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ORIGINAL ARTICLE

Decreased frequency of *FBN1* missense variants in Ghent criteria-positive Marfan syndrome and characterization of novel *FBN1* variants

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The diagnosis of Marfan syndrome (MFS) remains challenging despite the 2010 revision to Ghent nosology criteria, and there is a lack of published information regarding *FBN1* genotype associations in patients since the update in Ghent criteria. Applying revised Ghent criteria, we reviewed consecutive proband cases ($n=292$) submitted for *FBN1* sequencing. Testing yielded 207 pathogenic or likely pathogenic *FBN1* variants, with 114/207 (55%) missense, 67/207 (32%) non-sense or frameshift, and 28/207 (13%) splicing. There were 130 novel *FBN1* variants predicted as pathogenic or likely pathogenic ($n=109$) or variant of undetermined significance ($n=21$). Of the 104 patients who met 2010 revised Ghent criteria, 87/104 (82%) had a pathogenic or likely pathogenic variant. There was a significantly lower frequency of missense variants (41 vs 89%; $P<0.0001$) observed in the Ghent-positive (vs Ghent-negative) patients, and this association held true in age-based groupings. Previously described genotype associations with ectopia lentis and early onset/'neonatal' MFS were confirmed in our cohort. Overall, our study points to the imperfect nature of relying solely on clinical criteria to diagnose MFS as well as the potential importance of truncating/splicing variants in Ghent-positive cases. Furthermore, the description of numerous novel variants and associated clinical findings may be useful for future clinical interpretation of *FBN1* genotype in patients with suspected MFS.
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INTRODUCTION

Marfan syndrome (MFS), due to pathogenic variants in *FBN1*, is an autosomal dominant connective tissue disorder typically involving the ocular, skeletal and cardiovascular systems; but may less frequently can involve the skin, integument, lung, muscle and adipose tissue.[1] Mutations in *FBN1* are numerous and have been found across the gene. Many *FBN1* pathogenic variants are unique to individual families. Missense variants are the most common type of *FBN1* variant, with the majority of these being cysteine substitutions.[2] Approximately 25% of *FBN1* pathogenic variants are *de novo* variants.[3] There are very few strong genotype–phenotype correlations, with the exception of the association of early onset, rapidly progressive (previously termed 'neonatal') MFS and *FBN1* variants in exons 24 through 32. A higher probability of ectopia lentis (EL) in patients with missense variants substituting or creating a cysteine residue and a lower probability of EL in patients with a protein truncating variant has also been observed.[2,4–7]

Genetic analysis of *FBN1* is important to confirm a suspected diagnosis of MFS or related fibrillinopathy, as well as for potential elucidation of genotype–phenotype correlations. In the most recent diagnostic criteria published in 2010 (the revised Ghent Nosology criteria for MFS),[8] a diagnosis of MFS can be made when a causal *FBN1* variant is identified in the presence of aortic dissection or an aortic root Z-score of $\geqslant 2$. Alternatively, a causal *FBN1* variant in the setting of EL and known aortic root dilation also confirms the diagnosis. A diagnosis can be made in the absence of a causal *FBN1* variant as well, taking into account factors such as systemic score, aortic root dilation z-score, EL and family history. The type of *FBN1* variant identified and its likelihood of being pathogenic are recognized as important factors when making a diagnosis of MFS, with *de novo* (in the absence of family history), non-sense, frameshift, splicing and missense substitutions of conserved residues considered more likely to be pathogenic than other missense variants.

An improved or relatively unchanged diagnostic yield for MFS has been demonstrated in light of the 2010 revised Ghent criteria.[9,10] However, there is a scarcity of published information regarding genotype associations in patients who fulfill revised Ghent criteria. Furthermore, the enhanced genomic knowledge in recent years has allowed for improved classification of genetic variants with regards to pathogenicity. On the basis of these recent changes, we sought to investigate genotype and phenotype relationships in a cohort of consecutive probands who underwent *FBN1* analysis due to suspected or clinically confirmed MFS. The purpose of this study was three-fold: (1) to assess the relationship of *FBN1* analysis to MFS diagnosis in light of the revised Ghent criteria; (2) to explore and report *FBN1* genotype–phenotype relationships in our cohort; and (3) to describe novel *FBN1* variants associated with the MFS phenotype.

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MATERIALS AND METHODS

Patient samples and clinical information

Proband samples ($n=292$) with a suspected or confirmed diagnosis (utilizing 2010 revised Ghent criteria) of MFS were included in this study. Proband samples were received by our clinical reference laboratory (Mayo Medical Laboratories, Rochester, MN, USA) from local, national and international sources. Each patient was examined by his or her referring physician. For Mayo Clinic patients, phenotypic information was extracted from the patients' electronic medical record. For patients external to Mayo Clinic, phenotypic information was provided by the referring provider via a requisition form specific to MFS and related disorders that included age, gender, suspected diagnosis, family history and phenotypic features, including those related to the 2010 revised Ghent nosology criteria. Ghent-positive or -negative status was applied utilizing clinical and genotypic information. The study was approved by the Mayo Foundation Institutional Review Board.

FBN1 sequencing

Genomic DNA was extracted from EDTA-anticoagulated whole blood. All 65 exons of *FBN1* and a minimum of 20 basepairs (bp) of intronic DNA flanking each exon were amplified by PCR. Amplification was performed using a common master mix containing Platinum Taq DNA Polymerase, 10× PCRx Enhancer System, 10× PCR Buffer (-MgCl), $MgSO_4$ (all from Invitrogen, Carlsbad, CA, USA), and a 10 mM dNTP mixture (Roche, Indianapolis, IN, USA). Master mix, forward and reverse primers were combined with genomic DNA and amplified by 35 cycles of PCR (30 s at 95 °C; 30 s initially at 68 °C then decreased by 0.5 °C each cycle, with the last 20 cycles performed at 60 °C; and 1 min extension at 72 °C, with a final 10 min extension at 72 °C). Amplicons were bi-directionally sequenced using Big Dye Terminator technology on an ABI 3730 system (Applied Biosystems, Foster City, CA, USA). Sequence analysis was done using the Mutation Surveyor software (SoftGenetics, State College, PA, USA) and visual inspection.

Classification of sequence variants

FBN1 variant nomenclature was based on RefSeq NM_000249.3. Variants were classified based on a five-level classification system: pathogenic, likely pathogenic, variant of undetermined significance (VUS), likely benign or benign. *FBN1* variants were analyzed for pathogenicity based on criteria that included (a) whether or not they were previously reported with disease and/or had associated functional studies, (b) nature of variant (for example, missense and non-sense), (c) location of variant (for example, critical cysteine residue of calcium binding epidermal growth factor-like (cbEGF-like) domain), (d) frequency of variant in the Exome Variant Server (EVS) database and dbSNP.

