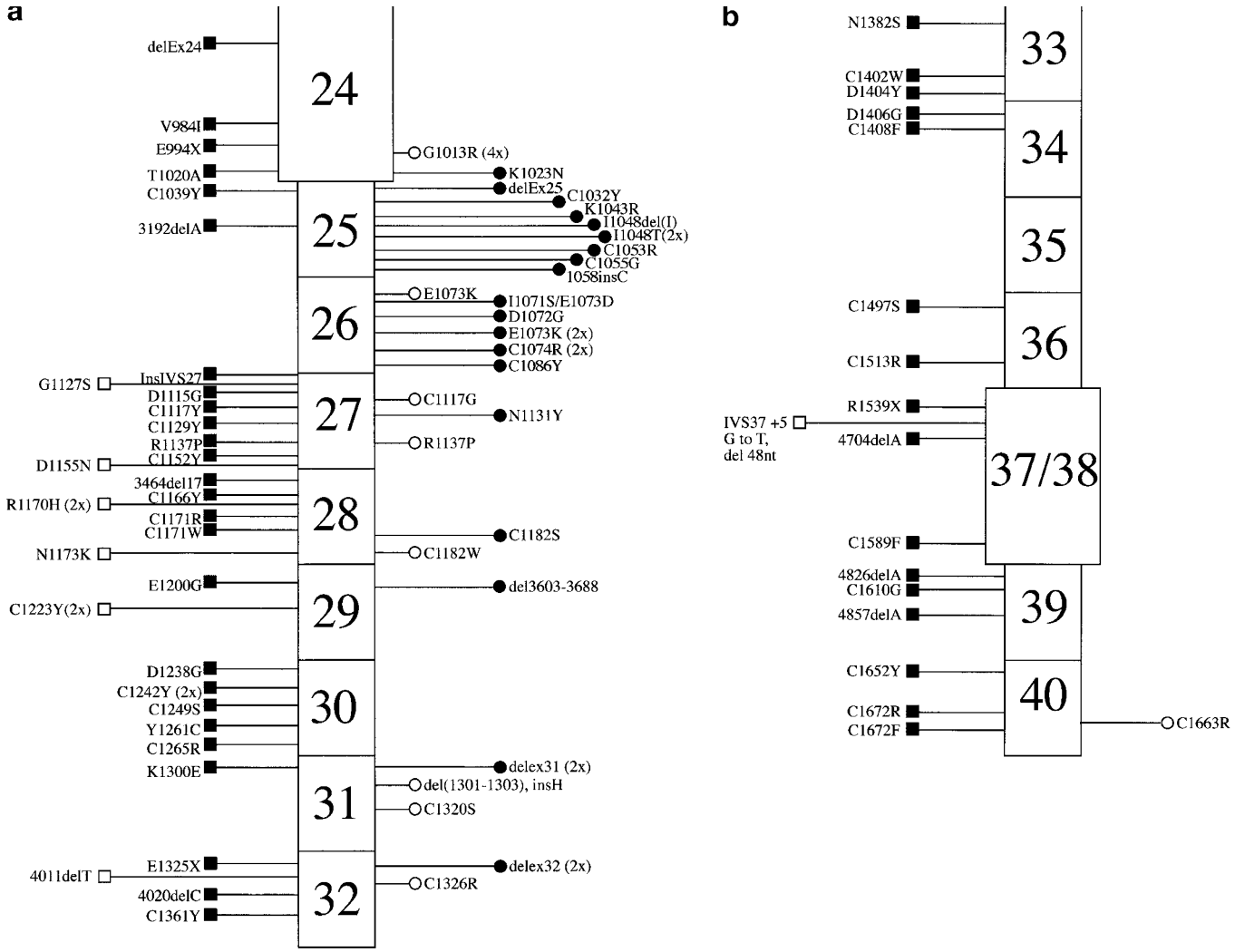

Figure 3 **a** and 3**b**. Summary of all published mutations in *FBN1* exons 24 to 40. Phenotypes were classified as neonatal MFS (•), atypically severe (○), classic MFS (■), and other fibrillinopathies (□). Neonatal phenotypes were defined as previously described,[9] and atypically severe phenotypes were defined as in the text. All mutations published as of December 1999 were included, original publications were consulted in every case. References can be found in the international fibrillin-1 mutation database[6] and in articles published subsequently[9,25–38].

