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ARTICLE

The clinical spectrum of complete *FBN1* allele deletions

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The most common mutations found in *FBN1* are missense mutations (56%), mainly substituting or creating a cysteine in a cbEGF domain. Other mutations are frameshift, splice and nonsense mutations. There are only a few reports of patients with marfanoid features and a molecularly proven complete deletion of a *FBN1* allele. We describe the clinical features of 10 patients with a complete *FBN1* gene deletion. Seven patients fulfilled the Ghent criteria for Marfan syndrome (MFS). The other three patients were examined at a young age and did not (yet) present the full clinical picture of MFS yet. Ectopia lentis was present in at least two patients. Aortic root dilatation was present in 6 of the 10 patients. In three patients, the aortic root diameter was on the 95th percentile and in one patient, the diameter of the aortic root was normal, the cross-section, however, had a cloverleaf appearance. Two patients underwent aortic root surgery at a relatively young age (27 and 34 years). Mitral valve prolapse was present in 4 of the 10 patients, and billowing of the mitral valve in 1. All patients had facial and skeletal features of MFS. Two patients with a large deletion extending beyond the *FBN1* gene had an extended phenotype. We conclude that complete loss of one *FBN1* allele does not predict a mild phenotype, and these findings support the hypothesis that true haploinsufficiency can lead to the classical phenotype of Marfan syndrome.
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INTRODUCTION

Marfan syndrome (MFS) is a dominant disorder mainly caused by mutations in the fibrillin-1 gene (*FBN1*) on chromosome 15. The estimated prevalence is about 1 in 10 000.[1] The disorder has a very variable intra- and interfamilial expression. Different tissues and organs can be affected, with main features in the cardiovascular, skeletal, and ocular systems. Revised international criteria for the diagnosis were published in 1996 to facilitate the clinical diagnosis.[2,3]

FBN1 mutations are detected in the majority of the patients fulfilling the clinical criteria, but also in incomplete phenotypes referred to as type 1 fibrillinopathies.[4] Mutations in other genes have been reported to cause Marfan syndrome-related disorders, such as *TGFBR1* and *2* in MFS type 2 (Mizuguchi *et al*[5]; Matyas *et al*[6]) and Loeys–Dietz syndrome[7,8] and *MYH11* and *ACTA2* in familial thoracic aortic aneurysms and dissections.[9,10]

To date, over 600 mutations have been published in the Universal Marfan Database (UMD-*FBN1*; http://www.umd.be), but only a minority are recurring mutations. Missense mutations substituting or creating a cysteine in one of the calcium-binding EGF domains are most prevalent. Other mutations are frameshift, splice-site and nonsense mutations.[11]

Deletions of single and multiple exons can be detected using appropriate methods, such as multiplex ligation-dependent probe amplification (MLPA), cDNA or Southern blot analyses. Most of these deletions are associated with a severe or classical Marfan phenotype.[12–17] Only four reports are known of a molecularly proven whole-gene deletion of *FBN1*.[18–21] We describe 10 patients, including a family with five patients with whole-gene deletions, and show that complete loss of one *FBN1* allele does not predict a mild phenotype. These findings support the hypothesis that true haploinsufficiency can lead to the classical phenotype of Marfan syndrome.

PATIENTS AND METHODS

Patients

We screened DNA samples of 300 patients with clinical features of MFS or a related phenotype by MLPA. All samples had been previously screened by DHPLC and no mutations in *FBN1* were found. In one patient, chromosome analysis and array CGH performed for mental retardation screening revealed a deletion, including the *FBN1* gene. In all patients, the size of the deletion was determined by SNP array analysis.

The clinical features of the patients are listed in Table 1. A more detailed description can be found in the Supplementary Material.

Multiplex ligation-dependent probe amplification

MLPA analysis was performed as described elsewhere[22] with the SALSA MLPA kits P065 and P066 that contain probes for 53 of the 65 *FBN1* exons (including

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Table 1 Clinical features of patients carrying a complete *FBN1* allele deletion described in this study

