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## Marfan syndrome and related connective tissue disorders

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## **Extensive clinical variability in Marfan syndrome patients with a single novel recurrent fibrillin-1 missense mutation**

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## **ABSTRACT**

### ***Background/Methods***

Marfan syndrome (MFS) is a heritable connective tissue disorder usually caused by a mutation in the fibrillin 1 (*FBN1*) gene. Typical characteristics of MFS that have been described are e.g. dolichostenomelia, ectopia lentis and aortic root dilatation. However, there is great clinical variability in the expression of the syndrome's manifestations, both between and within families. Here we discuss the clinical variability of MFS by describing a large four-generation Dutch family with MFS.

### ***Results***

Nineteen individuals of one family with a single missense *FBN1* mutation (c.7916A>G) were identified. The same mutation was found in one unrelated person. Clinical variability was extensive and not all mutation carriers fulfilled the diagnostic criteria for MFS. Some patients only expressed mild skeletal abnormalities, whereas aortic root dilation was present in eight patients, an acute type A aortic dissection was recorded in two other patients, and a mitral valve prolapse was present in eight patients. In some patients cardiac features were not present on initial screening, but did however develop over time.

### ***Conclusion***

MFS is a clinically highly variable syndrome, which means a meticulous evaluation of suspected cases is crucial. Mutation carriers should be re-evaluated regularly as cardiovascular symptoms may develop over time.

**INTRODUCTION**

Marfan syndrome (MFS) is a heritable connective tissue disorder primarily involving the ocular, skeletal and the cardiovascular system.<sup>1</sup> The diagnosis is made according to the Ghent nosology (Table 1).<sup>1</sup> Typical characteristics of MFS are dolichostenomelia (thin body habitus and long extremities), ectopia lentis (lens (sub-)luxation), pectus carinatum/excavatum, and aortic root dilatation.<sup>1,2</sup> There is, however, extensive variability in the phenotype of MFS patients, both between and within affected families.<sup>3-5</sup> The prevalence of MFS is relatively low, approximately 1:5,000, but considering the great clinical heterogeneity less typical patients may well remain undiagnosed.<sup>6</sup>

The syndrome is usually caused by a mutation in the fibrillin-1 (*FBN1*) gene on chromosome 15. In about 25% of the patients this is the result of a *de novo* mutation, in other cases the mutation is inherited in an autosomal dominant way.<sup>7,8</sup> In rare cases, MFS is caused by a mutation in the

**Table 1.** Diagnostic criteria for Marfan syndrome according to Ghent nosology.<sup>1</sup> At least two major criteria from two different organ systems + involvement of a third organ system is required for a diagnosis of Marfan syndrome.

Organ system	Major criteria	Minor criteria
<b>Cardiovascular</b>	<ul style="list-style-type: none"> <li>- aortic root dilatation</li> <li>- type A aortic dissection</li> </ul>	<ul style="list-style-type: none"> <li>- mitral valve prolapse calcification of the mitral annulus &lt;40 years</li> <li>- dilatation of pulmonary artery &lt;40 years</li> <li>- dilatation/dissection of descending aorta &lt;50 years</li> </ul>
<b>Skeletal*</b>	<ul style="list-style-type: none"> <li>(≥4 required)</li> <li>- pectus carinatum</li> <li>- pectus excavatum requiring surgery</li> <li>- pes planus</li> <li>- wrist and thumb sign</li> <li>- scoliosis &gt;20° or spondylololsthesis</li> <li>- arm span-height ratio &gt;1.05</li> <li>- protrusio acetabulae</li> <li>- diminished extension elbows (&lt;170°)</li> </ul>	<ul style="list-style-type: none"> <li>- moderate pectus excavatum</li> <li>- high narrowly arched palate</li> <li>- typical face</li> <li>- joint hypermobility</li> </ul>
<b>Ocular</b>	<ul style="list-style-type: none"> <li>- ectopia lentis</li> </ul>	<ul style="list-style-type: none"> <li>(≥2 required)</li> <li>- flat cornea, myopia, increased axial length of globe, hypoplastic iris</li> </ul>
<b>Family/Genetic history</b>	<ul style="list-style-type: none"> <li>- independent diagnosis in parent, child, sibling</li> <li>- mutation <i>FBN1</i></li> </ul>	<ul style="list-style-type: none"> <li>none</li> <li>none</li> </ul>
<b>Pulmonary</b>	<ul style="list-style-type: none"> <li>none</li> </ul>	<ul style="list-style-type: none"> <li>- spontaneous pneumothorax, apical bulla</li> </ul>
<b>Skin</b>	<ul style="list-style-type: none"> <li>none</li> </ul>	<ul style="list-style-type: none"> <li>- unexplained striae, recurrent or incisional herniae</li> </ul>
<b>Central nervous system</b>	<ul style="list-style-type: none"> <li>- lumbal sacral dural ectasia</li> </ul>	<ul style="list-style-type: none"> <li>none</li> </ul>