Clustering of mutations in FBN1 exons 24–32

In addition to the mutations described above, we identified eight mutations in exons encoding cbEGF motifs, including two cysteine substitutions, four substitutions affecting the calcium binding consensus sequence, and two nonsense mutations (Table 3). All of these mutations were associated with classic MFS, even those mutations identified in exons 25–32. These findings underline the difficulties of defining genotype–phenotype correlations in MFS. Mutations in exons 24–32 have been associated with classic MFS, nMFS, and atypically severe MFS. The type of residue affected within a given cbEGF module cannot be the only factor affecting phenotypic outcome: for instance, eight cysteine and seven noncysteine substitutions in these exons have been reported in nMFS (Figure 3a). At present, there is no way of predicting whether a given mutation in this region is more likely to be associated with classic, severe or neonatal presentations. However, missense mutations associated with nMFS are found primarily in *FBN1* exons 25 and 26. Missense mutations associated with classic MFS or severe MFS are found primarily in exons 24 and 27–32 (see Figure 3a). Epigenetic factors could potentially be responsible for exceptions to this pattern.

Including the mutations reported here, 64 mutations have been reported in exons 24–32 (Figure 3a), compared with only 17 mutations in exons 33–40 (Figure 3b). The clustering of mutations in exons 24–32 is statistically significant ($P < 0.001$). All fibrillin-2 mutations found to date in patients with CCA are located between *FBN2* exons 24 to 34, raising the question of whether other, milder phenotypes are associated with mutations in other portions of the gene.[20] Similarly, one may speculate that phenotypes associated with certain mutations in regions of the fibrillin-1 gene outside exons 24–32 may escape clinical detection, which could explain the relative paucity of mutations outside this region. It may be regarded as likely that these exons encode a functionally critical region in both *FBN1* and *FBN2*, so that mutations in these exons are more likely to have phenotypic consequences than mutations in other regions of the genes.