Patient	*Age*	*Gender*	*MR*	*Aortic root dilatation*	*Aortic root surgery (age)*	*Aorta dissection (age)*	*MVP*	*Eye (age)*	*Skeletal*	*Face*	*Skin*	*Pneumothorax*	*DE*	*Family*	*Ghent*	*Other*
1	41	F	No	Yes	No	No	Yes	EL, lens extraction (36)	Arachnodactyly, hypermobility	Normal	Normal	No	NP	Pos	Yes	
2	39	F	No	Yes	Yes (34)	No	Yes	Myopia	Arachnodactyly, hypermobility	High palate	Recurrent herniae	No	No	Pos	Yes	
3	16	M	No	Yes	No	No	No	Myopia	Pectus carinatum, flat feet, hypermobility	High palate	Normal	No	NP	Pos	Yes	
4	13	F	No	Yes	No	No	Yes	Dubious EL	Pectus carinatum, flat feet, hypermobility,	High palate	Normal	No	NP	Pos	Yes	
5	27	F	No	Yes	No	No	No	No	Flat feet, elbow contracture, scoliosis, pectus excavatum, hypermobility	High palate	Normal	No	NP	Pos	Yes	
6	21	F	No	No, but cloverleaf appearance	No	No	No	Mild EL right eye, left eye normal	Severe pectus excavatum, flat feet, scoliosis	High and narrow palate, marfanoid face	Normal	No	Yes	Neg	Yes	
7	34	M	No	Yes	Yes (27)	No	No	EL	Pectus excavatum, arachnodactyly, scoliosis, hypermobility	Long face, high palate	Stretch marks	Yes	NP	Neg	Yes	Congenital hip dislocation
8	5	F	Yes	On P95	No	No	Billowing	Translucent irides	Arachnodactyly, hypermobility, flat feet, long toes	Mild dysmorphisms	Normal	No	NP	Neg	No	Hypotonia,
9	13	F	Yes	On P95	No	No	Yes	Anisohypermetropia, amblyopia	Arachnodactyly, hypermobility, pectus excavatum, flat feet	Pale skin and hair, dysmorphisms	General hypo-pigmentation	No	No	Neg	No	Ataxia
10	8	M	No	On P95	No	No	No	No	Arachnodactyly, hypermobility, pectus carinatum, mild scoliosis	High palate	Normal	No	NP	Pos	No	

Abbreviations: DE, dural ectasia; EL, ectopia lentis; Ghent, Ghent criteria [ref]; MR, mental retardation; MVP, mitral valve prolapse; Neg, negative; NP, not performed; Pos, positive.

exons 1 and 65) and all the 7 *TGFBR2* exons, and reference probes widely spread over the genome (MRC-Holland, see http://www.mlpa.com).

PCR products were analyzed on a fluorescent capillary sequencer (ABI3130, Applied Biosystems, Torrence, CA, USA) using Genemarker software (Softgenetics Inc., State College, PA, USA).

Cytogenetic analysis (patients 8 and 9)

Conventional chromosome analysis was performed on phytohemagglutinin-stimulated lymphocytes from peripheral blood cultures using GTG banding according to standard protocols.

High-density microarray analyses, SNP arrays

The Affymetrix GeneChip Human Mapping 262K *NspI* array (Affymetrix, CA, USA) contains 262 000 25-mer oligonucleotides with an average spacing of ~12 kb. An amount of 250 ng DNA was processed according to the manufacturer's instruction (www.Affymetrix.com). SNP copy number was assessed using the software program Copy Number Analyzer for Genechip (CNAG) Version 2.0 (see http://www.genome.umin.jp).[23]

Fluorescence *in situ* hybridization analysis

Fluorescence *in situ* hybridization (FISH) analysis was performed following the manufacturer's instructions using the BAC clone RP11-42K15 (Children Hospital Oakland Research Institute, Oakland, CA, USA).

RESULTS

In nine patients, MLPA revealed reduced relative peak areas for all probes within the *FBN1* gene, indicating a deletion of the entire *FBN1* allele.

Five patients (patients 1–5) are part of one family (Figure 1). The parents of patients 1, 2 and 5 have no clinical features of MFS.

