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Increased frequency of *FBN1* truncating and splicing variants in Marfan syndrome patients with aortic events

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Purpose: Marfan syndrome is a systemic disorder that typically involves *FBN1* mutations and cardiovascular manifestations. We investigated *FBN1* genotype–phenotype correlations with aortic events (aortic dissection and prophylactic aortic surgery) in patients with Marfan syndrome.

Methods: Genotype and phenotype information from probands (n = 179) with an *FBN1* pathogenic or likely pathogenic variant were assessed.

Results: A higher frequency of truncating or splicing *FBN1* variants was observed in Ghent criteria–positive patients with an aortic event (n = 34) as compared with all other probands (n = 145) without a reported aortic event (79 vs. 39%; $P < 0.0001$), as well as Ghent criteria–positive probands (n = 54) without an aortic event (79 vs. 48%; $P = 0.0039$). Most probands with an early aortic event had a truncating or splicing variant (100% (n = 12) and 95% (n = 21) of patients younger than 30 and 40 years old, respectively). Aortic events occurred at a younger median age in patients with truncating/splicing variants (29 years) as compared with those with missense variants (51 years). A trend toward a higher frequency of truncating/splicing variants in patients with aortic dissection (n = 21) versus prophylactic surgery (n = 13) (85.7 vs. 69.3%; not significant) was observed.

Conclusion: These aortic event– and age-associated findings may have important implications for the management of Marfan syndrome patients with *FBN1* truncating and splicing variants.

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Key Words: aortic dissection; aortic surgery; *FBN1*; fibrillin-1; Marfan syndrome

INTRODUCTION

FBN1 mutations are most commonly associated with Marfan syndrome (MFS), an autosomal dominant connective tissue disorder typically involving the ocular, skeletal, and cardiovascular systems; MFS less frequently involves the skin, integument, lung, muscle, and adipose tissue. Cardiovascular manifestations, which are the major cause of morbidity and early mortality in MFS, include aortic dilatation at the level of the sinus of Valsalva, predisposition for aortic dissection, mitral valve and tricuspid valve prolapse, and enlargement of the proximal pulmonary artery.

In MFS, an age-dependent association with the occurrence of an aortic event (aortic dissection or prophylactic aortic surgery) has been demonstrated in 16% of 30–year-olds and 74% of 60-year-olds having an aortic event.[1] A sex-dependent association has also been observed: aortic events occur at an earlier age in males than in females.[1]

Hundreds of mutations have been identified in *FBN1*—many of them unique to individual families. Missense mutations are the most common type of *FBN1* mutation, the majority of which are cysteine substitutions. *FBN1* mutations have been shown to occur across the gene with limited genotype–phenotype correlations, with the exception of the association of early onset, severe (previously termed "neonatal") MFS and mutations in exons 24 through 32, as well as the association of ectopia lentis with missense mutations. A study investigating genotype–phenotype correlations with cardiovascular features did not observe significant differences with mutation type (e.g., frameshift versus missense) but did observe a higher probability of ascending aortic dilatation, aortic event, and mitral valve prolapse in patients with mutations altering a cysteine residue.[1]

The type of *FBN1* mutation identified and its likelihood of being pathogenic are recognized as important factors when making a diagnosis of MFS and later clinical decisions. Some have argued that truncating and splicing mutations may be associated with a milder disease course. Accordingly, we evaluated 179 consecutive probands with *FBN1* mutations to evaluate this important clinical issue.

MATERIALS AND METHODS

Study population

Proband samples (n = 179) with a pathogenic or likely pathogenic *FBN1* variant and detailed clinical information received over a 4.25-year period were included in this study. 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 the Mayo Clinic, phenotypic information was provided by the referring provider via a requisition form specific to *FBN1*, which included age, sex, suspected diagnosis, family history, and phenotypic features, including those related to the Ghent (2010 revised) nosology criteria.[2] The ethnicities of patients

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with reported aortic aneurysm/dissection and/or prophylactic aortic root surgery ($n = 34$) were European Caucasian ($n = 21$), African American ($n = 7$), Hispanic ($n = 2$), Arabic ($n = 1$), and unspecified ($n = 3$). The study was approved by the Mayo Foundation institutional review board.

FBN1 sequencing

Genomic DNA was extracted from ethylenediaminetetraacetic acid–anticoagulated whole blood. All 65 exons of *FBN1* (RefSeq NM_000249.3) and a minimum of 20 base pairs (bp) of intronic DNA flanking each exon were amplified by multiplexed polymerase chain reaction (PCR). Amplification was performed using a common master mix containing Platinum Taq DNA Polymerase, 10× PCRx Enhancer System, 10× PCR buffer (minus magnesium chloride), magnesium sulfate (all Invitrogen, Carlsbad, CA), and a 10 mmol/l deoxynucleotide triphosphate mixture (Roche, Indianapolis, IN). Master mix and 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 a 1-min extension at 72 °C, with a final 10-min extension at 72 °C). Amplicons were bidirectionally sequenced using Big Dye Terminator technology on an ABI 3730 system (Applied Biosystems, Foster City, CA). Sequence analysis was done using Mutation Surveyor software (SoftGenetics, State College, PA) and visual inspection.