\* 2 major or 1 major + 2 minor criteria required for the skeletal system to be involved

transforming growth factor beta (TGF- $\beta$ ) receptor 1 or 2 genes.<sup>9-11</sup>

Although MFS is often the result of a transmitted mutation, large families with MFS spanning multiple generations with a single founder mutation have rarely been reported. In this article series on recurrent and founder mutations we present a large, four-generation Dutch family and another unrelated patient with a single recurrent *FBN1* missense mutation that has not been reported before. To our knowledge this is one of the largest families described in the literature. On the basis of this family and the unrelated patient we discuss the clinical variability of the syndrome.

## PATIENTS AND METHODS

Clinical data were collected from the Marfan outpatients' clinics at the University Medical Center Groningen, Radboud University Nijmegen Medical Center, Leiden University Medical Center and Academic Medical Center Amsterdam.

### Mutation analysis

Genomic DNA was extracted from peripheral blood leukocytes and mutational analysis of the *FBN1* gene was performed by Denaturing High Performance Liquid Chromatography (DHPLC) with subsequent sequencing of fragments with aberrant patterns.<sup>12</sup>

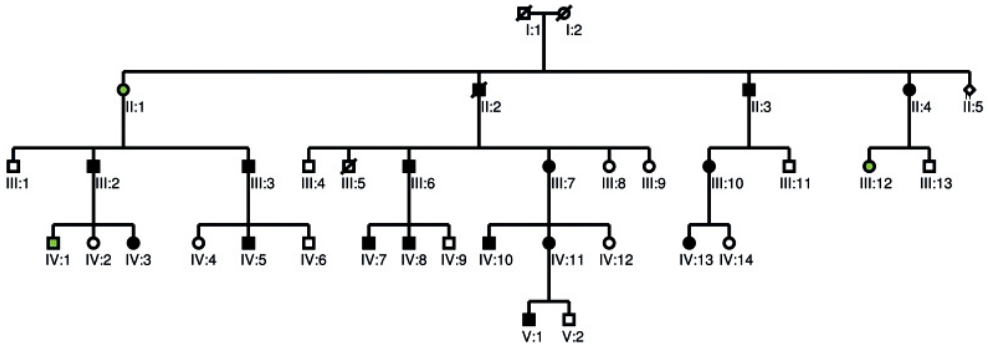
### Haplotype analysis

Seven repeat markers, D15S132, D15S123, D15S1024, D15S992, D15S1028, D15S126 and D15S119, in and around the *FBN1* gene were analyzed in three patients from the large pedigree and in the unrelated patient and his unaffected sister and mother (primers and conditions are available upon request).

## RESULTS

### Clinical

Four index patients were evaluated for MFS in four different centers. After diagnosing MFS, DNA analysis revealed an identical *FBN1* mutation (see below). Subsequent genealogical investigation revealed that three of the four index patients could be traced back to one ancestral couple. In Figure 1 the pedigree of the family is presented and in table 2 the phenotypic characteristics of the patients according to the Ghent nosology are displayed. Nineteen patients all carrying the same *FBN1* mutation (see below) could be linked to each other (Figure 1). One index patient was shown to harbour the identical *FBN1* mutation but he could not be linked to the pedigree. Most patients expressed important cardiovascular manifestations: eight patients had aortic root dilatation, two other patients had an acute type A aortic dissection, and seven had a mitral valve prolapse. In some patients cardiac manifestations were not present at the initial screening, but did develop over time. For example patient IV-7 showed no cardiac features at the age of ten, but eight years later he had an aortic root dilatation and a mitral valve prolapse. Skeletal symptoms varied widely