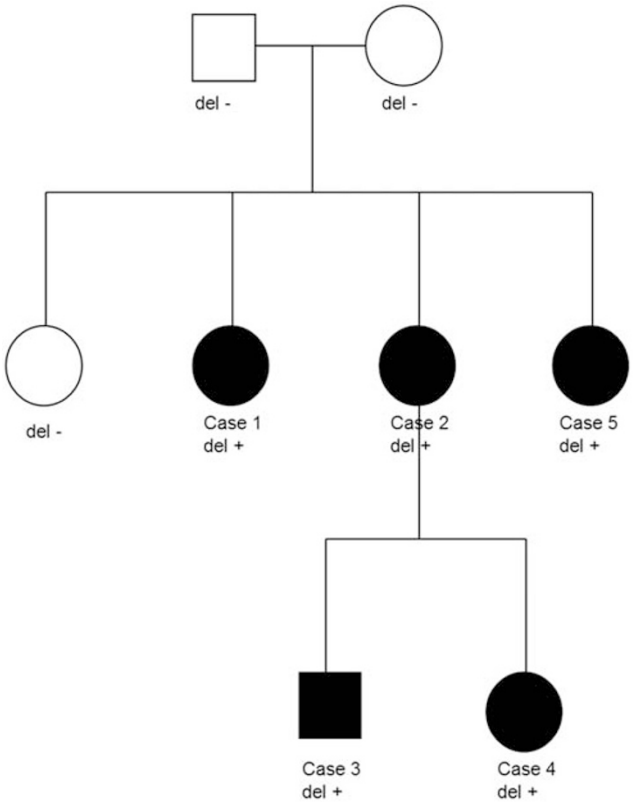

Figure 1 Pedigree of the family with five members carrying a complete deletion of a *FBN1* allele. *Del –*, no deletion present; *del +,* deletion present.

However, in the mother, both MLPA and SNP array analysis showed lower intensity signals for the probes in the deleted area but higher signals than in the patients, suggesting a mosaic deletion.

Figure 2 shows the MLPA results in patient 2 and her mother. The other eight patients have MLPA results comparable with the results of patient 2. FISH analysis with a probe within the *FBN1* gene confirmed the mosaic deletion in 21% of the totally 200 analyzed interphase nuclei. MLPA and FISH analysis in 200 interphase nuclei of the father showed a normal result (results not shown).

In three patients (patients 6, 8 and 9), the deletion occurred *de novo*. In one patient (patient 7), the parents were not tested for the deletion but appeared completely normal by clinical, ophthalmologic and cardiologic examination. The mother of patient 10 was not available for molecular testing.

In patient 8, the cytogenetic analysis revealed a *de novo* translocation between the long arms of chromosomes 12 and 15. Additional array CGH analysis, with a resolution of 1 Mb, detected a 4.9 Mb interstitial deletion at the translocation breakpoint of the long arm of chromosome 15 between the bands q21.1 and q21.2 (results not shown). The *FBN1* gene is located in this region. At the translocation breakpoint of chromosome 12, no deletion was detected by array CGH or SNP array analysis. Conventional karyotyping of case 9 was performed as part of the mental retardation screening, showing a normal female karyotype. In the other patients, no standard cytogenetic analysis was performed.

For all probands, the size of the deletion was characterized by SNP array analysis. The results are depicted in Figure 3 and Table 2.

The clinical features of the patients are summarized in Table 1. Except for patients 8, 9 and 10, all patients fulfilled the Ghent criteria for Marfan syndrome. The young age of patients 8, 9 and 10 could explain why they do not yet present the full clinical picture of MFS. Ectopia lentis was present in patients 1 and 7. Patient 4 had questionable lens subluxation, and patient 6 had very mild lens subluxation of her right eye. Aortic root dilatation was present in 6 of the 10 patients. In patients 8, 9 and 10, the aortic root diameter was on the 95th percentile. In patient 6, the diameter of the aortic root was on the 50th percentile. The cross-section, however, had a cloverleaf appearance. Patients 2 and 7 underwent aortic root surgery

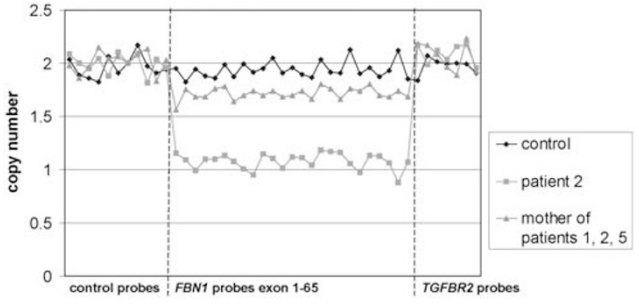

Figure 2 MLPA results of *FBN1* in mother and daughter (patient 2) compared with healthy control (MLPA kit P065, MRC-Holland). The control probes are normalized to two copies. The probe signals for *FBN1* relative to control probes and *TGFBR2* probes show a single copy for *FBN1* in patient 2. All other patients discussed in this paper show MLPA results comparable with patient 2. The healthy mother of patient 2 has reduced probe signals for all *FBN1* probes, indicative of somatic mosaicism for the deletion. The mean (±SD) signals for all *FBN1* probes were: 1.93 ± 0.08 (control), 1.08 ± 0.08 (case 2) and 1.71 ± 0.05 (mosaic mother; $P < 10^{-15}$ compared with control, according to a two-tailed *t*-test). The color reproduction of this figure is available on the html full text version of the manuscript.