Classification of alterations

FBN1 alteration nomenclature was based on RefSeq NM_000138.4. *FBN1* variants were analyzed for pathogenicity based on criteria that included (i) whether they were previously reported and had associated functional studies; (ii) the nature of the variant (e.g., missense, nonsense); (iii) the location of the variant (e.g., critical cysteine residue of calcium-binding epidermal growth factor-like (cbEGF-like) domain); and (iv) the frequency of the variant in the Exome Variant Server database and the Single Nucleotide Polymorphism Database. Variants were classified as pathogenic if they were nonsense point mutations, frameshift insertions/deletions, or mutations 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-β binding protein domain; if it affected a known consensus/critical residue (e.g., the critical glycine at position 3 between cysteine 2 and cysteine 3 in the cbEGF-like domain); 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.

Statistical analyses

Statistical analyses were performed when appropriate using Fisher's exact test with GraphPad Software (GraphPad is a free Web-based application available at http://www.graphpad.com).

RESULTS

A total of 179 probands with pathogenic and likely pathogenic variants fell into the following categories: 96 missense (53%), 59 (33%) nonsense or frameshift (truncating), and 24 (13%) splicing. Of these, 32 probands had a reported personal history of aortic events (aortic dissection (with or without surgical repair) or prophylactic aortic surgery), and two young Ghent-positive probands (15 and 21 years old) with aortic root dilatation had a detailed paternal family history of aortic events. All 34 of these individuals fulfilled the 2010 revised Ghent nosology criteria. Characteristics of patients with aortic events as compared with patients without a reported aortic event are described in **Table 1**. Detailed genetic and clinical information about the aortic event cohort is listed in **Table 2**.

Table 1 Characteristics of patients with aortic events as compared with patients without reported aortic events

	Prophylactic aortic surgery ($n = 13$)	(Median age at event (range))	Dissection (or aortic surgery and dissection) ($n = 21$)	(Median age at event (range))	Overall aortic event cohort ($n = 34$)	(Median age at event (range))	Patients without a reported aortic event ($n = 145$)	Patients older than 14 without a reported aortic event ($n = 71$)
Median age at time of testing (range)	36 (21–71)	(31 (14–71))	40 (15–65)	(30 (22–52))	38 (15–71)	(30 (14–71))	1 day–57 years	15–57
Male sex, %	62		38		47		47	51
Patients with missense mutation, %	30.7	(50 (14–71))	14.3	(52 (45–48))	20.6	(51 (14–71))	61.4	59.1
Patients with truncating mutation, %	61.6	(36 (20–50))	61.9	(40 (20–51))	61.8	(38 (20–51))	26.9	29.6
Patients with splicing mutation, %	7.7	(29 (NA))	23.8	(22 (21–43))	17.6	(23 (21–43))	11.7	11.3

All ages are in years unless otherwise specified.

NA, not applicable.

Table 2 Genetic and clinical information of patients with aortic events

Age at time of testing (years)	Age at time of event (years)	Sex	Ethnicity	Myopia[a] Retinal detachment[a]	Ectopia lentis[a] Mitral valve prolapse[a]	Aortic dilatation[a]	Aortic dissection[a]	Aortic surgery[a]	Pulmonary involvement[a] Epidermal features[a]	Skeletal features[a]	Aortic root *Z* score ≥2 Systemic score ≥7	Family history[a] Ghent criteria positive[b]	Fibrillin domain	Exon	Nucleotide	Amino acid	Type	Variant reference (or novel)	Family history/testing details	Cardiovascular details
22	14	M	Cauc		X	X		X	X	X	X	X Yes	EGF-like	4	c.401G>A	p.C134Y	M	Novel	Father diagnosed with MFS at age 50, two paternal uncles died from MFS complications, paternal aunt and paternal grandfather also diagnosed with MFS	MFS diagnosed at age 3 years, aortic valve–sparing root replacement at age 14 years
59	Unk	M	Caucasian			X	X				X	X Yes	cbEGF-like	13	c.1633C>T	p.R545C	M	29,30	Unknown family history	Abdominal aortic dissection
4	34	F	Caucasian		X			X		X	X	X Yes	cbEGF-like	13	c.1679G>A	p.G560D	m	Novel	Multiple siblings and children affected with MFS; sister (age 64) with ascending aortic dilatation	Aortic valve–sparing root replacement at sinus of Valsalva, 51 mm (age 34 years); bilateral ectopia lentis
71	71	M	Cauc		X	X		X		X	X	X Yes	cbEGF-like	14	c.1759T>G	p.C587G	M	Novel	63-Year-old sister tested positive for variant and has dilated ascending aorta	Aortic root replacement surgery (age 71); son with aortic root replacement at age 34; ectopia lentis from birth
52	48	F	Cauc	X	X	X	X	X	X	X	X	Yes	cbEGF-like	46	c.5782T>C	p.C1928R	M	31	No family history	MFS diagnosed at age 4; aortic root replacement surgery (sinus of Valsalva, 52 mm), aortic dissection during surgery (age 48)
55	Unk	F	Unk	X		X		X		X	X	Yes	cbEGF-like	47	c.5861T>G	p.F1954C	M	32	Father died suddenly at age 51; etiology not clear	Aortic valve–sparing root replacement surgery
50	50	M	Hisp		X	X		X		X	X	X Yes	cbEGF-like	52	c.6431A>G	p.N2144S	M	33	Mother and maternal uncle affected	Aortic valve–sparing root replacement (sinus of Valsalva, 49 mm), mitral valve repair (age 50)

Af Am, African American; Arab, Arabic; Cauc, Caucasian; cbEGF-like, calcium-binding epidermal growth factor-like; EGF, epidermal growth factor; FS, frameshift; ; Hisp, Hispanic; M, missense; MFS, Marfan syndrome; N, nonsense; S, splicing; Unk, unknown.