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References

1 Pyeritz RE: The Marfan syndrome. *Annu Rev Med* 2000; **51**: 481–510.
2 De Paepe A, Devereux RB, Dietz HC, Hennekam RC, Pyeritz RE: Revised diagnostic criteria for the Marfan syndrome. *Am J Med Genet* 1996; **62**: 417–426.
3 Pereira L, D'Alessio M, Ramirez F *et al*: Genomic organization of the sequence coding for fibrillin, the defective gene product in Marfan syndrome. *Hum Mol Genet* 1993; **2**: 1762.
4 Biery NJ, Eldadah ZA, Moore CS, Stetten G, Spencer F, Dietz HC: Revised genomic organization of FBN1 and significance for regulated gene expression. *Genomics* 1999; **56**: 70–77.
5 Robinson PN, Godfrey M: The molecular genetics of Marfan syndrome and related microfibrillopathies. *J Med Genet* 2000; **37**: 9–25.
6 Collod-Béroud G, Béroud C, Ades L *et al*: Marfan Database (3rd edn): new mutations and new routines for the software. *Nucleic Acids Res* 1998; **26**: 229–223.
7 Hayward C, Brock DJ: Fibrillin-1 mutations in Marfan syndrome and other type-1 fibrillinopathies. *Hum Mutat* 1997; **10**: 415–423.
8 Beighton P, de Paepe A, Danks D *et al*: International nosology of heritable disorders of connective tissue, Berlin, 1986. *Am J Med Genet* 1988; **29**: 581–594.
9 Booms P, Cisler J, Mathews KR *et al*: Novel exon skipping mutation in the fibrillin-1 gene: two 'hot spots' for the neonatal Marfan syndrome. *Clin Genet* 1999; **55**: 110–117.
10 Palz M, Tiecke F, Booms P *et al*: Clustering of mutations associated with mild Marfan-like phenotypes in the 3' region of *FBN1* suggests a potential genotype–phenotype correlation. *Am J Med Genet* 2000; **91**: 212–221.
11 Lerman LS, Silverstein K: Computational simulation of DNA melting and its application to denaturing gradient gel electrophoresis. *Methods Enzymol* 1987; **155**: 482–501.
12 Gille C, Gille A, Booms P, Robinson PN, Nurnberg P: Bipolar clamping improves the sensitivity of mutation detection by temperature gradient gel electrophoresis. *Electrophoresis* 1998; **19**: 1347–1350.
13 Robinson PN, Boddrich A, Peters H *et al*: Two recurrent nonsense mutations and a 4 bp deletion in a quasi-symmetric element in exon 37 of the NF1 gene. *Hum Genet* 1995; **96**: 95–98.
14 Pereira L, Levran O, Ramirez F *et al*: A molecular approach to the stratification of cardiovascular risk in families with Marfan's syndrome [see comments]. *N Engl J Med* 1994; **331**: 148–153.
15 Mathews K, Wang M, Corbit CK, Godfrey M: Fibrillin (FBN1) mutations in the 'neonatal region'. Toward genotype/phenotype correlations in neonatal Marfan syndrome. *Am J Hum Genet* 1995; **57**: abstract 1966.
16 Godfrey M, Raghunath M, Cisler J *et al*: Abnormal morphology of fibrillin microfils in fibroblast cultures from patients with neonatal Marfan syndrome. *Am J Pathol* 1995; **146**: 1414–1421.
17 Nijbroek G, Sood S, McIntosh I *et al*: Fifteen novel FBN1 mutations causing Marfan syndrome detected by heteroduplex analysis of genomic amplicons. *Am J Hum Genet* 1995; **57**: 8–21.
18 Yuan X, Downing AK, Knott V, Handford PA: Solution structure of the transforming growth factor β-binding protein-like module, a domain associated with matrix fibrils. *EMBO J* 1997; **16**: 6659–6666.
19 Kettle S, Yuan X, Grundy G, Knott V, Downing AK, Handford PA: Defective calcium binding to fibrillin-1: consequence of an N2144S change for fibrillin-1 structure and function. *J Mol Biol* 1999; **285**: 1277–1287.
20 Park ES, Putnam EA, Chitayat D, Child A, Milewicz DM: Clustering of FBN2 mutations in patients with congenital contractual arachnodactyly indicates an important role of the domains encoded by exons 24 through 34 during human development. *Am J Med Genet* 1998; **78**: 350–355.
21 Kainulainen K, Karttunen L, Puhakka L, Sakai L, Peltonen L: Mutations in the fibrillin gene responsible for dominant ectopia lentis and neonatal Marfan syndrome. *Nat Genet* 1994; **6**: 64–69.
22 Putnam EA, Cho M, Zinn AB, Towbin JA, Byers PH, Milewicz DM: Delineation of the Marfan phenotype associated with mutations in exons 23–32 of the *FBN1* gene. *Am J Med Genet* 1996; **62**: 233–242.

