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Homozygous and compound heterozygous mutations in the *FBN1* gene: unexpected findings in molecular diagnosis of Marfan syndrome

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ABSTRACT

Background Marfan syndrome (MFS) is an autosomal-dominant connective tissue disorder usually associated with heterozygous mutations in the gene encoding fibrillin-1 (*FBN1*). Homozygous and compound heterozygous cases are rare events and have been associated with a clinical severe presentation.

Objectives Report unexpected findings of homozygosity and compound heterozygosity in the course of molecular diagnosis of heterozygous MFS and compare the findings with published cases.

Methods and results In the context of molecular diagnosis of heterozygous MFS, systematic sequencing of the *FBN1* gene was performed in 2500 probands referred nationwide. 1400 probands carried a heterozygous mutation in this gene. Unexpectedly, among them four homozygous cases (0.29%) and five compound heterozygous cases (0.36%) were identified (total: 0.64%). Interestingly, none of these cases carried two premature termination codon mutations in the *FBN1* gene. Clinical features for these carriers and their families were gathered and compared. There was a large spectrum of severity of the disease in probands carrying two mutated *FBN1* alleles, but none of them presented extremely severe manifestations of MFS in any system compared with carriers of only one mutated *FBN1* allele. This observation is not in line with the severe clinical features reported in the literature for four homozygous and three compound heterozygous probands.

Conclusion Homozygotes and compound heterozygotes were unexpectedly identified in the course of molecular diagnosis of MFS. Contrary to previous reports, the presence of two mutated alleles was not associated with severe forms of MFS. Although homozygosity and compound heterozygosity are rarely found in molecular diagnosis, they should not be overlooked, especially among consanguineous families. However, no predictive evaluation of severity should be provided.

INTRODUCTION

Marfan syndrome (MFS) is an inherited autosomal-dominant disorder, with an estimated incidence of 1 in 5000 individuals, in which several systems (mainly cardiovascular, ocular, skeletal systems) are affected with great phenotypic variability.

Cardiovascular manifestations with thoracic aortic aneurysms or dissections are the most serious life-threatening complications of the syndrome. Clinical variability is high in MFS and diagnosis is established according to revised Ghent nosology.^{1,2} The Ghent criteria comprise a set of major and minor manifestations in different body systems and are recognised by international experts. Heterozygous mutations in the *FBN1* gene, encoding for fibrillin-1, an extracellular matrix protein, are found in the majority of patients with MFS.³ Besides severe neonatal cases with mutations between exons 24 and 32, no correlation between the type of mutation in the *FBN1* gene and severity of the various manifestations of the disease could be made.⁴

The first heterozygous mutations in the *FBN1* gene were published in 1991.⁵ In 1994, Karttunen *et al*⁶ described the first compound heterozygous case: a newborn boy with a very severe form of MFS, resulting in death from cardiac failure at the age of 4 months. Both of her parents were patients with MFS, each carrying a different *FBN1* mutation. An authentic case of homozygosity in the *FBN1* gene was later described in 2007 by de Vries *et al* in a Turkish consanguineous family.⁷ Subsequently, three other homozygous cases and two other compound heterozygous cases were reported. Since the original report in 1994, only seven cases of homozygous or compound heterozygous *FBN1* mutations are found in the literature. All of them present severe clinical features.

Homozygous mutations in inherited autosomal-dominant disorder are rare events. Here we have reported the experience of our team which performed systematic screening of the *FBN1* gene in patients suspected for MFS. The laboratory has performed diagnostic *FBN1* gene sequencing since 1998. More than 2500 probands have been tested for molecular diagnosis and 1400 heterozygous mutational events were found. Herein we have reported the surprising finding that nine probands carried homozygous or compound heterozygous mutations. From these molecular findings, complete clinical features were gathered in these nine families and were compared with what is described in the literature. In contrast to previous reports, this study

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reports on the finding of these events in a general patient population and not in selected severe forms of MFS alone.

METHODS

Patients

This study included all the patients followed by the ‘Centre National Maladies Rares—Syndrome de Marfan et apparentés’, the French National Reference centre located at Bichat Hospital (coordinator Professor Guillaume Jondeau) and by the seven affiliated ‘Competence centres’ located throughout the country.

Clinical diagnosis was established according to the revised Ghent nosology.¹ Patients were examined by several physicians: cardiologists, ophthalmologists, geneticists, rheumatologists or paediatricians (depending on their age) with specific evaluation of clinical features included in MFS. Systematic slit-lamp examination, cardiac ultrasonography and radiological investigations were also performed. Dural ectasia was looked for by imaging. Systemic score was calculated as described in the revised Ghent nosology.¹

Between 1998 and 2015, blood samples were obtained for 2500 consecutive unselected probands referred nationwide to our laboratory for molecular diagnosis because of suspected MFS, whatever the severity. Informed consent was provided for all patients in agreement with the requirements of French bioethics laws. Referral criteria for molecular testing of *FBN1* remained unchanged since 1998 and were the following: (1) patients must present features in at least two systems and one major feature as described in the first Ghent nosology⁸ and (2) an exception is made for patients with familial autosomal dominant or sporadic isolated ectopia lentis.