[a]"X" If feature was reported on form by ordering clinician, blank if feature not reported (does not indicate known absence of feature in patient). [b]2010 revised Ghent nosology criteria.

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45	Unk	M	Cauc					X	X				X	X		X	Yes	cbEGF-like	58	c.7298A>G	p.Y2433C	M	Novel	Father died from ascending aortic dissection; daughter likely affected	Dilatation and dissection of ascending and descending thoracic aorta
47	24	M	Cauc	X			X	X		X			X	X	X	X	Yes	EGF-like	5	c.493C>T	p.R165X	N	9	Mother with MFS died at age 65 from brain aneurysm; sister with MFS, aortic root repair at age 65; daughter, age 10, aortic dilatation (33 mm; *Z* score 3.9)	Aortic root and aortic valve replacement surgery at age 24 years
41	28	M	Cauc						X				X	X		X	Yes	Fibrillin	6	c.733C>T	p.Q245X	N	Novel	Mother and two children diagnosed with MFS	MFS diagnosed at age 11 years, aortic dissection at age 28 years, graft repair of ascending aorta; residual chronic dissection of aortic arch and right coronary artery dissection
37	Unk	F	Arab						X				X	X		X	Yes	Fibrillin	10	c.1285C>T	p.R429X	N	34	Son tested positive for variant; sister and brother tested negative for variant	Aortic dissection and aortic valve prolapse
36	32	F	Cauc			X		X		X	X		X	X	X		Yes	cbEGF-like	36	c.4567C>T	p.R1523X	N	35	No family history	MFS diagnosed at age 5; aortic valve–sparing root replacement at age 32 (sinus of Valsalva, 50 mm)
52	52	F	Cauc			X				X	X		X	X		X	Yes	Fibrillin	37	c.4615C>T	p.R1539X	N	36	Father died at age 47 from aortic dissection	Aortic dissection (age 52 years), aortic root repair, aortic valve replacement surgery
39	Unk	M	Af Am					X		X				X		X	Yes	Fibrillin	37	c.4615C>T	p.R1539X	N	36	Father died at age 30 from aortic dissection; brother, age 29, with aortic dilatation	Aortic root replacement surgery

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46	30	F	Cauc	X					X	X			X	X			Yes	Fibrillin	37	c.4621C>T	p.R1541X	N	37	No family history	Aortic dissection involving ascending aorta, arch, left subclavian, left carotid, and descending aorta extending to iliac bifurcation (age 30 years); abdominal aortic dilatation
40	Unk	F	Cauc						X	X		X	X	X		X	Yes	Fibrillin	37	c.4621C>T	p.R1541X	N	37	Mother, maternal grandmother, and siblings affected	Ascending aortic dissection; dilatation of ascending aorta; arterial tortuosity
45	Unk	F	Cauc			X		X	X	X		X	X	X			Yes	Matrix fibril-associated	38	c.4755del	p.Y1585X	N	Novel	No family history	Aortic dissection
35	Unk	M	Cauc					X	X				X	X	X	X	Yes	Fibrillin	50	c.6186T>G	p.Y2062X	N	Novel	Multiple affected family members including son, sister, and father. Familial deaths from aortic dissection include father (age 43), paternal uncle (age 39), and paternal uncle (age 3).	Ascending aortic dissection
36	Unk	M	Af Am	X				X	X			X	X	X			Yes	cbEGF-like	54	c.6735C>A	p.C2245X	N	Novel	No family history	Aortic dissection
30	29	F	Hisp	X			X	X		X			X	X	X		Yes	Fibrillin	57	c.7180C>T	p.R2394X	N	38	No family history	Mitral valve repair, aortic valve–sparing root replacement (sinus of Valsalva, 46 mm; age 29 years)
22	20	M	Cauc					X	X			X	X	X		X	Yes	cbEGF-like	7	c.768_769del	p.C257SfsX7	FS	Novel	Son, age 18 months, tested positive for variant, aortic *Z* score 4.1; sister and sister's 9-month-old daughter tested positive for variant	Aortic valve and root replacement at age 20 years (sinus of Valsalva, 50 mm)

Af Am, African American; Arab, Arabic; Cauc, Caucasian; cbEGF-like, calcium-binding epidermal growth factor-like; EGF, epidermal growth factor; FS, frameshift; ; Hisp, Hispanic; M, missense; MFS, Marfan syndrome; N, nonsense; S, splicing; Unk, unknown.