Variants were classified as pathogenic if they were non-sense point mutations, frameshift insertions/deletions, and variants involving the splice donor (intron +1G or +2T) or splice acceptor (intron −1G or −2A). Variants were classified as likely pathogenic if the variant affected or created a cysteine residue in a cbEGF-like or transforming growth factor-beta binding protein (TB) domain, if it affected a known consensus/critical residue (for example, the critical glycine at position 3 between cysteine 2 and cysteine 3 in the cb-EGF-like domain),[11–13] if there was a previous literature report describing a negative impact of the variant on protein function, and/or if the variant was determined to be *de novo* or otherwise likely pathogenic based on family studies. Variants were classified as benign or likely benign if they had frequency >0.39% (or sometimes lower depending on other factors) in the EVS database and dbSNP, had functional studies demonstrating no deleterious effect, were not at highly conserved residues or located in a highly variable region without a known function, were deeply intronic (> +/−20 bp from exon-intron boundary) and not predicted to impact splicing, and/or were not supported to be pathogenic based on *in silico* analyses. Variants were classified as VUS if they could not be categorized as one of the above and/or due to a lack of sufficient functional studies or frequency information.

Variants not definitively known to be pathogenic underwent *in silico* splicing analyses utilizing Alamut version 2.2 (Interactive Biosoftware, Rouen, France) integrated software tools GeneSplicer, MaxEntScan, NNSPLICE, SpliceSiteFinder-like and Human Splicing Finder. Additional online tools accessed through Alamut (MutationTaster, Align GVGD, PolyPhen-2 and SIFT) were utilized to analyze missense variants. Alamut was also utilized to derive allele frequency information via the EVS, dbSNP, 1000 Genomes and other online publicly available resources. Searches were performed online and in databases (for example, Human Gene Mutation Database) to aid in determination of novelty and significance of findings. Family testing was performed in some cases to aid in determination of clinical significance of specific variants.

Statistical analyses

Statistical significance was calculated by GraphPad software (www.graphpad.com), utilizing Fisher's exact test to calculate *P*-values.

RESULTS

Testing yielded 280 *FBN1* variants that were classified as pathogenic, likely pathogenic, VUS or likely benign (Table 1). Overall, 207 pathogenic and likely pathogenic variants were observed (Supplementary Table 1). The median age at testing for individuals with pathogenic or likely pathogenic variants was 17 years (average age at testing, 22 years). The frequency of the pathogenic and likely pathogenic variant type is depicted in Figure 1a and listed in Table 1.

Missense variants were the major type of pathogenic/likely pathogenic variant, and they were categorized based on potential functional impact (Figure 1b). The majority (75/114; 66%) of these 114 missense variants occurred in the cbEGF-like domains. For missense variants that occurred within the cbEGF-like domain, 91% of them impacted a known cbEGF-like highly conserved residue, such as altering one of the six critical cysteine (Cys) residues in the cbEGF-like domain. Specifically, in the cbEGF-like domain, 36% (31/87) variants altered one of the six critical cysteines, 21% (18/87) variants created a new Cys, 25% (22/87) altered one of the residues in the four amino-acid D-I/T/E/V/L-N/D-E consensus region, and 9% (8/87) affected another critical residue, such as a calcium binding residue or a critical glycine. In all, 11% of the missense variants (12/114) occurred in the TB domain and 58% of these (7/12) either created a cysteine or altered one of the 8 critical cysteines in a TB domain. Twenty-three percent of the missense variants (26/114) did not impact a known critical residue, but were considered as pathogenic or likely pathogenic due to previous reports that demonstrated functional impact and/or because of segregation with disease in the family.

Ghent criteria status

Taking into consideration phenotypic features and genotype, there were 104 patients who were determined to meet 2010 revised Ghent criteria. For genotype, pathogenic and likely pathogenic variants meeting criteria for causal variant as per the 2010 revised Ghent criteria were included in the categorization of Ghent status.[8] Of the patients whom met Ghent criteria, 87 (82%) had a pathogenic or likely pathogenic variant (Table 1). The majority of these variants (51/87; 59%) were protein truncating (that is, non-sense or frameshift) or splicing. The other 36 variants (41%) were missense. Overall, there was a lower frequency of missense variants (41 vs 89%; $P<0.0001$) observed in the Ghent-positive patients compared with the Ghent-negative patients (Table 1; Figure 2). The frequency of missense variants affecting critical cysteine residues was not statistically significantly different in Ghent-positive patients as compared with Ghent-negative patients (64 vs 47%, $P=0.1311$).

Since some MFS features may not be apparent in younger aged patients, patients were separated into two groups based on age. Adults were considered to be ⩾20 years old, whereas young individuals were categorized as <20 years old.[8] The pattern of variant type observed in

Table 1 Overview of observed *FBN1* Variants

Patient category	*Age*	*Type of variant*	*# Of variants*	*Missense,* n *(%)*	*Non-sense,* n *(%)*	*Frameshift,* n *(%)*	*Splicing,* n *(%)*	*Novel,* n *(%)*
Probands	All ages	All observed	280	187 (67)	33 (12)	32 (11)	28 (10)	130 (46)
	All ages	Pathogenic/Likely Pathogenic	207	114 (55)	33 (16)	32 (16)	28 (13)	109 (53)
	All ages	Variants of undetermined significance	33	25 (76)	0	0	8 (24)	21 (70)
Ghent criteria positive	All ages	Pathogenic/Likely Pathogenic	87	36 (41)	18 (21)	16 (18)	17 (20)	46 (53)
	<20 years	Pathogenic/Likely Pathogenic	25	13 (52)	2 (8)	3 (12)	7 (28)	20 (80)
	$\geqslant 20$ years	Pathogenic/Likely Pathogenic	62	23 (37)	16 (26)	13 (21)	10 (16)	27 (44)
Ghent criteria negative	All ages	Pathogenic/Likely Pathogenic	55	49 (89)	4 (7)	1 (2)	1 (2)	26 (47)
	<20 years	Pathogenic/Likely Pathogenic	38	36 (95)	1 (3)	1 (3)	0	18 (47)
	$\geqslant 20$ years	Pathogenic/Likely Pathogenic	17	13 (76)	3 (18)	0	1 (6)	8 (47)
Probands with ectopia lentis	All ages	Pathogenic/Likely Pathogenic	50	40 (80)	3 (6)	2 (4)	5 (10)	25 (50)

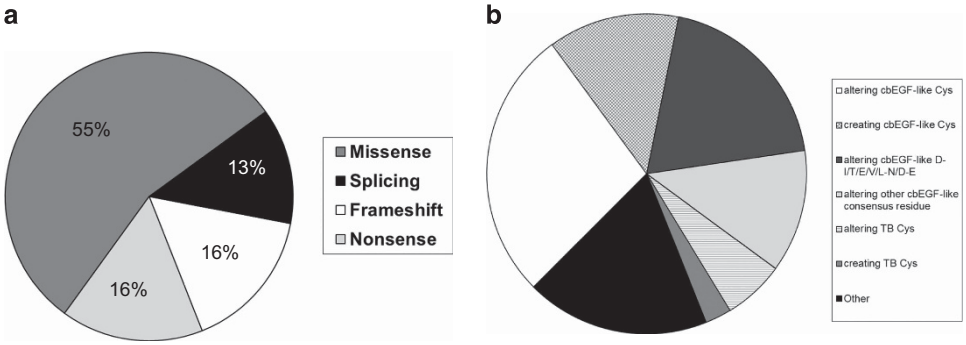

Figure 1 (**a**) Frequency of pathogenic/likely pathogenic variant type; (**b**) specific type of missense variant in relation to domain and impact on critical/conserved *FBN1* amino-acid residues. cbEGF-like, calcium binding EGF-like domain; Cys, cysteine residue; D-I/T/E/V/L-N/D-E, 4 amino-acid consensus region in 5′ cbEGF-like domain; TB, TGFβ binding protein domain.