DNA amplification and mutation detection

Genomic DNA was isolated from peripheral blood leucocytes by standard procedures already reported.⁹ The *FBN1* gene has been systematically screened in patients suspected of MFS by

bidirectional sequencing of all coding exons and closely flanking intronic sequences in each patient (Big Dye terminators kit, ABI 3100 Genetic Analyzer, Applied Biosystems, Warrington, Cheshire, UK; primers are available on request). Since 2014, the *FBN1* gene has been screened by Next-Generation Sequencing using MARFAN MASTR Assay (Multiplicom) on MiSeq (Illumina), with bioinformatics analysis of FASTQ files (CLC Genomics Workbench (CLCbio)). Once a mutation is found in this way, it is systematically confirmed by bidirectional Sanger sequencing of the altered exon. When the mutation alters the regional restriction map, the presence of the mutation is also checked by PCR/digestion using the appropriate restriction enzyme. In the case of a homozygous molecular event, it is ascertained with a minimum of two different primer pairs (primers available on request) and if possible on two different samples. When possible, familial segregation of mutations was investigated. Description of sequence variants is performed according to Human Genome Variation Society nomenclature.¹⁰ In brief, cDNA numbering with +1 corresponds to the A of ATG, the translation initiation codon in the reference sequence (*FBN1*: NM_000138). The initiation codon is codon 1. Exons numbering is historically made considering that exon 1 carries the initiation codon.

Molecular aspects of variants

Four traditional bioinformatics programs (Grantham score, Polyphen-2,¹¹ SIFT¹² via Alamut Visual 2.7 (Interactive Biosoftware, Rouen, France) and UMD-Predictor¹³) were used to predict pathogenicity of missense variants. Effect on splicing was analysed through different tools including Human Splicing Finder,¹⁴ splicing prediction algorithm NNSPLICE¹⁵ and MaxEntScan method.¹⁶ The locus-specific database UMD-FBN1³ was consulted for each identified variant. The existence of each molecular event was looked for in the Exome Aggregation Consortium (ExAC) database.

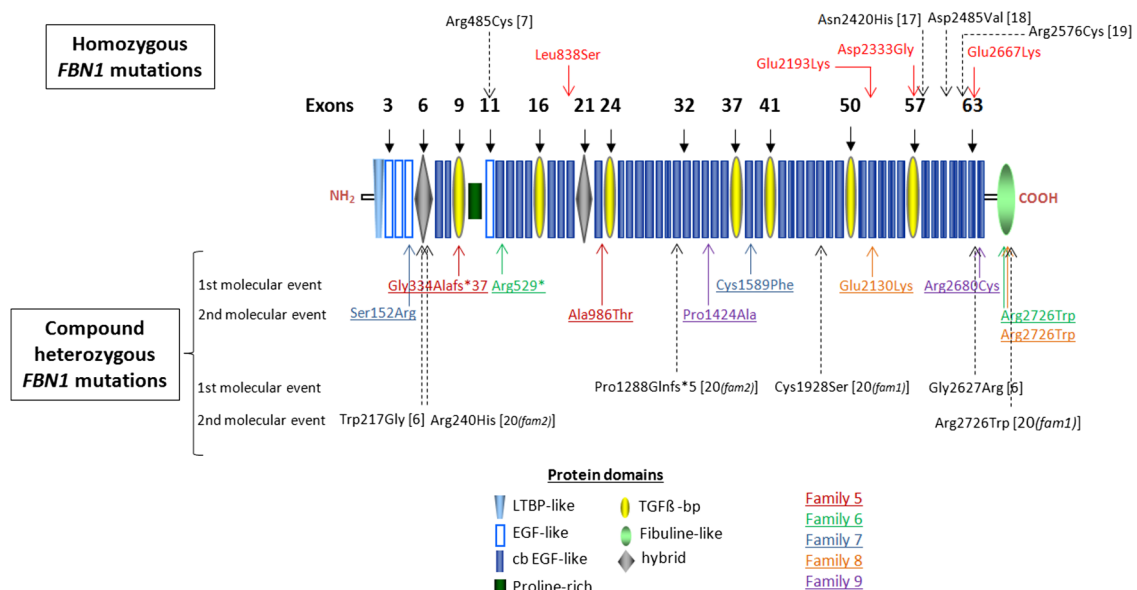


Figure 1 Representation of the localisation of homozygous and compound heterozygous mutations in fibrillin-1 protein. Exons encoding the different domains are numbered in bold. Homozygous mutations are represented above the figure (mutations reported here are in red). Compound heterozygous mutations are represented below the figure (mutations reported here are underlined and are colour-coded). Dashed arrows in black are used for previously reported mutations. Molecular events clearly in line with those usually observed in patients with Marfan syndrome (MFS) are named ‘1st molecular event’. cb-EGF, calcium-binding epidermal growth factor; EGF, epidermal growth factor; *FBN1*, gene encoding fibrillin-1; LTBP, latent TGF-β binding protein; TGFβ, transforming growth factor β.