**Figure 1.** Filled symbols: (obligate) carriers of the FBN1 mutation 7916A>G  
Index patients marked green

**Table 2.** Characteristics of the subjects according to the Ghent nosology

Patient (age in yrs)	Organ system						FBN1 mutation (age in yrs*)
	Cardiovascular	Skeletal	Ocular	Skin	Dural ectasia		
II-1 (80)	MVP	PP,S,AS,HP	-	striae	na	+ (74)	
II-2 (died at 52)	AAD	na	na	na	na	oc (na)	
II-3 (died at 58)	na	na	na	na	na	oc (na)	
II-4 (82)	-	-	-	herniae	-	oc (\$)	
III-2 (died at 39)	AAD	na	na	na	na	oc (na)	
III-3 (57)	-	AS, HP	-	striae	-	+ (\$)	
III-6 (58)	-	HP	-	striae	na	oc (\$)	
III-7 (53)	ARD, MVP	HP, PE,	-	striae	na	+ (37)	
III-10 (36)	MVP	WT, S, PE, HP	-	striae	na	+ (33)	
III-12 (53)	MVP	PE, PP, HP, typical face	minor	-	+	+ (44)	
IV-1 (24)	ARD, MVP	PC, PP, WT, typical face	EL	striae	na	+ (11)	
IV-3 (29)	ARD	HP	-	-	na	+ (11)	
IV-5 (34)	ARD	AS, DE, HP	-	striae	-	+ (27)	
IV-7 (25)	ARD	HP	-	striae	na	+ (\$)	
IV-8 (23)	ARD, MVP	PC, WT, HP,	EL	na	na	+ (13)	
IV-10 (27)	-	PP, mild PE, HP, DE	EL	na	na	+ (15)	
IV-11 (31)	na	WT, mild PE	-	striae	na	+ (19)	
IV-13 (8)	MVP	HP	-	-	na	+ (\$)	
V-1 (3)	ARD	PC, PP	EL	-	na	+ (2)	
- (33)	ARD,MVP	mild PE, JH, PP	EL	-	-	+ (23)	

**Cardiovascular:** ARD aortic root dilatation, AAD type A aortic dissection, MVP mitral valve prolapse

**Skeletal:** AS arm span-height ratio >1.05, DE diminished extension elbows, HP high arched palatum, JH joint hypermobility, PC pectus carinatum, PE pectus excavatum requiring surgery, PP pes planus, S scoliosis, WT wrist and thumb sign

**Ocular:** EL ectopia lentis na not available, oc obligate carrier, - no manifestations

\* age at diagnosis of Marfan syndrome, \$ not fulfilling Ghent criteria

between the family members, but none of our patients had major involvement of the skeleton. A high arched palate was one of the most frequent skeletal manifestations seen in our patients. Also, pectus excavatum or carinatum was seen in almost half of the patients. Scoliosis was only seen in two patients. In about half of the patients there were no ocular manifestations present. Striae were reported frequently as a skin manifestation (in 9 of 20 patients), whereas recurrent herniae were not. Lumbosacral MRIs to detect the presence of dural ectasia were performed only in five of the patients, of whom one had dural ectasias. None of the patients reported a spontaneous pneumothorax. There were five patients (II-4, III-3, III-6, IV-7 and IV-13) who did not completely satisfy the Ghent criteria for the diagnosis of MFS at ages 82, 57, 58, 25 and 8 years, respectively.

### Genetic analysis

A missense mutation in exon 63 (c.7916A>G) leading to a change of amino acid tyrosine into cysteine at position 2639 (p.Y2639C) was identified in all the tested family members (table 2). This amino acid concerns a highly conserved residue in calcium-binding epidermal growth factor (EGF)-like domain 42. The new cysteine residue is predicted to disturb disulphide bonding, which is essential for the stability of the EGF-like domains of fibrillin and will affect protein stability. The mutation was absent in an ethnically-matched healthy control population (n=1,000 chromosomes) and has not been described as a cause of MFS before.

One index patient (final patient in table 2) could not be linked to the large family, but carries the same *FBN1* mutation. Both his parents were examined for skeletal, cardiological and ocular symptoms but did not show any symptoms of MFS. His mother tested negative for the *FBN1* mutation. His father was not tested. An additional silent DNA variant was detected in exon 43 in this patient. This variant, c.5343G>A:[p.V1781V] was not found in the mother and hence is located on the paternal allele that contains the mutation in exon 63.

### Haplotype analysis

Haplotype analysis revealed an identical haplotype for affected individuals from the large family around the *FBN1* gene for seven markers located within a 2.3 Mb region on chromosome 15. This haplotype was absent in the unrelated patient who carried the same mutation (data not shown).