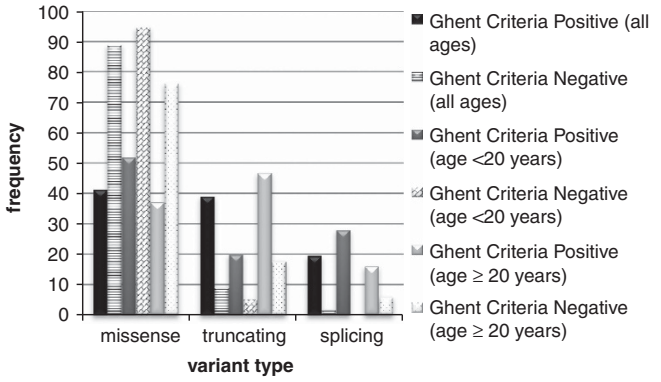

Figure 2 Frequency of pathogenic/likely pathogenic variant type observed in Ghent-positive and Ghent-negative patients overall as compared with adults $\geqslant 20$ years and young individuals <20 years. Non-sense and frameshift variants were combined into one category ('truncating').

adults vs young individuals with and without meeting Ghent criteria was very similar to what was observed in the Ghent-categorized cohort overall (Table 1; Figure 2). The highest frequency of missense variants was observed in patients <20 years old (whether Ghent criteria positive or negative). However, there were no statistically significant differences in missense variant frequency between age groups in the Ghent-positive or Ghent-negative patients.

There were 17 patients whom met Ghent criteria, but whom did not have an identifiable *FBN1* causal variant by sequence analysis. Of this group of patients, the majority (11/17; 65%) had aortic root *Z*-score $\geqslant 2$ and systemic score $\geqslant 7$ points (but no family history). Additionally, 4/17 had aortic root *Z*-score $\geqslant 2$ and systemic score $\geqslant 7$ points (plus family history); 1/17 had aortic root *Z*-score $\geqslant 2$ and EL, and 1/17 had systemic score $\geqslant 7$ points and family history.

Novel pathogenic and likely pathogenic variants

Of the 207 pathogenic and likely pathogenic variants, 53% (109/207) were novel, previously unreported variants (Table 2). For the novel variants, 52% (57/109) were missense, 15% (16/109) were non-sense, 19% (21/109) were frameshift and 14% (15/109) were splicing variants. Within the missense variant sub-grouping, the majority (81%; 46/57) of these occurred in the cbEGF-like domains, usually at critical residues. Seventeen of the novel missense variants were tested in family members of the proband to help determine whether the variant segregated with disease and/or was not present in unaffected family members (Tables 3 and 4).

Novel splicing variants

As described above, 14 novel splicing variants were observed, and most of them occurred in the critical intronic donor and acceptor splicing regions (one and two bases upstream and downstream of the exon, respectively). Two novel splicing variants that were considered to be likely pathogenic were observed outside this region. Given that these

Table 2 Novel pathogenic and likely pathogenic variants

Age at time of testing (year)	Gender	Ethnicity	Skeletal features[a]	Ocular features[a]	Cardio-vascular involvement[a]	Pulmonary involvement[a]	Epidermal features[a]	Aortic root Z-score ⩾2[a]	Ectopia Lentis[a]	Systemic score ⩾7[a]	Known aortic root dilation[a]	Family history[a]	Ghent criteria positive[b]	Exon	Nucleotide	Amino Acid	Variant Type	Missense classi-fication	Familial Testing
15	M	Af Am	x	x	x		x			x	x	x	Yes	Int 2	c.247+1G>T		s	C, PR, T, ISP	X
22	M	Cauc	x	x	x		x	x	x		x	x	Yes	4	c.401G>A	p.C134Y	m		X
13	M	Cauc	x				x					x	No	4	c.415G>T	p.G139X	n		X
21	M	Cauc	x	x	x		x		x			x	Yes	4	c.434G>A	p.C145Y	m		X
19	M	Arab	x		x						x		Unk	6	c.612C>A	p.C204X	n	C, PR, ISP	
63	F	Cauc	x	x	x							x	Unk	6	c.625T>C	p.C209R	m		X
51	F	Cauc	x			x						x	No	6	c.650G>A	p.W217X	n	C, ISP	
10	F	Arab											Unk	6	c.660_661del	p.C221X	fs		
41	M	Cauc	x		x			x			x	x	Yes	6	c.733C>T	p.Q245X	n	C, T, ISP	X
22	M	Cauc	x		x		x	x			x	x	Yes	7	c.768_769del	p.C257SfsX7	fs		
31	F	Af Am	x	x	x						x		Unk	7	c.821del	p.P274LfsX56	fs	C, ISP	
8	M	Hisp	x		x						x		Unk	8	c.983_984insTATG	p.I329MfsX20	fs	C, ISP	
18	M	Cauc	x	x									No	9	c.1030C>T	p.R344C	m	C, ISP	
12	M	Cauc	x		x								Unk	9	c.1090C>T	p.R364X	n		X
14	M	Af Am	x		x								No	11	c.1431_1432dup	p.K478TfsX102	fs	C, ISP	
11	F	Cauc	x									x	No	11	c.1468G>C	p.D490H	m	C, ISP	
14	M	Af Am	x		x				x		x		Yes	Int 11	c.1468+1G>A		s		X
23	M	Cauc										x	Yes	13	c.1646_1647del	p.T549RfsX9	fs		
34	F	Cauc	x	x	x			x	x		x	x	Yes	13	c.1679G>A	p.G560D	m	C, PR, ISP	
12	M	Asian	x	x					x			x	Yes	14	c.1715A>G	p.D572G	m	C, PR, ISP	
5	M	Cauc	x	x	x								Unk	14	c.1723G>T	p.E575X	n		X
27	F	Unk		x					x				No	14	c.1726T>C	p.C576R	m	C, ISP	
71	M	Cauc	x	x	x			x	x		x	x	Yes	14	c.1759T>G	p.C587G	m	C, ISP	
14	F	Af Am	x	x		x			x	x			No	14	c.1837G>A	p.D613N	m	C, PR, ISP	
21	M	Cauc	x		x								No	15	c.1897G>A	p.G633S	m		X
25	M	Cauc			x			x				x	Yes	16	c.2055C>G	p.C685W	m	C, ISP	
10	F	Cauc										x	Unk	16	c.2094del	p.C699VfsXC19	fs	C, PR, ISP	
2	M	Asian	x		x				x		x		Yes	Int 16	c.2114-3C>A		s		X
21	F	Cauc		x	x		x		x	x	x	x	Yes	Int 17	c.2167+1G>A		s		X
12	F	Cauc	x				x			x			Unk	18	c.2217_2218del	p.C739X	fs		
46	F	Unk											Unk	19	c.2393A>G	p.Y798C	m		
51	F	Unk											Unk	19	c.2393A>G	p.Y798C	m		X
6	F	Unk		x					x				Unk	19	c.2416_2419+2del	p.E806TfsX40	s	C, ISP	
10	F	Af Am	x	x	x		x		x		x		Yes	20	c.2431T>C	p.C811R	m		
2	M	Cauc	x	x	x				x				No	21	c.2562G>T	p.W854C	m	C, T, ISP	X
24	F	Cauc	x		x	x		x				x	Yes	22	c.2679delinsAA	p.D893EfsX6	fs		
5	F	Cauc	x	x					x				No	23	c.2762G>A	p.C921Y	m	C, DN, ISP	X
20	M	Cauc	x		x							x	Unk	Int 23	c.2854+1G>A		s		
16	F	Unk		x	x	x			x			x	Yes	24	c.2986T>G	p.C996G	m	C, T, ISP	X
11	M	Cauc	x		x						x		Unk	24	c.3012C>A	p.Y1004X	n		X
5 days	M	Unk	x				x						Unk	26	c.3220T>A	p.C1074S	m		
24	M	Unk	x		x				x		x		Yes	26	c.3250G>C	p.G1084R	m		
10	F	Cauc	x		x						x		No	28	c.3476G>A	p.C1159Y	m	C, ISP	
22	M	Hisp	x		x	x		x				x	Yes	28	c.3489_3490insGC	p.H1164AfsX10	fs	C, ISP	
3	F	Unk	x									x	Unk	28	c.3489_3490insGC	p.H1164AfsX10	fs	C, T, ISP	X
15	F	Hisp	x		x						x		No	28	c.3545G>A	p.C1182Y	m		
21	M	Unk											Unk	29	c.3670C>T	p.Q1224X	n	C, PR, ISP	
14	F	Unk											Unk	30	c.3794G>C	p.C1265S	m		X
10	F	Arab										x	Unk	31	c.3885del	p.C1296VfsX117	fs	C, ISP	