RESULTS

Molecular aspects

In the context of molecular screening of the *FBN1* gene in patients with confirmed or potential diagnosis of heterozygous MFS or related allelic diseases, 2500 probands have been tested and 1400 heterozygous mutational events were found. Four probands presented homozygosity and 22 presented potential compound heterozygosity. Family investigations were possible in 12/22 cases and were performed to determine the respective locations of the events (in *cis* or *trans*). Five probands were shown to be compound heterozygotes. Thus, nine individuals carrying two mutated *FBN1* alleles were identified, representing 14 molecular events (figure 1, table 1). Four probands carried

missense homozygous *FBN1* mutations: two were absent from both the ExAC and the UMD-FBN1 databases (p.Leu838Ser and p.Asp2333Gly) and two were reported in heterozygotes with an overall frequency of below 5/120 000 in ExAC (p.Glu2667Lys and p.Glu2193Lys (the last was also found in the UMD-FBN1 database)). Three of them (p.Leu838Ser, p.Glu2193Lys and p.Glu2667Lys) affect highly conserved residues located in calcium-binding epidermal growth factor-like (cb-EGF) domains #9, #33 and #63, respectively (figure 1). The fourth mutation p.Asp2333Gly is located in transforming growth factor β -binding protein #7 domain. It affects a highly conserved position which is the first nucleotide of exon 57; therefore, it may also affect splicing. Prediction tools

Table 1 Predictions of pathogenicity of homozygous and compound heterozygous gene encoding fibrillin-1 (*FBN1*) mutations in the report and in the literature

Family no./reference	<i>FBN1</i> mutations	Grantham distance (0–215)	Polyphen human variant (0–1)	SIFT (1–0)	UMD-predictor (0–100)	Allele frequency in ExAC	No. of probands in UMD-FBN1 database*
<i>Homozygous FBN1 mutations from the present report</i>							
Family 1	c.2513T>C p.Leu838Ser	L (145)	D (0.992)	Del (0)	Path (96)	0	0
Family 2	c.6577G>A p.Glu2193Lys	S (56)	B (0.146)	T (0.35)	PM (63)	3/121 248	1
Family 3	c.6998A>G p.Asp2333Gly	M (94)	B (0.433)	Del (0)	Path (90)	0	0
Family 4	c.7999G>A p.Glu2667Lys	S (56)	B (0.183)	Del (0.03)	Path (75)	4/121 340	0
<i>Homozygous FBN1 mutations from the literature</i>							
de Vries <i>et al</i> ⁷	c.1453C>T p.Arg485Cys	L (180)	P (0.719)	Del (0)	Path (96)	0	2
Khan <i>et al</i> ¹⁷	c.7258A>C p.Asn2420His	S (68)	D (0.972)	Del (0)	Path (78)	0	0
Hilhorst-Hofstee <i>et al</i> ¹⁸	c.7454A>T p.Asp2485Val	L (152)	P (0.787)	Del (0)	Path (93)	5/120 870	0
Hogue <i>et al</i> ¹⁹	c.7726C>T p.Arg2576Cys	L (180)	D (0.998)	Del (0)	Path (96)	1/120 864	2
<i>Compound heterozygous FBN1 mutations from the present report</i>							
Family 5							
First molecular event	c.1001_1073del p.Gly334Alafs*37	–	–	–	–	0	0
Second molecular event	c.2956G>A p.Ala986Thr	S (58)	B (0.190)	T (0.18)	Path (90)	183/121 370	0
Family 6							
First molecular event	c.1585C>T p.Arg529*	–	–	–	–	0	7
Second molecular event	c.8176C>T p.Arg2726Trp	M (101)	B (0.001)	Del (0.01)	P Path (68)	89/121 410	15
Family 7							
First molecular event	c.4766G>T p.Cys1589Phe	L (205)	D (0.994)	Del (0)	Path (100)	0	1
Second molecular event	c.456T>A p.Ser152Arg	M (110)	B (0.040)	T (0.21)	Path (99)	0	0
Family 8							
First molecular event	c.6388G>A p.Glu2130Lys	S (56)	D (0.993)	Del (0)	P Path (72)	0	6
Second molecular event	c.8176C>T p.Arg2726Trp	M (101)	B (0.001)	Del (0.01)	P Path (68)	89/121 410	15
Family 9							
First molecular event	c.8038C>T p.Arg2680Cys	L (180)	P (0.873)	Del (0)	Path (96)	0	4
Second molecular event	c.4270C>G p.Pro1424Ala	S (27)	P (0.578)	T (0.07)	Path (90)	22/121 398	10
<i>Compound heterozygous FBN1 mutations from the literature</i>							
Karttunen <i>et al</i> ⁶							
First molecular event	c.7879G>A p.Gly2627Arg	M (125)	D (1.000)	Del (0)	Path (100)	0	1
Second molecular event	c.649T>G p.Trp217Gly	L (184)	D (1.000)	Del (0)	Path (96)	1/121 278	0
Van Dijk <i>et al</i> ²⁰							
Family 1							
First molecular event	c.5783G>C p.Cys1928Ser	M (112)	P (0.492)	Del (0)	Path (100)	0	1
Second molecular event	c.8176C>T p.Arg2726Trp	M (101)	B (0.001)	Del (0)	P Path (68)	89/121 410	15
Family 2							
First molecular event	c.3861del p.Pro1288Glnfs*5	–	–	–	–	0	1
Second molecular event	c.719G>A p.Arg240His	S (29)	D (0.987)	Del (0)	Path (78)	1/120 934	0

*Number of probands in the database out of 3146 probands, excluding the present case.