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36	Unk	M	Unk	X		X		X	X	X		X	Yes	cbEGF-like	8	c.937del	p.C313AfsX17	FS	4	Daughter with many skeletal features but not formally evaluated	Aortic root surgery (52 mm)
25	25	F	Af Am			X	X		X	X		X	Yes	cbEGF-like	34	c.4255dup	p.Q1419PfsX12	FS	Novel	Newborn daughter with MFS features tested positive for variant	Dissection of ascending aorta at age 25 (sinus of Valsalva, 46 mm); type B aortic dissection, age 26, 28 weeks pregnant
15	Unk	M	Af Am				X		X	X	X	X	Yes	cbEGF-like	35	c.4374dup	p.G1459WfsX13	FS	Novel	Father died from biventricular dilatation and dissection at age 25; brother, age 17, has aortic dilatation and is positive for mutation	Ascending aortic dilatation
50	40	F	Af Am	X	X		X			X	X			Yes	cbEGF-like	55	c.6769_6733del	p.D2257YfsX2	FS	Novel	Unknown family history	Ascending aortic dissection (age 49 years)
50	50	M	Af Am				X	X		X	X	X	X	Yes	Matrix fibril-associated	57	c.7039_7040del	p.M2347VfsX19	FS	39	Siblings and parents with reported early deaths (as early as 20s to 30s); etiology unclear	Aortic root replacement (sinus of Valsalva. 51 mm) at age 50
29	Unk	M	Cauc				X	X		X	X	X	X	Yes	cbEGF-like	62	c.7760del	p.G2587AfsX95	FS	Novel	Family history details not provided	Aortic dissection
40	40	F	Unk	X			X	X		X	X			Yes	Fibrillin	65	c.8556del	p.Y2853TfsX10	FS	Novel	No family history	Ascending aortic dissection and aortic valve–sparing root replacement at age 40; mitral valve prolapse
22	Unk	F	Cauc				X		X	X	X			Yes	Intronic	Intron 11	c.1468+5G>A		S	2,30; de novo	No family history; both asymptomatic parents tested negative for the variant (de novo variant)	Abdominal aortic dissection
43	Unk	F	Cauc				X			X	X			Yes	Intronic	Intron 11	c.1468+5G>A		S	2,30	No family history	Aortic dissection

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21	Unk	F	Cauc		X	X					X	Yes	Intronic	Intron 17	c.2167+1G>A		S	Novel	Father died from aortic dissection at age 35 (aortic valve replacement at age 25); paternal aunt had aortic dissection at age 36; paternal grandmother died from aortic dissection at age 40	MFS diagnosed at age 2 years; aortic dilatation (sinus of Valsalva, 43 mm)
29	29	F	Cauc			X	X	X		X	X	Yes	Intronic	Intron 34	c.4336+1G>A		S	40	Mother diagnosed with MFS in 30s, aortic root replaced at age 42; brother had aortic dissection at age 27; brother age 20 with aortic dilatation; sister (twin), age 27, diagnosed with MFS, normal aorta; sister (twin), age 27, with aortic dilatation; sister, age 24, with aortic dilatation	MFS diagnosed at age 3; aortic valve–sparing root replacement at age 29 (sinus of Valsalva, 48 mm); mitral valve replacement at age 18
24	24	F	Cauc				X		X	X		Yes	Intronic	Intron 34	c.4337-2A>G		S	Novel	No family history	Ascending aortic dissection led to death 15 hours after giving birth; evaluated at age 12 but considered not to have MFS
22	22	M	Af Am			X	X		X	X	X	Yes	Intronic	Intron 36	c.4583-5A>G		S	Novel, de novo	No family history, both asymptomatic parents tested negative for variant (de novo)	Aortic dissection at age 22; aortic dilatation (sinus of Valsalva, 47 mm)

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Table 3 Potential functional impact of *FBN1* splicing variants

Intron	Nucleotide	Impact on fibrillin-1 protein
11	c.1468+5G>A	Activation of latent splice donor site within exon 11, possibly creating a frameshift[2]
17	c.2167+1G>A	Possible skipping of exon 17; unknown impact
34	c.4336+1G>A	Possible skipping of exon 34; unknown impact
34	c.4337-2A>G	Possible frameshift (p.Asp1446ValfsX21 observed with c.4337-1G>A)[2]
36	c.4583-5A>G	Predicted to create new cryptic splice acceptor site at c.4583–4, resulting in 4-bp insertion, frameshift, and stop codon at +27 from activated cryptic splice acceptor site[a]

bp, base pair.

[a]In silico splice prediction tools (SpliceSiteFinder-like, MaxEntScan, NNSPLICE, GeneSplicer, Human Splicing Finder; accessed 23 January 2014).

The majority (79%) of the observed variants in this aortic event group were protein truncating (62%: nonsense (12/34; 35%) and frameshift (9/34; 27%)) or splicing (6/34; 18%), and only 21% (7/34) of them were missense mutations (**Table 1**). In comparison, protein truncating or splicing variants occurred at a frequency of 39% (truncating, 27%; splicing, 12%) in mutation-positive patients without a reported aortic event. An analysis of mutation-positive patients without a reported aortic event who fulfilled the 2010 revised Ghent nosology criteria demonstrated that 52% (28/54) of these patients had a missense variant. Thus, among Ghent-positive patients, there was also a significantly lower frequency of truncating/splicing variants in those who did not have a reported aortic event (48 vs. 79%; $P = 0.0039$). Because our aortic event cohort did not contain patients younger than 15 years of age, and because younger patients are much less likely to have aortic events, we performed a secondary analysis of the cohort, excluding patients younger than 15 years of age. Patients without a reported aortic event who were 15 years of age or older had a similar frequency of truncating/splicing variants (41%) as patients without a reported aortic event at all ages (39%) (**Table 1**), and this frequency was still statistically significantly lower than the 79% observed in patients with aortic events ($P = 0.0003$).

Détaint et al.[1] previously reported on cardiovascular manifestations in 1,013 probands with pathogenic *FBN1* mutations. They observed that the probability of an aortic event before the age of 30 years in their cohort was 16% in men and 11% in women; before the age of 40 years this probability was ~40–45% in men and 25% in women. Because our aortic event cohort had a wide age range (15–71 years) at the time of testing, we performed additional age-dependent analyses. There were 12 patients with aortic events (6 male and 6 female) younger than the age of 30, and all of them (12/12; 100%) had a truncating or splicing variant. When we included patients 40 years of age or younger with an aortic event, 20 of 21 patients (95%; 9 males, 12 females) had a truncating or splicing variant. The one patient in this group who did not have a truncating or splicing variant had aortic root replacement surgery at the age of 34 years (aortic root diameter, 51 mm), and she harbored a novel, likely pathogenic p.G560D variant in a cbEGF-like domain (**Table 2**).