Table 2 (Continued)

Age at time of testing (year)	Gender	Ethnicity	Skeletal features[a]	Ocular features[a]	Cardio-vascular involvement[a]	Pulmonary involvement[a]	Epidermal features[a]	Aortic root Z-score ⩾2[a]	Ectopia Lentis[a]	Systemic score ⩾7[a]	Known aortic root dilation[a]	Family history[a]	Ghent criteria positive[b]	Exon	Nucleotide	Amino Acid	Variant Type	Missense classi-fication	Familial Testing
43	F	Unk											Unk	31	c.3902G>T	p.G1301V	m		
14	M	Unk	x		x						x	x	No	33	c.4122C>G	p.C1374W	m	C, ISP	
20	F	Af Am	x										No	33	c.4210G>C	p.D1404H	m		
7	F	Cauc	x	x	x				x				Unk	Int33	c.4210+1G>A		s	T, ISP	
25	F	Af Am	x		x		x	x			x	x	Yes	34	c.4255dup	p. Q1419PfsX12	fs		
24	F	Cauc	x		x			x			x		Yes	Int 34	c.4337-2A>G		s		
35	F	Unk			x								Unk	35	c.4337A>G	p.D1446G	m	C, ISP	
38	M	Cauc										x	Unk	35	c.4337A>T	p.D1446V	m	C, ISP	
15	M	Af Am	x		x		x			x	x	x	Yes	35	c.4374dup	p. G1459WfsX13	fs		
8	M	Cauc	x		x						x	x	No	35	c.4388A>G	p.N1463S	m	C, ISP	
8	F	Cauc		x	x	x			x		x		Yes	35	c.4424G>A	p.G1475D	m	C, PR, ISP	
10	F	Cauc	x	x					x				No	36	c.4582G>A	p.D1528N	m	C, PR, ISP	
22	M	Af Am	x	x	x		x			x	x		Yes	Int 36	c.4583-5A>G		s		
65	F	Af Am	x		x		x	x				x	Yes	Int 36	c.4583-9G>A		s		
4	F	Hisp	x		x			x				x	Yes	37	c.4640_4641del	p.T1547SfsX5	fs		
19	F	Af Am	x	x	x		x			x			Yes	Int 37	c.4748-1G>C		s		
45	F	Cauc	x	x	x		x	x	x		x		Yes	38	c.4755del	p.Y1585X	n		
10	M	Cauc	x		x						x	x	Unk	38	c.4813_4816+7del	p.E1605IfsX34	s		
34	F	Cauc	x		x			x				x	Yes	40	c.4974T>A	C1658X	n	C, ISP	
3	M	Cauc		x	x				x		x		Yes	40	c.5060G>T	p.C1687F	m		
2	F	Hisp										x	Unk	Int 40	c.5065+1G>T		s	C, ISP	
14	M	Cauc	x		x						x	x	Unk	41	c.5084G>A	p.C1695Y	m	C, ISP	
8	F	Hisp	x	x	x		x	x	x				Yes	44	c.5470T>G	p.C1824G	m	C, ISP	
17	M	Hisp	x		x		x				x		No	44	c.5518C>T	p.R1840C	m	C, T, ISP	
8	M	Arab			x							x	Unk	45	c.5588G>A	p.G1863E	m		
53	M	Cauc		x					x			x	Yes	46	c.5680G>T	p.E1894X	n	C, PR, ISP	
49	M	Cauc	x		x	x	x				x		Unk	46	c.5719A>C	p.N1907H	m		
5	M	Unk											Unk	47	c.5869C>T	p.Q1957X	n	C, PR, ISP	
6	M	Cauc	x	x	x				x		x	x	Yes	48	c.5992T>C	p.C1998R	m	C, DN, ISP	
34 mo	F	Cauc	x		x		x	x					Yes	49	c.6119G>A	p.C2040Y	m	C, PR, ISP	
7	F	Cauc	x		x						x		No	49	c.6158G>A	p.C2053Y	m		
35	M	Cauc	x		x			x		x	x	x	Yes	50	c.6186T>G	p.Y2062X	n	C, PR, ISP	
10	M	Unk		x					x				Unk	51	c.6379G>A	p.D2127N	m	C, T, ISP	
8	M	Hisp	x		x						x	x	Unk	52	c.6386A>T	p.D2129V	m		
6	M	Hisp	x		x			x				x	Yes	52	c.6397G>T	p.E2133X	n		
8	M	Cauc	x		x					x			Unk	52	c.6495dup	p.D2166RfsX3	fs	C, T, ISP	
36	F	Af Am			x								Unk	53	c.6575G>A	p.C2192Y	m		
36	M	Af Am	x	x	x		x	x			x		Yes	54	c.6735C>A	p.C2245X	n		
51	F	Unk			x		x				x		No	Int 54	c.6739+1G>T		s		
50	F	Af Am	x	x	x			x			x		Yes	55	c.6769_6733del	p.D2257YfsX2	fs	C, ISP	
5	F	Hisp	x		x			x		x			Yes	57	c.7087T>C	p.C2363R	m	C, ISP	
45	M	Cauc	x		x			x			x	x	Yes	58	c.7298A>G	p.Y2433C	m	C, PR, ISP	
17	M	Cauc	x		x		x				x		No	60	c.7454A>G	p.D2485G	m		
3	F	Af Am/ Cauc	x		x			x		x			Yes	60 (and 58)	c.7497_7498del (and c.7315G>A)	p.Val2501X (and p. G2439R)	fs	C, T, ISP	
4	M	Cauc	x		x						x		No	61	c.7604G>T	p.C2535F	m	C, ISP	
33	M	Cauc	x	x	x				x		x		Yes	61	c.7694G>T	p.C2565F	m	C, ISP	
27	F	Cauc	x	x								x	No	61	c.7699G>A	p.D2567N	m	C, T, ISP	
31	F	Cauc	x										No	62	c.7705G>T	p.D2569Y	m		
29	M	Cauc	x		x		x	x			x	x	Yes	62	c.7760del		fs		