Grantham distance: L, large; M, moderate; S, small. Polyphen¹¹: B, benign; D, probably damaging; P, possibly damaging. SIFT¹²: Del, deleterious; T, tolerated. UMD-predictor¹³: Path, pathogenic; P Path, probably pathogenic; PM, probable polymorphism. Prediction score is indicated between brackets. UMD-FBN1 database was consulted in April 2016.

were mainly in favour of the pathogenicity of these mutations (table 1). When possible, the study of family members showed good segregation with the disease (figure 2, table 2). Four homozygous *FBN1* mutations are described in the literature.^{7 17–19} Three of them are also located in cb-EGF domains (#37, #39 and 41) and one is in EGF-like domain #4 (figure 1). In two instances, the mutation introduces a new cysteine residue,^{7 19} a mutation known to affect proper disulfide bond formation and disrupts domain conformation. Taken together, the eight homozygous mutations (this report and the literature) are all missense and are potentially associated with a dominant negative effect. Moreover, no homozygous mutation was found in exons 24–32, the hotspot of mutations found in cases of neonatal MFS.⁴ Finally, 5/8 homozygous mutations strikingly cluster at the 3' end of the *FBN1* gene (between exons 57 and 63) (figure 1).

In the five compound heterozygous probands, we arbitrarily named 'first molecular event' the mutations that are comparable with those most often seen in patients with MFS: premature termination codon (PTC) mutations, loss or gain of cysteine mutations or mutations affecting a highly conserved residue in a given domain. The other mutation observed in a given proband was thus named 'second molecular event'. The mutations found in the five compound heterozygotes from this study are listed in table 1 and figure 1. Among the 'second molecular events' identified, p.Ser152Arg is absent from both the ExAC and the UMD-*FBN1* databases. The mutation is located in EGF#3 and affects a highly conserved residue. Three other variants were found in four patients (p.Ala986Thr, p.Pro1424Ala and p.Arg2726Trp) and are reported in the ExAC database. However their frequency is globally low and algorithms are generally in favour of pathogenicity. Furthermore, two have already been reported in UMD-*FBN1* database.

Interestingly, none of the homozygous or compound heterozygous carriers from the present report and from the literature carried two PTC molecular events in the *FBN1* gene.

Clinical aspects

Three out of four homozygous carriers (from families 2, 3 and 4) were adults who displayed the classical MFS features usually found in heterozygous carriers (table 2). The fourth homozygous subject (family 1) was discovered through systematic clinical and molecular family screening for MFS. At discovery, he was aged 8 years, had a low systemic score and no cardiovascular or ocular feature. He is the nephew of the family proband (subject III-1, figure 2) in whom the diagnosis of MFS was made. The existence of a consanguineous mating was documented in families 1, 3 and 4. These three families were of Algerian origin. Family 2 originated from the eastern part of France, no consanguinity was known and history taking revealed no other case of MFS or related disease. In this family, the proband's parents were not available for investigation.

In two families, heterozygous carriers of the family mutation were available for investigation: five in family 1 and three in family 4 (table 2 and figure 2). A diagnosis of MFS was made in 3/5 from the first family and 2/3 in the second family. The remaining carriers in each family were women who displayed only mild skeletal features. The same clinical observation was made in the parents of the proband in family 3. This would be in line with an autosomal recessive form of MFS as that reported by de Vries *et al.*⁷

All the five compound heterozygous subjects presented the classical MFS features found in heterozygous carriers (table 2). Relatives heterozygous for one of the two mutations were only available in two families. In family 5 (figure 3), two of the

Figure 2 Representation of pedigrees of the homozygous carriers from the present report. Full square/circle=homozygous carrier. Half-full square/circle=heterozygous carrier. Empty square/circle=data not available. Arrow=proband.