23 Lipscomb KJ, Clayton-Smith J, Harris R: Evolving phenotype of Marfan's syndrome. *Arch Dis Child* 1997; **76**: 41–46.
24 Liu W, Qian C, Comeau K, Brenn T, Furthmayr H, Francke U: Mutant fibrillin-1 monomers lacking EGF-like domains disrupt microfibril assembly and cause severe Marfan syndrome. *Hum Mol Genet* 1996; **5**: 1581–1587.
25 Grau U, Klein HG, Detter C *et al*: A novel mutation in the neonatal region of the fibrillin (FBN) 1 gene associated with a classical phenotype of Marfan syndrome (MfS). *Hum Mutat* 1998; **12**: 137.
26 Karttunen L, Ukkonen T, Kainulainen K, Syvanen AC, Peltonen L: Two novel fibrillin-1 mutations resulting in premature termination codons but in different mutant transcript levels and clinical phenotypes. *Hum Mutat* 1998; Suppl: S34–37.
27 Ng DK, Chau KW, Black C, Thomas TMM, Mak KL, Boxer M: Neonatal Marfan syndrome: a case report. *J Paediatr Child Health* 1999; **35**: 321–323.
28 Schrijver I, Liu W, Brenn T, Furthmayr H, Francke U: Cysteine substitutions in epidermal growth factor-like domains of fibrillin-1: distinct effects on biochemical and clinical phenotypes. *Am J Hum Genet* 1999; **65**: 1007–1020.
29 Bresters D, Nikkels PG, Meijboom EJ, Hoorntje TM, Pals G, Beemer FA: Clinical, pathological and molecular genetic findings in a case of neonatal Marfan syndrome. *Acta Paediatr* 1999; **88**: 98–101.
30 El-Aleem AA, Karck M, Haverich A, Schmidtke J, Arslan-Kirchner M: Identification of 9 novel FBN1 mutations in German patients with Marfan-Syndrome. *Hum Mutat* 1999; **14**: 181.
31 Francke U, Berg MA, Tynan K *et al*: A Gly1127Ser mutation in an EGF-like domain of the fibrillin-1 gene is a risk factor for ascending aortic aneurysm and dissection. *Am J Hum Genet* 1995; **56**: 1287–1296.
32 Perez AB, Pereira LV, Brunoni D, Zatz M, Passos-Bueno MR: Identification of 8 new mutations in Brazilian families with Marfan syndrome. Mutations in brief no. 211. Online. *Hum Mutat* 1999; **13**: 84.
33 Montgomery RA, Geraghty MT, Bull E *et al*: Multiple molecular mechanisms underlying subdiagnostic variants of Marfan syndrome. *Am J Hum Genet* 1998; **63**: 1703–1711.
34 Pepe G, Giusti B, Attanasio M *et al*: A major involvement of the cardiovascular system in patients affected by Marfan syndrome: novel mutations in fibrilin 1 gene. *J Mol Cell Cardiol* 1997; **29**: 1877–1884.
35 Rantamaki T, Kaitila I, Syvanen AC, Lukka M, Peltonen L: Recurrence of Marfan syndrome as a result of parental germ-line mosaicism for an FBN1 mutation. *Am J Hum Genet* 1999; **64**: 993–1001.
36 Weidenbach M, Brenner R, Rantamäki T, Redel DA: Acute mitral regurgitation due to chordal rupture in a patient with neonatal Marfan syndrome caused by a deletion in exon 29 of the FBN1 gene. *Pediatr Cardiol* 1999; **20**: 383–385.
37 Gibson MA, Ellis SL, Ades LC, Haan E, Cleary EG: Preferential pre-mRNA utilisation of an upstream cryptic 5' splice site created by a single base deletion mutation in exon 37 of the FBN-1 gene. *Eur J Biochem* 1998; **256**: 221–228.
38 McGrory J, Cole WG: Alternative splicing of exon 37 of FBN1 deletes part of an 'eight-cysteine' domain resulting in the Marfan syndrome. *Clin Genet* 1999; **55**: 118–121.
39 Milewicz DM, Pyeritz RE, Crawford ES, Byers PH: Marfan syndrome: defective synthesis, secretion, and extracellular matrix formation of fibrillin by cultured dermal fibroblasts. *J Clin Invest* 1992; **89**: 79–86.
40 Dietz HC, Cutting GR, Pyeritz RE *et al*: Marfan syndrome caused by a recurrent *de novo* missense mutation in the fibrillin gene. *Nature* 1991; **352**: 337–339.
41 Dietz HC, Saraiva JM, Pyeritz RE, Cutting GR, Francomano CA: Clustering of fibrillin (FBN1) missense mutations in Marfan syndrome patients at cysteine residues in EGF-like domains. *Hum Mutat* 1992; **1**: 366–374.