Table 2 (Continued)

Age at time of testing (year)	Gender	Ethnicity	Skeletal features[a]	Ocular features[a]	Cardio-vascular involvement[a]	Pulmonary involvement[a]	Epidermal features[a]	Aortic root Z-score ⩾2[a]	Ectopia Lentis[a]	Systemic score ⩾7[a]	Known aortic root dilation[a]	Family history[a]	Ghent criteria positive[b]	Exon	Nucleotide	Amino Acid	Variant Type	Missense classification	Familial Testing
36	F	Hisp	x				x					x	No	62	c.7784G>C	p.G2587AfsX95		C, PR, ISP	
17	F	Cauc	x		x					x		x	Yes	62	c.7814G>T	p.G2595A	m		
37	F	Unk										x	Unk	Int 62	c.7820-2A>T	p.C2605F	m	C, ISP	
32	F	Cauc	x		x		x	x	x			x	Yes	63	c.7832G>A		s		
43	M	unk		x	x							x	Unk	63	c.7845dup	p.C2611Y	m	C, ISP	
14	M	Cauc	x										No	63	c.7949A>G	p.I2616HfsX25	fs		
6	F	Unk	x		x					x			Yes	63	c.7999G>T	p.N2650S	m	C, ISP	
4 mo	F	Af Am	x									x	No	63	c.8005G>T	p.E2667X	n		
3 mo	F	Cauc	x				x					x	Unk	64	c.8219_8225dupATATCGA	p.G2669C	m	T, ISP	
15	F	Cauc	x		x					x	x	x	Yes	65	c.8333T>C	p.D2743YfsX6	fs		
40	F	Unk	x	x	x			x			x		Yes	65	c.8556del	p.L2778P	m	C, ISP	
																p.Y2853TfsX10	fs		

Abbreviations: Af Am, African American; C, critical residue; Cauc, Caucasian (unspecified); DN, *de novo*; F, female; f, frameshift; Hisp, Hispanic; Int, intron; ISP, *in silico* analyses indicate variant is pathogenic; M, male; m, missense; n, non-sense; PR, previously reported with different amino-acid change; s, splicing; T, tracks in family (present in affected, not present in unaffected); Unk, unknown.
[a]'X' if feature was reported on form by ordering clinician, blank if feature not reported (does not indicate the absence of feature in patient).
[b]2010 revised Ghent nosology criteria.

two variants would questionably impact splicing, we performed familial and *in silico* analyses to help ascertain the pathogenicity of the variants, as detailed below. We have also included the clinical details of these two probands below in order to further provide support for the potential pathogenicity of these two variants.

The first novel splicing variant outside the critical splice junction region, in patient 29, a 2-year-old Asian (Indian) male, was observed to harbor the c.2114-3C>A variant. This patient had bilateral EL, bicuspid aortic valve with mild insufficiency, reduced upper to lower segment ratio, armspan to height ratio >1.05, pes planus, joint hypermobility and highly arched palate with crowded teeth. There was no family history of MFS and his unaffected parents both tested negative for the variant thus making this variant a likely *de novo* variant in the proband, assuming correct assignment of paternity. Four *in silico* splice prediction programs (SpliceSiteFinder-like, MaxENTScan, GeneSplicer and Human Splicing Finder) predict that this variant reduces splicing at the canonical splice acceptor site for intron 16.

The second splicing variant outside the critical splice junction region was c.4583-5A>G in a 22-year old Caucasian male (patient 64). This patient met Ghent criteria and had a positive wrist sign, pectus carinatum, hindfoot valgus deformity, pes planus, facial features, skin striae, possible myopia and a dilated aortic root. Similar to the previous patient described above, there was no family history of MFS and his unaffected parents both tested negative for the variant, thus making this variant a likely *de novo* occurrence in the proband, assuming correct assignment of paternity. Five *in silico* splice prediction programs (SpliceSiteFinder-like, MaxENTScan GeneSplicer, NNSPLICE and Human Splicing Finder) predict that this variant creates a new splice acceptor site in intron 36.

Novel VUSs

Thirty-three probands had VUSs identified (Table 3). Some of the VUSs were observed in multiple probands; therefore, there were 30 unique VUSs observed, of which 21 were novel, previously unreported variants. The majority of the VUSs (70%; 21/30) were missense variants with eight of the VUSs occurring in the intronic regions. There were four exonic missense VUSs that were predicted to potentially impact splicing: p.G899V (c.2696G>T), p.N1168S (c.3503A>G), p.G2506S (c.7516G>A) and p.G2618R (c.7852G>A).

Familial testing

Nineteen probands with novel missense variants and novel or previously reported VUSs had additional family testing performed to help elucidate the significance of the variant identified (Table 4). Seventeen of these variants were missense and two were potential splicing variants. As described above, the two potential splicing variants were both determined to be *de novo*, assuming correct assignment of paternity. Of the seventeen missense variants, family testing in nine of the cases supported the classification of likely pathogenic. There were eight inconclusive cases (p.C209R, p.M977R, p.N1168S, p.I1175V, p.G2116A, p.P2471R, p.D2569Y and p.L2778P) where either the variant did not track with symptomatic family members, or not enough information was provided to determine whether the variant tracked with disease, or too few family members were tested to substantiate pathogenicity or lack thereof.

Neonatal region

We tested nine patients with a severe form of MFS present from birth, previously termed neonatal MFS. The age range of patients tested was 9–525 days old (mean, 91 days old). As previously reported, most patients with this presentation have causal variants in exons 24–32,