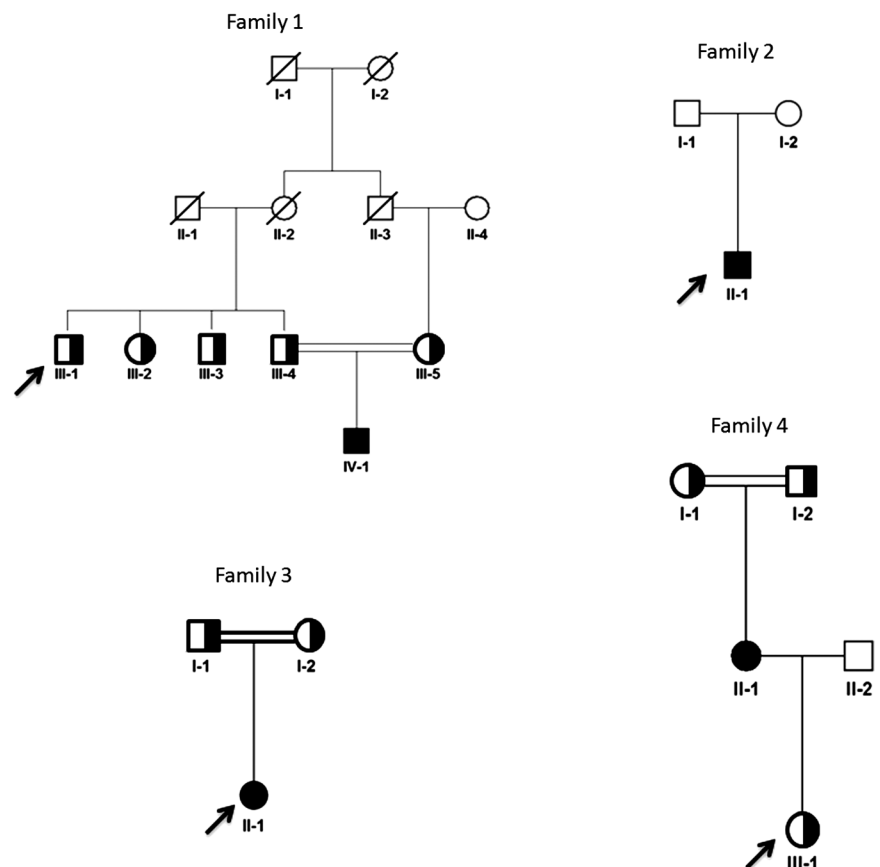


Table 2 Clinical features of homozygous and compound heterozygous gene encoding fibrillin-1 (*FBN1*) mutation carriers and their family members in the report and in the literature

Family no.	Individual no.	Sex	<i>FBN1</i> mutations	Age at diagnosis	Age at the last physical examination	Cardiovascular system (age at surgery)	Ocular system (age at surgery)	Systemic score (Ghent 2)	Geographical origin
Homozygous vs heterozygous carriers from the present report									
Family 1	IV-1	Male	Leu838Ser (homozygous)	8	8	0	0	3	Algeria
	III-1*	Male	Leu838Ser	63	63	ao dil	0	9	
	III-2	Female	Leu838Ser	47	56	0	0	8	
	III-3	Male	Leu838Ser	48	48	ao dil	0	4	
	III-4	Male	Leu838Ser	40	40	ao diss (34)	0	3	
	III-5	Female	Leu838Ser	39	39	0	0	4	
Family 2	II-1*	Male	Glu2193Lys (homozygous)	32	34	ao dil	EL	2	France
Family 3	II-1*	Female	Asp2333Gly (homozygous)	26	28	ao dil	EL (4)	14	Algeria
	I-1	Male	Asp2333Gly	66	66	0	0	7	
	I-2	Female	Asp2333Gly	59	59	0	0	5	
Family 4	II-1	Female	Glu2667Lys (homozygous)	51	53	ao dil	0	11	Algeria
	I-1	Female	Glu2667Lys	76	76	0	NA	2	
	I-2	Male	Glu2667Lys	78	79	ao dil	NA	0	
	III-1*	Female	Glu2667Lys	12	15	ao dil	0	7	
Homozygous vs heterozygous carriers from the literature									
de Vries <i>et al</i> ⁷	III-1*	Male	Arg485Cys (homozygous)	22	NA	ao diss	EL	5	Turkey
	III-4	Female	Arg485Cys (homozygous)	13	NA	0	EL	3	
	II-1	Male	Arg485Cys	NA	NA	0	0	0	
	II-2	Female	Arg485Cys	NA	NA	0	0	0	
	II-3	Female	Arg485Cys	NA	NA	MVP	0	2	
	II-4	Male	Arg485Cys	NA	NA	0	0	0	
Khan <i>et al</i> ¹⁷	II-1*	Female	Asn2420His (homozygous)	3	NA	NA	EL	NA (at least 1)	Saudi Arabia
	I-1	Male	Asn2420His	NA	NA	NA	0	NA	
	I-2	Female	Asn2420His	NA	NA	NA	0	NA	
Hilhorst-Hofstee <i>et al</i> ¹⁸	II-15	Male	Asp2485Val (homozygous)	NA	9	ao dil	EL	6	Turkey
	III-1*	Female	Asp2485Val (homozygous)	NA	22	ao dil	EL	7	
	III-3	Male	Asp2485Val (homozygous)	NA	10	ao dil	EL	5	
	I-1	Male	Asp2485Val	NA	61	0	0	0	
	I-5	Male	Asp2485Val	NA	57	0	0	0	
	I-6	Female	Asp2485Val	NA	56	0	0	0	
	II-1	Male	Asp2485Val	NA	44	ao dil	0	1	
	II-2	Male	Asp2485Val	NA	41	0	0	0	
	II-3	Female	Asp2485Val	NA	37	0	0	1	
	II-4	Male	Asp2485Val	NA	35	0	0	1	
	II-5	Female	Asp2485Val	NA	33	0	0	0	
	II-6	Male	Asp2485Val	NA	42	0	0	0	
	II-7	Female	Asp2485Val	NA	43	0	0	6	
II-8	Female	Asp2485Val	NA	55	0	0	2		
II-9	Female	Asp2485Val	NA	48	0	0	1		
II-12	Female	Asp2485Val	NA	37	0	0	0		
Hogue <i>et al</i> ¹⁹	II-1*	Female	Arg2576Cys (homozygous)	20	NA	ao diss (20)	EL	11	Mexico
	I-1	Male	Arg2576Cys	NA	NA	NA	0	0	
	I-2	Female	Arg2576Cys	NA	NA	NA	0	0	
	III-1	Male	Arg2576Cys	NA	NA	0	0	1	
Compound heterozygous vs heterozygous carriers from the present report									
Family 5	I-2*	Male	Gly334Alafs*37/Ala986Thr	25	29	ao diss (25)	flat corneas	6	
	II-2	Male	Gly334Alafs*37	6	6	ao dil	EL	2	
	II-3	Male	Gly334Alafs*37	3	3	ao dil	NA	5	
	II-1	Male	Ala986Thr	8	10	ao dil	EL	0	
Family 6	III-3*	Male	Arg529*/Arg2726Trp	8	18	ao dil	EL	6	
	II-1	Male	Arg529*	30	51	ao diss (46)	EL	5	
	I-1	Male	Arg529* mosaic	76	76	0	0	2	
	II-2	Female	Arg2726Trp	45	45	0	0	4	
	II-3	Male	Arg2726Trp	43	43	0	flat corneas	2	
	III-1	Male	Arg2726Trp	5	22	0	0	1	
	III-2	Male	Arg2726Trp	3	19	0	0	5	
Family 7	II-2*	Female	Cys1589Phe/Ser152Arg	53	55	ao dil (Bentall at 51)	EL (40)	7	
	III-1	Female	Cys1589Phe	26	28	ao dil	EL (20)	3	