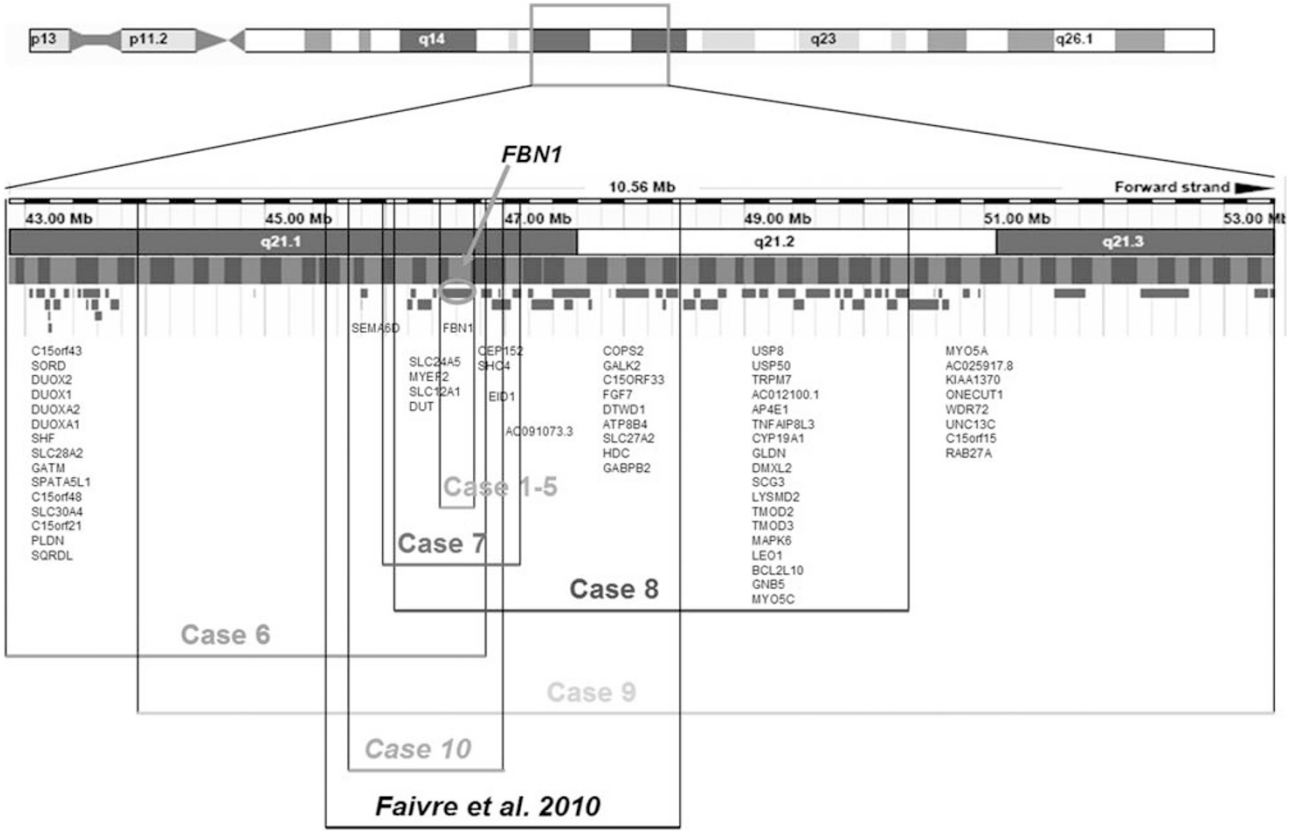

Figure 3 Position of the deletions on chromosome 15 (Ensemble release 53, March 2009) including the deletion described by Faivre *et al*[19] The red bars underneath the chromosome depict the known protein-coding genes according to Ensemble release 53, March 2009. The names of the genes are written below. The horizontal colored lines show the size of the different deletions and their overlap. The color reproduction of this figure is available on the html full text version of the manuscript.