Table 3 *FBN1* variants of undetermined significance

Age at time of testing (year)	Gender	Ethnicity	Skeletal features[a]	Ocular feautres[a]	Cardio-vascular involvement[a]	Pulmonary involvement[a]	Epidermal features[a]	Aortic root Z-score ⩾2[a]	Ectopia lentis[a]	Systemic score ⩾7[a]	Known aortic root dilation[a]	Family history[a]	Ghent criteria positive[b]	Protein domain	Exon	Nucleotide	Amino acid	In silico analyses[c]	EVS frequency (Eur Am; Af Am)[d]	Familial testing[e]	References
8	M	Hisp	x	x	x								Unk	Fibrillin	1	c.32T>A	p.L11Q	Ben (A); Path (S, MT,P)			Novel
62	F	Cauc	x		x			x			x	x	Unk	EGF-like	4	c.398A>C	p.H133P	Path (A, S,MT,P)			Novel
50	F	Cauc	x		x						x		Unk	Fibrillin	11	c.1345G>A	p.V449I	Ben (A,S, MT,P)	0.02%; 0.00%		Rommel *et al.* (2002), EVS, dbSNP
29	M	Af Am	x										Unk	Intronic	Int 14	c.1838-23A>G		Spl (SS, M,N,G,H)	0.00%; 0.02%		EVS
5	F	Arab		x					x			x	Unk	Fibrillin	22	c.2696G>T	p.G899V	Ben (A, S); Path (MT, P)		SP	Novel
3	F	South Am	x									x	Unk	cbEGF-like	23	c.2828T>C	p.L943S	Path (A, S,MT,P)			Novel
7	M	Cauc			x							x	No	TB	24	c.2930T>G	p.M977R	Path (A, S,MT,P)		SP, SN	dbSNP
14	M	Cauc	x										Unk	TB	25	c.3197G>C	p.R1066T	Path (A, S,MT,P)	0.01%; 0.00%		EVS
17	M	N Eur, E Eur	x	x	x							x	No	cbEGF-like	28	c.3503A>G	p.N1168S	SPL (SS, M,H)		AP, AN (Parents)	Ogawa *et al.* (2011)
14	F	Hisp	x		x		x						No	cbEGF-like	28	c.3523A>G	p.I1175V	Path (S, MT,A); Ben (P)		SP, ?P, ?N	Novel
16	M	Hisp	x										Unk	cbEGF-like	31	c.3890A>G	p.E1297G	Ben (A); Path (S, MT,P)			dbSNP
28	F	Hisp			x			x			x		Unk	cbEGF-like	31	c.3890A>G	p.E1297G	Ben (A); Path (S, MT,P)			dbSNP
26	M	Unk										x	Unk	cbEGF-like	31	c.3931T>A	p.Y1311N	Ben (A); Path (S, MT,P)		SP	Novel
18	M	Cauc	x		x								No	cbEGF-like	32	c.4040A>T	p.K1347I	Ben (A); Path (S, MT,P)			Novel
25	M	Unk											Unk	cbEGF-like	33	c.4190G>T	p.G1397V	Ben (A); Path (S, MT,P)			Novel
65	F	Af Am	x		x	x		x			x	x	Unk	Intronic	Int 36	c.4583-9G>A		Spl (SS, M,N,G,H)			Novel
10	F	Hisp	x									x	No	Intronic	Int 39	c.4943-9G>A		Spl (SS, M,N,G,H)			Novel
21	M	Unk	x			x	x						No	Intronic	Int 42	c.5297-19A>G		Spl (H)	0.01%; 0.00%		EVS
13	M	Hisp			x	x					x		Unk	Fibrillin	51	c.6347G>C	p.G2116A	Ben (A, P); Path (S,MT)		AP, AN (Parents)	Novel
38	F	Af Am											Unk	Intronic	Int 51	c.6380-3A>G		Spl (SS, M,N,G,H)			Novel
17	M	Cauc	x										No	Intronic	Int 52	c.6496+5G>A		Spl (SS, M,N,H)			Novel
60	M	Cauc	x		x		x						No	Intronic	Int 54	c.6739+10C>T		Spl (G)			Novel
8	M	Native Am	x				x			x			No	Intronic	Int 55	c.6871+5G>C		Spl (SS, M,N,G,H)			Novel
16	M	Indian				x							Unk	Fibrillin	57	c.7000A>T	p.N2334Y	Path (A, S,MT,P)			Novel

Table 3 (Continued)

Age at time of testing (year)	Gender	Ethnicity	Skeletal features[a]	Ocular feautres[a]	Cardio-vascular involvement[a]	Pulmonary involvement[a]	Epidermal features[a]	Aortic root Z-score ⩾ 2[a]	Ectopia lentis[a]	Systemic score ⩾ 7[a]	Known aortic root dilation[a]	Family history[a]	Ghent criteria positive[b]	Protein domain	Exon	Nucleotide	Amino acid	In silico analyses[c]	EVS frequency (Eur Am; Af Am)[d]	Familial testing[e]	References
4	M	Cauc										x	Unk	cbEGF-like	58	c.7210G>A	p.D2404N	Ben (A); Path (S, MT,P)		SP, ?P, ? P	Novel
14	F	Hisp			x							x	Unk	cbEGF-like	59	c.7412C>G	p.P2471R	Ben (A); Path (S, MT,P)		SP,?N	dbSNP
11	F	Hisp	x										No	cbEGF-like	60	c.7516G>A	p.G2506S	Ben (A,S, P); Path (MT); Spl (M,H)			Novel
12	M	Hisp	x		x								Unk	cbEGF-like	62	c.7790T>A	p.L2597H	Ben (A); Path (S, MT,P)			Novel
16	M	Cauc	x		x								Unk	cbEGF-like	63	c.7852G>A	p.G2618R	Path (A, S,MT,P); Spl (S,M, H)	0.01%; 0.02%		Matyas *et al.* (2002), EVS, dbSNP
8	F	Hisp	x		x								Unk	cbEGF-like	63	c.7852G>A	p.G2618R (observed with likely pathogenic variant p. N2144S)	Path (A, S,MT,P); Spl (S,M, H)	0.01%; 0.02%		Matyas *et al.* (2002), EVS, dbSNP
19	F	Cauc	x	x	x		x				x	x	No	cbEGF-like	63	c.7964C>T	p.A2655V	Ben (A, MT);Path (S,P)			dbSNP
19	F	Cauc	x	x									No	cbEGF-like	63	c.7964C>T	p.A2655V	Ben (A, MT);Path (S,P)			dbSNP
15	F	Cauc	x		x			x			x		No	cbEGF-like	63	c.8012T>C	p.L2671P	Path (A, S, MT,P)			Novel

Abbreviations: Af Am, African American; Cauc, Caucasian (unspecified); E Eur, Eastern European; F, female; Hisp, Hispanic; Int, intron; M, male; MFS, Marfan syndrome; Native Am, Native American; N Eur, Northern European; South Am, South American; TB, transforming growth factor-beta binding protein; unk, unknown.

[a]'x' if feature was reported on form by ordering clinician, blank if feature not reported (does not indicate the absence of feature in patient).

[b]2010 revised Ghent nosology criteria.

[c]All *in silico* analyses accessed 9/19/2013; Ben, benign; Path, pathogenic; Spl, splice site creation/disturbance; A, Align GVGD; S, SIFT; MT, MutationTaster; P, PolyPhen-2; SS, SpliceSiteFinder-like; M, MaxEntScan; N, NNSPLICE; G, GeneSplicer; H, Human Splicing Finder.

[d]EVS, NHLBI GO Exome Sequencing Project, Exome Variant Server, accessed 06/06/2014.

[e]SP, symptomatic family member positive for variant; SN, symptomatic family member negative for variant; AP, asymptomatic family member positive for variant; AN, asymptomatic family member negative for variant; ?, questionable MFS features present. See text for more details.