We compared the mutation type and age at event for patients with prophylactic aortic surgery versus dissection (**Table 1**). Patients with dissection ($n = 21$) were less likely to have a missense mutation (14%) compared with patients who had prophylactic aortic surgery ($n = 13$; 31%; not statistically significant). The median age at the time of aortic event was younger for patients with truncating or splicing variants as compared with patients with missense variants. The median age for aortic dissection was 52 years in those with a missense mutation ($n = 3$), 40 years in those with a truncating mutation ($n = 12$), and 22 years in those with a splicing mutation ($n = 5$). A similar trend was observed in the prophylactic aortic surgery group (**Table 1**). Overall, the median age for an aortic event was 51 years for patients with a missense mutation as compared with 29 years for patients with a truncating/splicing mutation.

There were five different splicing variants in six of the probands in our aortic event cohort. In general, the mechanism whereby *FBN1* splicing mutations exert their effect is unclear. Accordingly, we examined these five splicing variants to ascertain their potential impact on fibrillin-1 protein (**Table 3**). Three of the variants (in four probands) were likely to create a frameshift and two of the novel splicing variants were predicted to cause exon skipping with an unknown impact on fibrillin-1. We identified two different splicing variants that did not occur in the universal GT splice donor or AG splice acceptor sites in three probands in the aortic event cohort. We observed an intron 11 variant, c.1468+5G>A, in two probands. Ogawa et al.[3] found that this variant was associated with the activation of a latent splice donor site within exon 11, thereby creating a frameshift. The second intronic variant in the aortic event cohort that occurred outside of the GT or AG sites was c.4583-5A>G in intron 36. This variant was found to be a likely de novo variant in the proband (who fulfilled Ghent criteria) and therefore was likely pathogenic. Furthermore, five in silico splice prediction programs indicated that this alteration activates a cryptic splice acceptor site in intron 36 (**Table 3**).

Four novel missense variants were identified in the aortic event cohort: p.C134Y, p.G560D, p.C587G, and p.Y2433C. The first three of these novel missense variants occurred at critical residues in the cbEGF-like domain for which different amino acid substitutions had been previously reported (p.C134G and p.C134S[4,5]; p.G560S[6]; and p.C587Y and p.C587R[7,8]). The fourth novel missense variant, p.Y2433C, also occurred in a cbEGF-like domain; it created an additional (seventh) cysteine residue, which likely disrupts fibrillin folding.

We observed three mutations (p.R1539X, p.R1541X, and c.1468+5G>A) that each occurred in two apparently unrelated patients (**Table 2**). All three of these mutations have been observed numerous (9–12) times in the *FBN1* Universal

Mutation Database (http://www.umd.be/FBN1/; accessed 11 April 2014).

DISCUSSION

The *FBN1* gene is large, comprising 65 exons in which numerous alterations have been previously reported, but strong genotype–phenotype correlations have been elusive. Our data are novel in that we observed a high frequency of truncating and splicing *FBN1* mutations in Ghent-positive patients with aortic events (aortic dissection and/or surgery) as compared with *FBN1* mutation-positive patients without reported aortic events (79 vs. 39%, respectively; $P < 0.0001$). Considering only Ghent-positive patients, the difference was also significant: 48% of patients without reported aortic events had a truncating or splicing variant (versus 79% of Ghent-positive patients with an aortic event; $P = 0.0039$). We also observed that in patients who had an early aortic event, all or nearly all (12/12 (100%) of patients younger than age 30 and 20/21 (95%) of patients age 40 or younger) had a protein-truncating or -splicing variant. We characterized these age groups as having early aortic events because of a previous publication detailing aortic events in only 16% of men and 11% of women younger than 30 years, and ~40–45% of men and 25% of women younger than 40 years, in a large MFS cohort.[1] We also observed that patients with aortic dissection had a higher frequency of a protein-truncating or -splicing variant (86%) as compared with patients with prophylactic aortic surgery (69%), although this observation was not statistically significant. In addition, we noted a trend in association between genotype and age at aortic surgery, dissection, or both. Overall, aortic dissection or surgery occurred at a younger age in patients with a truncating or splicing variant (median age, 29 years) as compared with patients with missense mutations (median age, 51 years).

Some previous publications have observed a nonsignificant trend toward aortic dissection occurring more frequently in patients with an *FBN1* truncating or splicing mutation versus a missense or cysteine-substituting mutation.[9,10] Specifically, Schrijver et al.[10] found that dissection was the predominant indication for ascending aortic replacement in patients with a truncating mutation (58%; 15/26), whereas this was the case for only 32% (6/19) of patients with a missense mutation ($P = 0.08$). Rommel et al.[9] likewise observed a nonsignificant trend: dissection occurred more often in their protein truncation group (5/25; 20%) than in their cysteine substitution group (3/30; 10%). A recent study observed an association with truncating and splicing mutations in Chinese patients with cardiovascular defects (dilatation, aneurysm, and dissection).[11] Others have not reported an association with truncating mutations or cysteine mutations and cardiovascular manifestations.[6,12–14] One reason that others may not have observed an association is because of the way the data were analyzed, for example, lumping dilatation and dissection and/or mitral valve prolapse together, including in the analysis patients who are too young to manifest cardiovascular events, and/or including patients who were older and more likely to manifest cardiovascular events. Détaint et al.[1] observed a higher probability of ascending aortic dilatation, aortic event, and mitral valve prolapse in patients with mutations altering a cysteine residue. However, their published analysis did not split out aortic dissection or aortic surgery alone and genotype.