Table 2 Size of the deletions and number of genes deleted. The location of the SNPs are derived from Ensemble release 53, March 2009

Patient	*Starting SNP*	*Ending SNP*	*SNPs*	*Starting bp*	*Ending bp*	*Max size (bp)*	*Genes*
2	SNP_A-1876769	SNP_A-1938857	44	46.434.718	46.742.196	307478	1
6	SNP_A-2169073	SNP_A-2251237	429	42.867.884	46.950.476	4082592	23
7	SNP_A-2099749	SNP_A-1871856	107	46.098.636	47.145.902	1047366	9
8	SNP_A-2055581	SNP_A-1811386	366	46.116.834	50.383.848	4267009	36
9	SNP_A-2127245	SNP_A-2283948	2007	44.000.164	53.427.159	9426995	46
10	SNP_A-1826755	SNP_A-1823624	141	45.715.212	46.963.495	1248283	9

Abbreviations: bp, basepair; SNP, single-nucleotide polymorphism.

at a relative young age (27 and 34 years, respectively). Mitral valve prolapse was present in 4 of the 10 patients, and billowing of the mitral valve in one. All patients had facial or skeletal features of MFS.

The two children with larger deletions (patients 8 and 9) had an extended phenotype with psychomotor retardation and additional features. Patient 8 was a 5-year old girl who presented with psychomotor retardation and hypotonia with severe motor delay at the age of 2.5 years. Patient 9 presented with psychomotor retardation with non-progressive ataxia. Apart from her marfanoid features, she had a very pale skin and hair without other ectodermal manifestations. She had facial dysmorphisms consisting of a brachycephalic skull, long philtrum, broad nose and prognathism.

Further details about the clinical manifestations of the 10 described patients are found in the Supplementary Information.

DISCUSSION

There are several reports of deletions of the long arm of chromosome 15 involving chromosome band q21.1. However, in most of these reports, the deletion of *FBN1* or the presence of marfanoid features are not discussed[24–29]. In four reports, the deletion of *FBN1* is confirmed by molecular techniques, with marfanoid features in three cases[18,19,21] and absence of marfanoid features in one case, which could be due to the young age of this patient.[20]

In this study, we describe 10 patients with a deletion of an entire *FBN1* allele. To our knowledge, this is the first series of complete *FBN1*

allele deletions published so far. These patients and three previously described sporadic patients[18,19,21] have a Marfan phenotype due to pure haploinsufficiency. The phenotype of the patients in our series varies from mild features of MFS to the classical MFS phenotype. One family (patients 1–5) has a deletion encompassing only the *FBN1* gene, whereas patients 6, 7, 8, 9 and 10 have much larger deletions spanning 1–9.4 Mb, with 9–46 genes, respectively (Figure 3 and Table 2). Patients 6, 7 and 10 have no other features than those that can be attributed to the deletion of *FBN1*. Patients 8 and 9 have psychomotor retardation and dysmorphic features. In addition, patient 9 has an extended phenotype with more severe neurological impairment, and lack of skin and hair pigmentation. The deleted genes Myosin 5A (*MYO5A*, MIM 160777) and RAS-associated protein (*RAB27A*, MIM 603868) could play a role in the phenotype of this girl. Mutations in *MYO5A* and *RAB27A* cause Griscelli syndrome types 1 and 2, respectively. These rare autosomal recessive disorders are characterized by partial albinism, immunological problems and/or neurological impairment. Further studies of these genes on the normal allele are pending. The three previously published case studies[18,19,21] also have a deletion extending beyond the *FBN1* gene. Faivre *et al*[19] describes a teenage girl with a deletion of 2.97 Mb with some skeletal features of MFS and mitral valve prolapse, but absence of aortic root dilatation and ectopia lentis. Apart from language disabilities, she was not mentally retarded. The size of the deletion was characterized by array CGH, and 13 genes were found to be deleted including *FBN1*. In Figure 3, the size and position of this deletion is compared with the deletions described in this study. The patients described by Adès *et al*[18] and Hutchinson *et al*[21] have psychomotor retardation with additional features, probably due to haploinsufficiency of other genes. The size of the published deletions is unknown, but in the patient described by Hutchinson *et al*, the *MFAP1* locus was deleted. That means that this deletion is extending more centromeric than our deletions. No further information is available about the breakpoints in these patients. Hutchinson *et al*[21] found that in the deletion patient, the fibrillin-1 protein and mRNA levels were significantly higher than expected for a single *FBN1* allele. They suggest that the clinical variability in MFS could be due to variable *FBN1* expression from the normal allele. They compared their results with three members of one family with a premature termination codon (PTC) mutation, and showed that the variable expression in these individuals appeared to correlate with variability in *FBN1* expression of the normal allele and not with variable rates of nonsense-mediated decay (NMD).