Table 4 Familial testing for novel missense variants and variants of uncertain significance

Exon	*Nucleotide*	*Amino acid*	*Variant details*	*Proband features*	*Family member 1*	*Family member 2*	*Family member 3*	*Family member 4*
6	c.625T>C	p.C209R	Critical cysteine residue in TB domain	Female, 36 years old, Caucasian, bilateral ectopia lentis, dilated aortic root and mid-ascending aorta, mitral valve and tricuspid valve prolapse, mild pectus carinatum, positive wrist and thumb sign, facial features	Positive in mother, 59 years old, bilateral ectopia lentis, abdominal aortic aneurysm	Positive in sister, 33 years old, myopia, normal echocardiogram, no skeletal features	Negative in nephew (sister's son), 11 years old, protrusion acetabulae, flat feet, tall stature	
14	c.1715A>G	p.D572G	Critical residue in cbEGF-like domain (D-I/T/E/V/L-N/D-E consensus region)	Male, 12 years old, Asian, pectus carinatum, wrist and thumb signs, ectopia lentis	Negative in sister, 8 years old, no features			
14	c.1759T>G	p.C587G	Critical cysteine in cbEGF-like domain	Male, 71 years old, Caucasian, ectopia lentis from birth, aortic dilatation with aortic root replacement, history of hernias, mild pectus excavatum	Positive in daughter, 34 years old, highly arched palate with crowded teeth, ectopia lentis, dilated ascending aorta			
Int 16	c.2114-3C>A		*In silico* prediction to impact splicing	Male, 2 years old, Asian (Indian) male, bilateral ectopia lentis, bicuspid aortic valve with mild insufficiency, reduced upper to lower segment ratio, armspan to height ratio >1.05, pes planus, joint hypermobility, highly arched palate with crowded teeth	Negative in mother, no features	Negative in father, no features		
22	c.2696G>T	p.G899V		Male, 5 years old, Arab, ectopia lentis	Positive in mother, 30 years old, affected (features unspecified)			
24	c.2930T>G	p.M977R		Male, 7 years old, Caucasian, >95th percentile for height, mild hypotonia, hyperreflexivity	Positive in father, 46 years old, tall stature, joint hypermobility, pectus excavatum, aortic graft	Negative in paternal male cousin, wrist and thumb signs, joint hypermobility, highly arched palate with crowded teeth		
28	c.3503A>G	p.N1168S		Male, 17 year old, Caucasian, severe pectus excavatum, wrist and thumb signs, pes planus, highly arched palate, facial features, striae, mild dilated aortic root	Positive in father, 53 years old, no features	Negative in mother, 51 years old, no features		
28	c.3523A>G	p.I1175V		Female, 14 year old, Hispanic, 75th to 90th percentile for height, armspan to height ratio 0.996, upper to lower segment ratio 0.77	Positive in mother, affected (features unspecified)	Positive in brother, greater than 95th percentile for height, normal aortic root, tricuspid regurgitation, wrist sign, borderline thumb sign, mild hyperextensible elbows, striae	Negative in brother, age 9, greater than 95th percentile for height, armspan to height ratio 1.04, upper to lower segment ratio 0.86	
31	c.3931T>A	p.Y1331N		Male, 26 years old, unspecified features of MFS	Positive in infant son, 5 months old, mild pectus deformity, low weight gain, patent foramen ovale			
45	c.5588G>A	p.G1863E	Critical glycine in cbEGF-like domain between Cys2 and Cys3	Male, 8 years old, Arab, aortic dilatation	Positive in father, 35 years old, affected (features unspecified)	Positive in sister, 8 years old, affected (features unspecified)	Positive in sister, 12 years old, affected (features unspecified)	Negative in brother, 11 years old, no features
51	c.6347G>C	p.G2116A		Male, 13 years old, Hispanic, aortic root dilatation, pneumothorax	Positive in mother, only suggested feature was being tallest in her family of seven siblings (5′7″)			

Table 4 (Continued)

Exon	Nucleotide	Amino acid	Variant details	Proband features	Family member 1	Family member 2	Family member 3	Family member 4
52	c.6386A>T	p.D2129V	Critical residue in cbEGF-like domain (D-I/T/E/V/L-N/D-E consensus region)	Male, 8 years old, Hispanic, joint hypermobility, highly arched palate, height in the 99th percentile, upper to lower segment ratio=0.84	Positive in mother, aortic and mitral valve repair, joint hypermobility, long narrow facies, highly arched palate, positive thumb sign	Negative in brother, 11 years old		
53	c.6575G>A	p.C2192Y	Critical cysteine in cbEGF-like domain	Female, 36 years old, African American, affected but features unspecified	Positive in daughter, 4 years old, pectus carinatum, pes planus	Negative in daughter, 10 years old, no features	Negative in son, 2 years old, no features	
58	c.7210G>A	p.D2404N	Occurs at 3rd residue in 4 amino-acid consensus region of cbEGF-like domain (D-I/T/E/V/L-N/D-E consensus region)	Male, 4 years old, Caucasian, affected but features unspecified	Positive in father, clinical diagnosis of MFS (features unspecified)	Positive in brother, affected (features unspecified)	Positive in paternal grandmother, affected (features unspecified)	
59	c.7412C>G	p.P2471R		Female, 14 years old, Hispanic, minor cardiovascular findings (unspecified)	Positive in paternal grandmother, affected (features unspecified)	Negative in brother, 16 years old, pectus excavatum of moderate severity, arachnodactyly		
Int 36	c.4583-5G>A		*In silico* prediction to impact splicing	Male, 22 years old, Caucasian male, Ghent criteria positive, wrist sign pectus carinatum, hindfoot valgus deformity, pes planus, facial features, skin striae, possible myopia, and dilated aortic root	Negative in mother, no features	Negative in father, no features		
61	c.7604G>T	p.C2535F	Critical cysteine in cbEGF-like domain	Male, 4 years old, Caucasian, wrist and thumb signs, dilatation of ascending aorta with aortic regurgitation, mitral valve prolapse, joint hypermobility, facial features	Negative in mother, no features			
62	c.7705G>T	p.D2569Y	Critical residue in cbEGF-like domain (D-I/T/E/V/L-N/D-E consensus region)	Female, 37 years old, Caucasian, pectus carinatum, reduced upper to lower segment ratio, armspan to height ratio >1.05, wrist and thumb signs, highly arched palate with crowded teeth, facial features	Positive in father, 57 years old, aortic root dilatation	Negative in paternal uncle, 60 years old, only reported feature was pectus carinatum		
65	c.8333T>C	p.L2778P		Female, 15 years old, Caucasian, marfanoid habitus, aortic root dilatation	Positive in father, aortic valve and ascending aortic repair, club foot	Positive in nephew, 25 months old, greater than 97th percentile for height, not evaluated for ophthalmologic or cardiovascular features		

Abbreviations: MFS, Marfan syndrome; TB, transforming growth factor-beta binding protein.

especially missense variants in exons 24–32 and variants resulting in exon skipping of exon 31 or 32.[14,15] Of the nine patients that we tested for variants in exons 24–32, four of these patients were observed to have a pathogenic variant. One of the variants occurred in exon 25 (p.I1048T), two of the variants occurred at cysteine residues in exon 26 (p.C1074S (novel) and p.C1086Y) and one variant occurred at the splice donor site of intron 32 (c.4087+1G>A). As is typical with early onset, rapidly progressive MFS, no family history of MFS was reported for any of these patients.