Continued

Table 2 Continued

Family no.	Individual no.	Sex	<i>FBN1</i> mutations	Age at diagnosis	Age at the last physical examination	Cardiovascular system (age at surgery)	Ocular system (age at surgery)	Systemic score (Ghent 2)	Geographical origin
Family 8	II-2*	Female	Glu2130Lys/Arg2726Trp	41	49	ao dil (Bentall at 29)	EL	10	
	I-2	Female	Arg2726Trp	67	67	0	0	1	
	III-1	Male	Arg2726Trp	16	16	0	0	1	
Family 9	I-2*	Female	Arg2680Cys/Pro1424Ala	30	30	ao dil	myopia	8	
	II-1	Male	Arg2680Cys	2	2	NA	EL	0	
Compound heterozygous vs heterozygous carriers from the literature									
Karttunen <i>et al</i> ⁶	II-1*	Male	Gly2627Arg/Trp217Gly	1 week	3 months	NA	NA	4	
	I-2	Female	Gly2627Arg	teenager	33	0	myopia	5	
	I-1	Male	Trp217Gly	NA	35	ao dil	EL	6	
Van Dijk <i>et al</i> ²⁰	II-1*	Male	Cys1928Ser/Arg2726Trp	12	20	ao dil (Bentall at 19)	EL	9	
Family 1	I-2	Female	Cys1928Ser	20	NA	ao dil (Bentall at 35)	EL	7	
	I-1	Male	Arg2726Trp	NA	54	0	0	3	
	II-2	Male	Arg2726Trp	NA	17	0	0	4	
Van Dijk <i>et al</i> ²⁰	II-1*	Female	Pro1288Glnfs*5/Arg240His	5	19	0	EL	7	
Family 2	II-2	Male	Pro1288Glnfs*5/Arg240His	5	15	ao dil (Bentall at 15)	EL	5	
	I-2	Female	Pro1288Glnfs*5	31	NA	0	EL	5	
	I-1	Male	Arg240His	NA	NA	0	0	1	

Homozygous or compound heterozygous carriers are in bold.

*Proband in the family.

ao dil, aortic dilatation; ao diss, aortic dissection; EL, ectopia lentis; MVP, mitral valve prolapse; NA, not available.

proband's sons (II-2 and II-3) inherited p.Gly334Alafs*37 and both displayed a dilated aortic root at the age of 6 and 3 years, respectively. Conversely their brother carried the second paternal mutation (p.Ala986Thr). At the clinical level, he also displayed dilated aortic root at 8 years. In family 6 (figure 3), the proband (III-3) inherited p.Arg529* from his father (II-1) in whom diagnosis of MFS was made. Molecular screening in the grandfather (I-1) revealed a somatic (blood cells) mosaicism and no clinical manifestation at 76 years. The second molecular event was inherited from the maternal family of the proband. Both the mother (II-2) and her brother (II-3) carried p.Arg2726Trp and displayed mild skeletal features. Interestingly, this mutation was also found in family 8 (compound heterozygosity) where it is present as a heterozygous event in two subjects (I-2 and III-1). Both also displayed mild skeletal features. Finally in the two remaining families (figure 3), heterozygous carriers of only one of the two family mutations were available for investigation: one p.Cys1589Phe carrier in family 7 with a definite diagnosis of MFS at age 26 years and one p.Arg2680Cys carrier in family 9 with ectopia lentis operated on at age 2 years (figure 3).