It is commonly thought that patients with a truncating mutation have a milder course of disease because of nonsense-mediated decay of the mutant transcript.[15–17] Our data suggest that this is not the case, specifically our observation that the vast majority of early aortic events occurred in patients with a truncating (or splicing) variant. Therefore, instead of having a milder course of disease, as is commonly thought, patients with truncating or splicing variants may have a less clinically apparent MFS phenotype as compared with patients with missense variants, which also has been previously suggested by Schrijver et al.[10] For example, one patient in our cohort was a 24-year-old woman with a splicing variant (predicted to result in protein truncation; c.4337-2A>G; **Table 3**) who died from an aortic dissection shortly after giving birth. When she was evaluated at the age of 12, she had some features of MFS but was considered not to have a diagnosis of MFS based on the Ghent criteria. The lack of diagnosis at age 12 may have been because of her young age, or it may have been because of her overall lack of clinically apparent MFS presentation, regardless of age.

The mechanism whereby *FBN1* truncating or splicing mutations exert their effect is not totally clear, and both dominant negative and loss-of-function mechanisms have been identified. Although mechanistic studies of *FBN1* splicing mutations have been performed on only a very limited basis, some *FBN1* splicing mutations have been demonstrated to lead to a truncated protein, thereby having a similar impact as a nonsense or frameshift mutation.[11,16,18,19] Other *FBN1* splicing mutations cause in-frame exon deletions that disrupt microfibril assembly,[20] theoretically having the same impact as a truncating mutation in a dominant negative effect model. Three of the five splicing variants in our cohort were likely to create a frameshift (leading to truncation), and two of the novel splicing variants were predicted to cause in-frame exon deletions with an unknown effect on fibrillin-1.

It has been observed that premature termination codon mutations in *FBN1* can lead to preferentially degraded messenger RNA via nonsense-mediated messenger RNA decay pathways.[15,16,21,22] Whereas rapid decay of the mutant transcript would be expected to create a loss-of-function phenotype, synthesis of a truncated transcript would be expected to create a dominant negative effect. This dominant negative effect would be due to integration of the truncated fibrillin-1 molecules into the microfibrils during microfibrillar assembly, thereby creating structurally inferior connective tissues. The dominant negative effect model was supported by the observation that most patients with classic MFS harbor missense mutations and the findings of patients with nonsense mutations and mild clinical presentation, in which they failed to meet the Ghent criteria.[10,16,17] However, several others have stressed the importance of haploinsufficiency in the pathogenesis of MFS, as observed

in mouse model studies and because of the identification of patients with classic MFS and full-gene deletions.[23,24] It was also observed that variable disease expression in patients with premature termination codon mutations appeared to correlate with variable expression of the normal *FBN1* allele and not with variable rates of nonsense-mediated decay.[24]

In recent years, it has come to light that the fibrillin-1-related mechanisms leading to MFS pathogenesis include not only its effect on extracellular matrix structure through microfibrillar formation and elastin association but also its effect on transforming growth factor (TGF)-β regulation and homeostasis. The discovery of Loeys-Dietz syndrome, a disorder phenotypically overlapping MFS (but generally with more aggressive aortic dilatation (and greater involvement of the arterial tree) and without ocular involvement), and mutations in genes encoding for TGF-β receptors (*TGFBR1* and *TGFBR2*) helped to springboard the understanding of the importance of TGF-β signaling in MFS and related connective tissue disorders.[25] It is now known that mutations in genes affecting MFS, Loeys-Dietz syndrome, and some other related connective tissue disorders lead to an increase in TGF-β signaling, and activation of both canonical (TGF-β/SMAD) and noncanonical (TGF-β/extracellular signal-regulated kinase (ERK)) pathways that have been shown to play a role in aneurysm development.[26] These discoveries have also helped enhance the understanding of the efficacy of losartan (an angiotensin II type I receptor blocker) and RDEA-119 (an ERK antagonist) in attenuating aortic enlargement in MFS through the inhibition of TGF-β signaling.[26–28] Whether *FBN1* premature termination codon mutations have a stronger influence on TGF-β activation as compared with missense variants is unknown. The interplay between *FBN1* mutation type and TGF-β signaling, dominant negative versus haploinsufficiency mechanisms, and dosage of normal fibrillin-1, as well as the effect on aneurysm progression and severity, deserves further investigation. Further elucidating these mechanisms has important potential for the treatment and management of patients with MFS and related disorders.

A limitation to our observations is related to the sometimes incomplete phenotypic information provided to us. Therefore, we cannot rule out the possibility that a patient with a pathogenic variant (missense, truncating, or splicing) who we categorized as not having a reported aortic event truly did not have aortic dissection/surgery. However, it should be noted that the frequency of aortic events in our cohort was similar to that reported by another larger study. Specifically, Détaint et al.[1] observed a frequency of aortic events in 279 of 965 (28.9%) *FBN1* mutation-positive patients ages 11 and older. We observed a similar aortic event frequency of 27.4% (34 of 124) in our cohort, considering patients ages 11 and older. Moreover, we were likely not biased by having a skewed percentage of mutation type because the missense mutation frequency (53.3%) in our overall cohort was similar to that observed in previous studies (50–59%).[6,13,17] Furthermore, 18% of the patients in the cohort displayed mutations in exons 1–10, which is in agreement with previous works.[12,16] Another potential limitation to our study is that our data may have been biased when younger or older patients were included in the analyses because of lower and higher frequencies of cardiovascular events, respectively. Furthermore, our data represent a mixture of ethnicities and therefore may unknowingly create a bias because of the potential for the presence of ethnicity-based differences in frequency of mutation type. Overall, it would be beneficial to use a more systematic method to better flesh out the association between protein-truncating/splicing variants and well-characterized cardiovascular features, including *Z* score, as well as other parameters such as patient age, sex, ethnicity, and presence of specific Ghent criteria features.