Apart from the PTC mutations where the phenotype will be due to partial haploinsufficiency caused by NMD and a dominant-negative effect of the fibrillin-1 molecules that escape NMD, few other mutations have been described, leading to a haploinsufficiency state. Milewicz *et al*[30] described a patient with only skeletal features of MFS and a missense mutation in the *FBN1* gene. This mutation cosegregated with tall stature in the family. The mutation disrupted the normal processing of one-half of the secreted profibrillin in fibrillin. Half the normal amount of fibrillin was shown to be deposited in structurally normal-appearing microfibrils. They hypothesized that this mutation mimics a null allele of *FBN1* and leads to a milder phenotype, analogous to the null allele of *COL1A1* which leads to the milder form of osteogenesis imperfecta.[31,32] Our results, however, show that haploinsufficiency of *FBN1* is sufficient for the development of the full clinical expression of MFS with some carriers exhibiting severe features. For instance, two of our patients (patients 2 and 7) needed aortic surgery at a relatively young age (age 27 and 34, respectively). Additional evidence supporting the haploinsufficiency model are the two patients with classical MFS described by Mátyás *et al*[16] with a deletion of the putative regulatory and promoter region of *FBN1*, resulting in complete loss of transcription of the corresponding allele. Both patients fulfilled the Ghent criteria with major manifestations in the skeletal and cardiovascular systems, but no ectopia lentis. The authors conclude that these two patients represent true haploinsufficiency.

Although no mouse model is known with a complete deletion of one *FBN1* allele, the mouse model of Pereira suggests that there is a threshold of expression of the normal allele below which the abnormal phenotype will develop.[33,34] Mice with a heterozygous-targeted mutation leading to 15% expression of a normal product have no abnormal phenotype, whereas the mice with the same mutation on both alleles have severe abnormalities comparable with the neonatal MFS phenotype.

Judge *et al*[35] used yeast artificial chromosome-based transgenesis to overexpress a disease-associated mutant form of human fibillin-1 (C1663R) on a normal mouse background. These mice showed no abnormalities, whereas a heterozygous comparable cysteine mutation in mice leads to the Marfan phenotype and histological changes as seen in heterozygous human. They showed that haploinsufficiency for the WT protein can be a significant factor in the pathogenesis of MFS when combined with an abnormal *FBN1* allele. In keeping with the hypothesis of the critical contribution of haploinsufficiency, introduction of a wild-type transgene in the heterozygous mouse rescues the aortic phenotype.

How the lower production and deposition of fibrillin-1 will affect the TGFβ signaling pathway, and how it leads to the aortic and skeletal features is subject for debate. Recent evidence of the role of the TGFβ signaling pathway in the pathogenesis of MFS shows that fibrillin has a stabilizing effect on the latent TGFβ-binding protein-1 (LTBP-1) in the extracellular matrix (ECM)[34–37] *LTBP1* plays a role in the release of TGFβ in the ECM. Mice with a Marfan phenotype and a centrally deleted *FBN1* allele showed marked dysregulation of TGFβ activation and enhanced signaling.[38] They hypothesize that deficiency of fibrillin-1 causes excessive amounts of active TGFβ to be liberated from the matrix. This might as well be the case in the patients with a deletion of the *FBN1* gene. Increased TGFβ signaling is also shown in aortic tissue of patients with Loeys–Dietz syndrome.[8] The exact mechanism, how changes in TGFβ signaling lead to such a specific phenotype, has still to be elucidated.

Ectopia lentis was present in at least two of our patients. The published patients with molecularly proven complete *FBN1* allele deletions did not exhibit ectopia lentis.[18–21] None of the patients with a *TGFBR2* or *TGFBR1* mutation have ectopia lentis. We hypothesize that ectopia lentis in our patients is caused by the lower production of fibrillin-1 and not by perturbation of the TGFβ signaling. This is in keeping with the observation that mutations in *LTBP2* (latent-transforming growth factor-β-binding protein 2) cause recessive eye abnormalities including ectopia lentis.[39,40] LTBP2 is the only member of the LTBP family not to bind to latent forms of TGFβ, and is thought to have an important structural role in the ciliary body together with fibrillin-1.[41]

In conclusion, our patients with a complete *FBN1* allele deletion show that haploinsufficiency has a major contribution to the pathogenesis of MFS and can lead tot the whole spectrum of MFS. We hypothesize that the skeletal and aortic phenotype are caused by aberrant TGFβ signaling and the ocular phenotype by the lower production of the fibrillin-1 microfibrils.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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