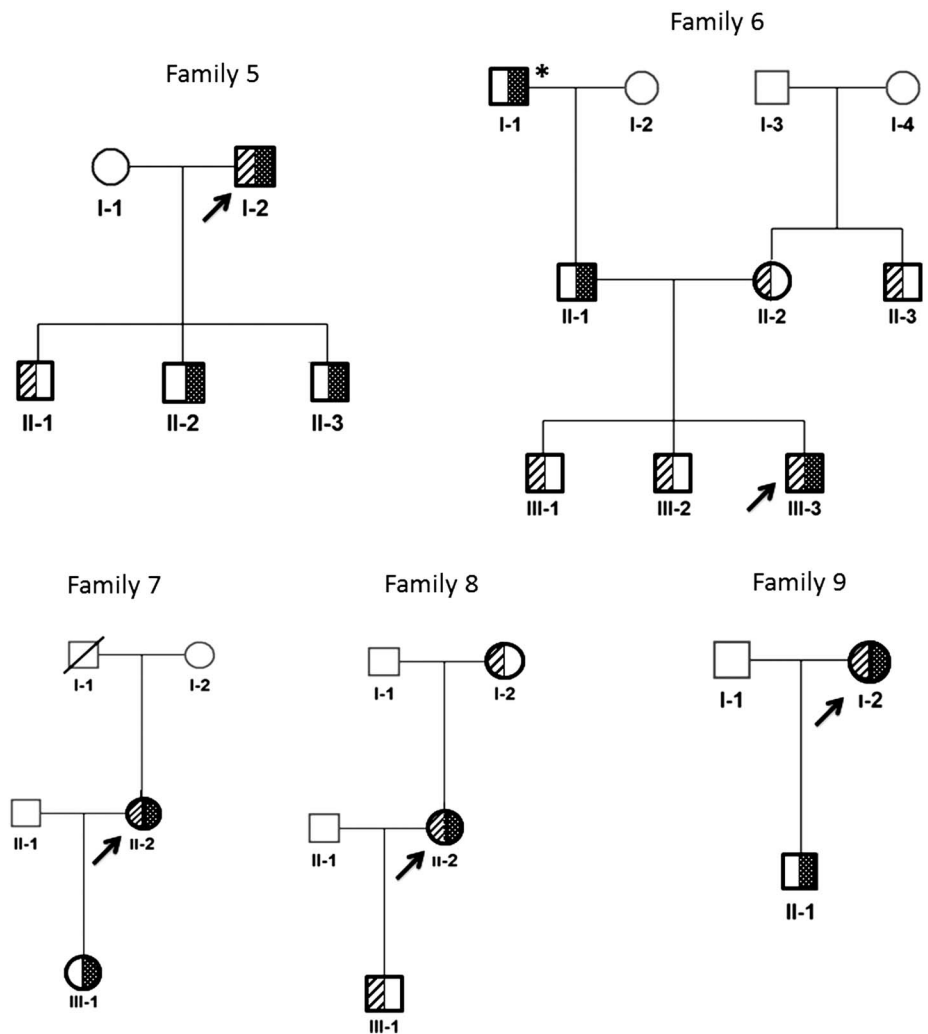
DISCUSSION

In the context of molecular diagnosis of MFS and related disorders, our laboratory has performed systematic sequencing of the entire *FBN1* gene in over 2500 probands leading to the identification of over 1400 mutations. In the course of this systematic work, completed by familial screening, we identified 26 subjects carrying two molecular events in the *FBN1* gene: homozygosity was observed in four, while two distinct events were found in the remaining 22. Through family investigation, compound heterozygosity was confirmed in five probands. These findings of homozygous and compound heterozygous subjects were

unexpected since they were found in probands or subjects sampled through family investigation for classic MFS. Therefore, heterozygosity for molecular events in the *FBN1* gene was expected. Overall, we identified four homozygous and five compound heterozygous subjects. This is a rare finding since it represents 0.64% of our series. It is also rare worldwide since the UMD-FBN1 database (the most comprehensive list of mutations) contains information of 3146 probands and only five are homozygotes and seven are compound heterozygotes. In the literature, only seven comparable cases (four homozygotes and three compound heterozygotes) have been reported (tables 1 and 2). In four reports,^{7, 17–19} seven homozygous individuals among four families were described. These were severe cases of MFS with an age at diagnosis ranging from 3 to 22 years. All of them presented with ectopia lentis, two of them had an ascending aortic dissection and one underwent preventive aortic surgery. In two other reports,^{6, 20} four compound heterozygous individuals among three families were described. These were also very severe MFS cases diagnosed between the first week of life and 12 years. In brief, these cases were identified either because of great clinical severity^{6, 19, 20} recessive MFS^{7, 18} or consanguineous families.¹⁷ Therefore, they cannot be compared with the ones we report since our findings were in a wider molecular context but in a very different clinical setting, that is, diagnosis of classic form of MFS.