In summary, our findings are significant and novel in that they demonstrate a strong association of MFS patients with truncating/splicing *FBN1* mutations with both age-independent and age-dependent (i.e., early) aortic events. We observed a novel trend among those patients who had an aortic event of the event occurring at a earlier median age in the presence of a truncating/splicing variant. Overall, the results of this study may have important implications for the management of patients with *FBN1* truncating and splicing variants, especially in light of the fact that current practice generally considers such patients to have a more mild disease course. Our data suggest that although all patients with MFS may benefit from *FBN1* gene sequencing for the purpose of confirming the diagnosis, affected individuals should be monitored similarly for aortic dilatation and its complications, regardless of the *FBN1* mutation type.

DISCLOSURE

The authors declare no conflict of interest.

REFERENCES

1. Détaint D, Faivre L, Collod-Beroud G, et al. Cardiovascular manifestations in men and women carrying a FBN1 mutation. *Eur Heart J* 2010;31:2223–2229.
2. Loeys BL, Dietz HC, Braverman AC, et al. The revised Ghent nosology for the Marfan syndrome. *J Med Genet* 2010;47:476–485.
3. Ogawa N, Imai Y, Takahashi Y, et al. Evaluating Japanese patients with the Marfan syndrome using high-throughput microarray-based mutational analysis of fibrillin-1 gene. *Am J Cardiol* 2011;108:1801–1807.
4. Howarth R, Yearwood C, Harvey JF. Application of dHPLC for mutation detection of the fibrillin-1 gene for the diagnosis of Marfan syndrome in a National Health Service Laboratory. *Genet Test* 2007;11:146–152.
5. Sakai H, Visser R, Ikegawa S, et al. Comprehensive genetic analysis of relevant four genes in 49 patients with Marfan syndrome or Marfan-related phenotypes. *Am J Med Genet A* 2006;140:1719–1725.
6. Loeys B, Nuytinck L, Delvaux I, De Bie S, De Paepe A. Genotype and phenotype analysis of 171 patients referred for molecular study of the fibrillin-1 gene FBN1 because of suspected Marfan syndrome. *Arch Intern Med* 2001;161:2447–2454.
7. Booms P, Cisler J, Mathews KR, et al. Novel exon skipping mutation in the fibrillin-1 gene: two 'hot spots' for the neonatal Marfan syndrome. *Clin Genet* 1999;55:110–117.
8. Liang C, Fan W, Wu S, Liu Y. Identification of a novel FBN1 mutation in a Chinese family with isolated ectopia lentis. *Mol Vis* 2011;17:3481–3485.
9. Rommel K, Karck M, Haverich A, et al. Identification of 29 novel and nine recurrent fibrillin-1 (FBN1) mutations and genotype-phenotype correlations in 76 patients with Marfan syndrome. *Hum Mutat* 2005;26:529–539.
10. Schrijver I, Liu W, Odom R, et al. Premature termination mutations in FBN1: distinct effects on differential allelic expression and on protein and clinical phenotypes. *Am J Hum Genet* 2002;71:223–237.