## DISCUSSION

MFS is diagnosed according to the Ghent nosology (see table 1 for the diagnostic criteria) and is usually caused by a mutation in the *FBN1* gene.<sup>1,7</sup> Fibrillin-1 is an extracellular matrix protein regulating *TGF-β* activity, which is a cytokine that plays an important role in cell proliferation and differentiation, apoptosis and extracellular matrix formation.<sup>13,14</sup> Increased *TGF-β* signalling plays an important role in the pathogenesis of MFS, causing deleterious effects on, for example the aortic wall, the mitral valve, and pulmonary tissue.<sup>13,15,16</sup>

More than 1,000 different mutations, spread throughout the entire gene, have been identified

in the *FBN1* gene.<sup>17,18</sup> The types of mutations are diverse, although missense mutations are the most prevalent, in particular a missense mutation affecting a cysteine residue, leading to a disruption of the tertiary structure of fibrillin.<sup>19</sup> Most mutations are unique for a certain MFS family, and only approximately 10% of mutations are recurrent in different families.<sup>20</sup> In about 25% of individual Marfan patients, the syndrome is caused by a *de novo* mutation in the *FBN1* gene. In other cases, MFS is the result of an autosomal dominantly transmitted mutation.<sup>8</sup> Here we have both situations: a recurrent missense mutation which spread through four generations in one family and an identical mutation which is believed to be *de novo* in a single MFS patient. Because the different index patients were ascertained in different centres and were not aware of each other and the fact that this is a large four generation family, we suggest that this mutation can be considered a founder mutation. Large MFS families spanning multiple generations are rare and the MFS family presented here constitutes an exception as few such large families have been described in the literature.<sup>21</sup> The *FBN1* mutation described here, was found in one unrelated individual, who had a neutral variant on the mutant allele that was not present in the large family. Haplotype analysis of repeat markers in and around the *FBN1* gene showed no common haplotype. Consequently this mutation does not appear to be a founder mutation in this particular index patient.

The potentially aggressive nature of MFS could explain the rare occurrence of large MFS families. Patients with severe (cardiovascular) complications early in life might not produce offspring, making multiple-generation families less likely to occur.

MFS is known for its clinical variability, both between families and within families, as also evident in the family we describe. For example, patient II-4, an 82-year old female, and patient II-1, an 80-year-old female only demonstrated mild manifestations of MFS (herniae and mitral valve prolapse with some skeletal abnormalities, respectively). Whereas their brother (patient II-2) developed an acute type A aortic dissection at the age of 52 years, which was fatal. The son of patient II-1 (patient III-2) developed a type A aortic dissection at age 37 years, which was operated on successfully. In contrast, her other son (patient III-3), aged 57 years, only expressed mild skeletal abnormalities. Due to this clinical variability, 'mild' cases may well go unnoticed, as these people will only be recognized as having MFS by a meticulous clinical and genetic examination according to the Ghent nosology. It is, however, important to recognize these cases, as important (cardiovascular) manifestations and complications can develop over time. Family members not completely fulfilling diagnostic criteria for MFS should have confirmatory or presymptomatic genetic testing for a possible *FBN1* mutation. If a *FBN1* mutation is present, regular cardiological follow-up is necessary to discover and treat possible cardiovascular manifestations in a timely way.

It is uncertain what causes the clinical variability of MFS. Hutchinson et al. have suggested that allelic variation of normal *FBN1* expression (of the non-mutated *FBN1* allele) might contribute to the clinical variability, particularly in patients with premature termination codon mutations, where fibrillin-1 is predominantly derived from the normal allele (non-mutated allele).<sup>5</sup> In addition, Van Dijk et al. described two families with MFS, in which certain individuals had two *FBN1* mutations,



and suggested that the additional mutations might have a modifying role as a cause of intrafamilial variability.<sup>22</sup> Giusti et al. found a relationship between severity of cardiovascular manifestations and elevated homocysteine levels.<sup>4</sup> It should further be kept in mind that many genes are involved in the complicated process of extracellular matrix formation, many parts of which have yet to be discovered. These genes are likely to play an important role in modifying the phenotypic expression of MFS.

## CONCLUSION

We have described a unique, large four-generation Marfan syndrome family, in which 19 individuals have a single missense founder mutation in the *FBN1* gene (c.7916A>G). We also found the same mutation in one other, unrelated individual, proving that this mutation can also be recurrent. The mutation carriers showed extensive clinical variability in symptoms and signs of MFS and not all mutation carriers fulfilled diagnostic criteria for MFS, even at advanced ages. The variability in the phenotypic expression makes a careful clinical and genetic evaluation of suspected cases crucial. Mutation carriers should undergo regular follow-up with regards to the cardiovascular system as symptoms may develop over time.

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