Ectopia lentis

EL has been reported as more likely associated with missense variants involving cysteine residues than with other types of missense, exon skipping and premature truncating variants.[2,4–7,16] It has also been reported that patients with EL have pathogenic variants clustering in the first 15 exons of *FBN1*,[6,17] although this was not observed in another study.[4] In our cohort, there were 65 individuals with reported EL, and 50 of them tested positive for a pathogenic or likely pathogenic variant, with 39/50 (78%) being a missense variant (Table 1). Overall, 26/50 (52%) either altered or created a cysteine residue (vs 14% for cohort without reported EL; $P<0.0001$), 10/50 (20%) were truncating variants (vs 51% cohort without reported EL; $P<0.0001$), and 17/50 (34%) occurred in exons 1–15 (vs 20% cohort without reported EL; $P=0.0027$). To reduce the risk of bias due to incomplete reporting of phenotype in our cohort, we excluded

mutation-positive patients whom did not have accompanying phenotypic information ($n=27$) from this analysis.

DISCUSSION

With the revision to Ghent criteria in 2010, few studies have been performed to assess the association with *FBN1* genetic analysis and MFS diagnosis in light of the revised criteria. Before the revised Ghent criteria, it had been observed that for individuals who met Ghent criteria, the *FBN1* causal variant detection rate ranged from 66 to 93%.[4–6,18,19] A more recent smaller study observed an 80% *FBN1* causal variant detection rate in 24/30 patients who fulfilled 2010 revised Ghent criteria.[20] Another study observed that genotype information changed the final diagnosis in more patients who met revised Ghent criteria as compared with original Ghent criteria, thus pointing to the importance of genotype analysis in suspected MFS cases.[21]

To help address the question of extent of correlation with *FBN1* genotype and revised Ghent criteria, we reviewed 292 proband cases that underwent *FBN1* sequence analysis. In our cohort, there were 104 patients who were determined to meet 2010 revised Ghent criteria, and 87 (82%) had a pathogenic or likely pathogenic *FBN1* variant. Thus, our causal variant detection rate in Ghent-positive patients was similar to that reported in previous studies. Additionally, of the 207 patients with a pathogenic or likely pathogenic variant, 55 of them did not meet Ghent criteria. This speaks to the imperfect nature of relying solely on clinical criteria to diagnose MFS, and the importance of the genetic evaluation of patients with suspected MFS.

An *FBN1* causal variant was not identified in 16% of Ghent-positive patients in our study. This may be due, at least in part, to the phenotypic overlap with other genetic disorders such as Loeys-Dietz syndrome. Additionally, we did not perform large deletion/duplication analysis of *FBN1* that may have impacted our mutation detection rate slightly. On the other hand, 55/207 (27%) of the patients in this cohort whom tested positive for a pathogenic or likely pathogenic *FBN1* variant did not fulfill 2010 Ghent criteria. The observation of *FBN1* causal variants in Ghent-negative and, on the other hand, lack of *FBN1* causal variants in Ghent-positive patients is likely due to the largely heterogenous nature of the disorder as well as the fact that the criteria used to establish MFS are not completely specific or sensitive. Therefore, our data support that *FBN1* genetic testing can be beneficial to help substantiate a diagnosis of MFS, and also to provide a basis for potentially ruling out the disorder or considering another, overlapping genetic disorder.

Current practice generally considers that *FBN1* missense variants are associated with more classic MFS, and patients with non-missense *FBN1* variants may have a milder disease presentation. However, the frequency of missense variants in our Ghent-positive patients was significantly lower than the Ghent-negative patients (41 vs 89%; $P<0.0001$). We observed a similar lower frequency of missense variants between Ghent-positive and -negative patients in adults and young individuals. As compared with other studies examining variant type in Ghent-positive patients, the frequency of missense variants observed in our cohort (41%) was lower than in previous reports (59%;[19] 50%;[22] and 51%[23]). However, these previous studies occurred before the 2010 change to the Ghent nosology criteria, which may have impacted the differences observed between our study and the previous studies. Furthermore, the criteria used to establish variant pathogenicity is more robust than in previous years due to increased knowledge about human genetic variation and access to large variant databases. A more recent smaller study of 24 Ghent-positive patients, utilizing Ghent 2010 criteria, observed a 54% missense variant frequency, although a comparison was not made with patients who were Ghent criteria negative.[20] Our novel observation that Ghent-positive patients have a lower frequency of missense variants is contrary to current practice (which would anticipate that most Ghent-positive patients would have missense variants). We have observed a similar phenomenon with a lower frequency of missense variants in MFS patients with aortic events.[24] Future studies examining mechanism of missense vs non-missense *FBN1* variants in pathogenesis of MFS could help to further our understanding of this topic.

FBN1 is a large gene, comprising 65 exons, in which numerous alterations have been previously reported. Here, we add to that body of literature by describing an additional 130 novel *FBN1* variants that fall into the categories of pathogenic, likely pathogenic or VUS. In the pathogenic or likely pathogenic variant category ($n=207$), 109 of these variants were novel. Similar to previous reports, we observed that the majority of *FBN1* variants that occurred in our overall cohort were missense. Those missense variants that were pathogenic or likely pathogenic were likely to occur in highly conserved regions of the gene, for example, impacting a critical cysteine residue in a cbEGF-like or TB domain.

Familial studies have been known to be beneficial in helping to elucidate the pathogenicity of novel and previously reported VUSs. In our study, we were able to evaluate family members of 19 probands with novel missense variants and novel or previously reported VUSs. For 11 of these cases, familial testing helped to determine or substantiate variant pathogenicity. There were eight inconclusive cases where either the variant did not track with suspected disease in family members, or not enough information was provided to determine whether the variant tracked with disease, or too few family members were tested to substantiate pathogenicity or lack thereof.

It has been previously suggested that there is a relationship between severity of ocular involvement and the presence of a cysteine substitution or other *FBN1* pathogenic variant in specific regions of the gene.[2,6,17,25] Consistent with what others found, we observed that individuals with EL were significantly more likely to have missense variants involving a cysteine residue and pathogenic variants in exons 1–15, and were less likely to have truncating variants. We also confirmed previous findings of patients with early onset, rapidly progressing MFS and pathogenic variants in exons 24–32.

In MFS suspected cases where an *FBN1* genetic variant is identified, the results may sometimes be difficult to interpret in terms of their impact on protein function, especially if they are novel missense or intronic variants (outside the critical splice junction region). It is well known that interpretation of the clinical significance of genetic variants is often dependent on knowledge of previously reported specific variants, known gene regions/residues critical for protein function, familial studies and genotype–phenotype correlations. Additionally, it is known that MFS can present variably, and is sometimes undiagnosed or unsuspected in patients presenting with isolated features of MFS. Recent strides in large databases containing human genetic variation, such as the EVS, have greatly enhanced our ability to properly classify genetic variants. As we move down the path of increased utilization of large-scale genetic testing, it is likely that scenarios identifying *FBN1* variants will increase in frequency, thus substantiating the need to publish new variants with associated clinical data.

With our report, we have added to this body of literature by reporting multiple novel *FBN1* variants, as well as familial testing information and phenotypic details in the patients who harbor these variants. A novel observation reported here is the lower frequency of

missense variants occurring in patients with positive Ghent criteria status. The implications for this novel observation could be important since it conflicts with the general mindset that patients with non-missense variants have a milder course of disease. Our study additionally points to the imperfect nature of relying solely on clinical criteria to establish a diagnosis of MFS, and the benefit of utilizing genetic testing to aid in diagnosis. Overall, the information detailed here can be utilized as an aid in the clinical interpretation of *FBN1* genetic findings and may act as a springboard for further studies of *FBN1* and MFS.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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