11. Wang WJ, Han P, Zheng J, et al. Exon 47 skipping of fibrillin-1 leads preferentially to cardiovascular defects in patients with thoracic aortic aneurysms and dissections. *J Mol Med (Berl)* 2013;91:37–47.
12. Comeglio P, Johnson P, Arno G, et al. The importance of mutation detection in Marfan syndrome and Marfan-related disorders: report of 193 FBN1 mutations. *Hum Mutat* 2007;28:928.
13. Arbustini E, Grasso M, Ansaldi S, et al. Identification of sixty-two novel and twelve known FBN1 mutations in eighty-one unrelated probands with Marfan syndrome and other fibrillinopathies. *Hum Mutat* 2005;26:494.
14. Loeys B, De Backer J, Van Acker P, et al. Comprehensive molecular screening of the FBN1 gene favors locus homogeneity of classical Marfan syndrome. *Hum Mutat* 2004;24:140–146.
15. Aoyama T, Francke U, Dietz HC, Furthmayr H. Quantitative differences in biosynthesis and extracellular deposition of fibrillin in cultured fibroblasts distinguish five groups of Marfan syndrome patients and suggest distinct pathogenetic mechanisms. *J Clin Invest* 1994;94:130–137.
16. Dietz HC, McIntosh I, Sakai LY, et al. Four novel FBN1 mutations: significance for mutant transcript level and EGF-like domain calcium binding in the pathogenesis of Marfan syndrome. *Genomics* 1993;17:468–475.
17. Rand-Hendriksen S, Tjeldhorn L, Lundby R, et al. Search for correlations between FBN1 genotype and complete Ghent phenotype in 44 unrelated Norwegian patients with Marfan syndrome. *Am J Med Genet A* 2007;143A:1968–1977.
18. Guo D, Tan FK, Cantu A, Plon SE, Milewicz DM. FBN1 exon 2 splicing error in a patient with Marfan syndrome. *Am J Med Genet* 2001;101:130–134.
19. Dietz HC, Valle D, Francomano CA, Kendzior RJ Jr, Pyeritz RE, Cutting GR. The skipping of constitutive exons *in vivo* induced by nonsense mutations. *Science* 1993;259:680–683.
20. Liu W, Qian C, Comeau K, Brenn T, Furthmayr H, Francke U. Mutant fibrillin-1 monomers lacking EGF-like domains disrupt microfibril assembly and cause severe marfan syndrome. *Hum Mol Genet* 1996;5:1581–1587.
21. Schrijver I, Liu W, Brenn T, Furthmayr H, Francke U. Cysteine substitutions in epidermal growth factor-like domains of fibrillin-1: distinct effects on biochemical and clinical phenotypes. *Am J Hum Genet* 1999;65:1007–1020.
22. Karttunen L, Ukkonen T, Kainulainen K, Syvänen AC, Peltonen L. Two novel fibrillin-1 mutations resulting in premature termination codons but in different mutant transcript levels and clinical phenotypes. *Hum Mutat* 1998(suppl 1):S34–S37.
23. Hilhorst-Hofstee Y, Hamel BC, Verheij JB, et al. The clinical spectrum of complete FBN1 allele deletions. *Eur J Hum Genet* 2011;19:247–252.
24. Hutchinson S, Furger A, Halliday D, et al. Allelic variation in normal human FBN1 expression in a family with Marfan syndrome: a potential modifier of phenotype? *Hum Mol Genet* 2003;12:2269–2276.
25. Loeys BL, Chen J, Neptune ER, et al. A syndrome of altered cardiovascular, craniofacial, neurocognitive and skeletal development caused by mutations in TGFBR1 or TGFBR2. *Nat Genet* 2005;37:275–281.
26. Holm TM, Habashi JP, Doyle JJ, et al. Noncanonical TGFβ signaling contributes to aortic aneurysm progression in Marfan syndrome mice. *Science* 2011;332:358–361.
27. Brooke BS, Habashi JP, Judge DP, Patel N, Loeys B, Dietz HC 3rd. Angiotensin II blockade and aortic-root dilation in Marfan's syndrome. *N Engl J Med* 2008;358:2787–2795.
28. Nataatmadja M, West J, Prabowo S, West M. Angiotensin II receptor antagonism reduces transforming growth factor beta and Smad signaling in thoracic aortic aneurysm. *Ochsner J* 2013;13:42–48.
29. Comeglio P, Evans AL, Brice G, Cooling RJ, Child AH. Identification of FBN1 gene mutations in patients with ectopia lentis and marfanoid habitus. *Br J Ophthalmol* 2002;86:1359–1362.
30. Hayward C, Porteous ME, Brock DJ. Mutation screening of all 65 exons of the fibrillin-1 gene in 60 patients with Marfan syndrome: report of 12 novel mutations. *Hum Mutat* 1997;10:280–289.
31. Nijbroek G, Sood S, McIntosh I, et al. Fifteen novel FBN1 mutations causing Marfan syndrome detected by heteroduplex analysis of genomic amplicons. *Am J Hum Genet* 1995;57:8–21.
32. Hung CC, Lin SY, Lee CN, et al. Mutation spectrum of the fibrillin-1 (FBN1) gene in Taiwanese patients with Marfan syndrome. *Ann Hum Genet* 2009;73(Pt 6):559–567.
33. Hewett DR, Lynch JR, Smith R, Sykes BC. A novel fibrillin mutation in the Marfan syndrome which could disrupt calcium binding of the epidermal growth factor-like module. *Hum Mol Genet* 1993;2:475–477.
34. Mátyás G, De Paepe A, Halliday D, Boileau C, Pals G, Steinmann B. Evaluation and application of denaturing HPLC for mutation detection in Marfan syndrome: Identification of 20 novel mutations and two novel polymorphisms in the FBN1 gene. *Hum Mutat* 2002;19:443–456.
35. Youil R, Toner TJ, Bull E, et al. Enzymatic mutation detection (EMD) of novel mutations (R565X and R1523X) in the FBN1 gene of patients with Marfan syndrome using T4 endonuclease VII. *Hum Mutat* 2000;16:92–93.
36. Tiecke F, Katzke S, Booms P, et al. Classic, atypically severe and neonatal Marfan syndrome: twelve mutations and genotype-phenotype correlations in FBN1 exons 24-40. *Eur J Hum Genet* 2001;9:13–21.
37. Halliday D, Hutchinson S, Kettle S, Firth H, Wordsworth P, Handford PA. Molecular analysis of eight mutations in FBN1. *Hum Genet* 1999;105:587–597.
38. Halliday DJ, Hutchinson S, Lonie L, et al. Twelve novel FBN1 mutations in Marfan syndrome and Marfan related phenotypes test the feasibility of FBN1 mutation testing in clinical practice. *J Med Genet* 2002;39:589–593.
39. Körkkö J, Kaitila I, Lönnqvist L, Peltonen L, Ala-Kokko L. Sensitivity of conformation sensitive gel electrophoresis in detecting mutations in Marfan syndrome and related conditions. *J Med Genet* 2002;39:34–41.
40. Stheneur C, Collod-Béroud G, Faivre L, et al. Identification of the minimal combination of clinical features in probands for efficient mutation detection in the FBN1 gene. *Eur J Hum Genet* 2009;17:1121–1128.