Taken together, our results and literature reports have identified eight cases of homozygous *FBN1* mutations associated with MFS. In 7/8 instances, subjects belonged to pedigrees with consanguineous loops from geographical regions where inbreeding is well documented (Algeria (this report), Turkey,^{7, 18} Saudi Arabia,¹⁷ Mexico¹⁹ (table 2)). The clinical presentations of published probands tend to affect all systems (notably ocular and cardiovascular) and appear at an early age. Conversely, the

Figure 3 Representation of pedigrees of the compound heterozygous carriers from the present report. Half-dot square/circle=heterozygous carrier for the first molecular event. Half-hatched square/circle=heterozygous carrier for the second molecular event. Empty square/circle=data not available. * Probable mosaicism. Arrow=proband.



homozygous French patients have milder phenotypes, not different from phenotypes observed in the patients carrying only one mutation in the *FBN1* gene. At the molecular level, the eight molecular events are all missense and strikingly cluster at the 3' end of the *FBN1* gene (5/8 between exons 57 and 63 (figure 1)). In this region (exons 59–63), Palz *et al*²¹ described a cluster of mutations associated with milder phenotypes with less aortic involvement. The mutations found in the French probands provide further support to this observation which could partly explain the lack of a severe MFS presentation in homozygous carriers. None of the homozygous or compound heterozygous cases carried two molecular events usually observed in patients with MFS, that is to say PTC mutations, loss or gain of cysteine mutations or mutations affecting a highly conserved residue in a given domain. Comparatively, in our cohort, approximately 30% of all mutations are PTC mutations and 35% are loss or gain of cysteine.

Regarding compound heterozygous *FBN1* mutations associated with MFS, a total of nine cases have been identified combining our results and the literature. The clinical presentations of the three published probands are generally severe and appear at a much earlier age (1 week to 12 years) as compared with the French compound heterozygotes (8 to 53 years) and the homozygous probands described above. Interestingly, the p.Arg2726Trp mutation is found in three probands and is

associated with p.Arg529* in family 6 (diagnosis at 8 years), with p.Glu2130Lys in family 8 (aortic surgery at 29 years) and with p.Cys1928Ser as reported in a 12-year-old proband by Van Dijk *et al*.²⁰ The pathogenicity of p.Arg2726Trp was demonstrated in vitro where it was shown to inhibit furin/PACE processing of profibrillin.²² At the clinical level, the mutation was described in heterozygous patients with isolated skeletal features of MFS and/or high stature.^{22–23} Interestingly, cases of incomplete penetrance have been reported for heterozygous carriers of this mutation.²³ This is in line with the heterozygous carriers of families 6 and 8 who display few clinical features as well as one reported by Van Dijk *et al*²⁰ (table 2). Therefore, it can be speculated that p.Arg2726Trp displays lack of penetrance to explain the lack of a severe phenotype in compound heterozygotes who carry this mutation. This conclusion could also apply to mutations p.Ala986Thr (family 5) and p.Pro1424Ala (family 9). Indeed, in our series as well as in the UMD-*FBN1* database, heterozygote carriers are known for these two mutations and they display features in all systems, thus presenting an unequivocal diagnosis of MFS. Therefore, the lack of an aggravated phenotype in compound heterozygotes from families 5 and 9 is surprising and can only be explained this far through incomplete penetrance. Finally, the last proband (family 7) carries a mutation comparable with those usually found in patients (p.Cys1589Phe) and a second event has never been reported in any database and predicted to be

deleterious (p.Ser152Arg). The lack of an aggravated disease in the proband again is in favour of an overall lack of penetrance due to an unexplained mechanism.

In conclusion, we report nine new patients with homozygous or compound heterozygous mutations in the *FBN1* gene. This is the first report evaluating the frequency of occurrence of these events in the Marfan population in relation to mutation in the *FBN1* gene (0.64%), as it is the first report of these findings during systematic screening of the complete gene in this population. These patients complete the clinical data available for these rare molecular occurrences. Contrary to previous reports, these probands were not identified through early and very severe clinical presentations but unexpectedly in the course of molecular diagnosis of patients presenting classic form of MFS. Therefore, these rare events should not be overlooked, especially among consanguineous families. Finally, no prediction of disease severity should be extrapolated as evidenced by the lack of an aggravated phenotype in carriers.

Web resources

The URLs for data presented herein are as follows:

HGVS, Nomenclature for the description of sequence variants:

<http://www.hgvs.org/mutnomen/>

ExAC database: <http://exac.broadinstitute.org>

PolyPhen-2: <http://genetics.bwh.harvard.edu/pph2/>

UMD-Predictor: <http://umd-predictor.eu/>

UMD-FBN1 mutations database: <http://www.umd.be/FBN1/>

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