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Effect of Mutation Type and Location on Clinical Outcome in 1,013 Probands with Marfan Syndrome or Related Phenotypes and *FBN1* Mutations: An International Study

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Mutations in the fibrillin-1 (*FBN1*) gene cause Marfan syndrome (MFS) and have been associated with a wide range of overlapping phenotypes. Clinical care is complicated by variable age at onset and the wide range of severity of aortic features. The factors that modulate phenotypical severity, both among and within families, remain to be determined. The availability of international *FBN1* mutation Universal Mutation Database (UMD-*FBN1*) has allowed us to perform the largest collaborative study ever reported, to investigate the correlation between the *FBN1* genotype and the nature and severity of the clinical phenotype. A range of qualitative and quantitative clinical parameters (skeletal, cardiovascular, ophthalmologic, skin, pulmonary, and dural) was compared for different classes of mutation (types and locations) in 1,013 probands with a pathogenic *FBN1* mutation. A higher probability of ectopia lentis was found for patients with a missense mutation substituting or producing a cysteine, when compared with other missense mutations. Patients with an *FBN1* premature termination codon had a more severe skeletal and skin phenotype than did patients with an inframe mutation. Mutations in exons 24–32 were associated with a more severe and complete phenotype, including younger age at diagnosis of type I fibrillinopathy and higher probability of developing ectopia lentis, ascending aortic dilatation, aortic surgery, mitral valve abnormalities, scoliosis, and shorter survival; the majority of these results were replicated even when cases of neonatal MFS were excluded. These correlations, found between different mutation types and clinical manifestations, might be explained by different underlying genetic mechanisms (dominant negative versus haploinsufficiency) and by consideration of the two main physiological functions of fibrillin-1 (structural versus mediator of TGFβ signalling). Exon 24–32 mutations define a high-risk group for cardiac manifestations associated with severe prognosis at all ages.

Marfan syndrome (MFS [MIM 154700]) is a connective-tissue disorder, with autosomal dominant inheritance and a prevalence of 1 in 5,000–10,000 individuals.[1] The cardinal features of MFS involve the ocular, cardiovascular, and skeletal systems.[2] The skin, lung, and dura may also be involved. MFS is notable for its variability in age at onset, tissue distribution, and severity of clinical manifestations, both among and within affected families. Because of the high population frequency and the nonspecific nature of many of the clinical findings for MFS, clinical diagnostic criteria for this disorder have been established,[3–4] the latest being the Ghent criteria, which superseded the previous so-called Berlin criteria.

Study of the molecular determinants of phenotypical variations in MFS has been possible only since the identification of the causative fibrillin-1 (*FBN1*) gene[5] (MIM 134797). Fibrillin-1 has a modular structure, with 47 repeats of six-cysteine epidermal-growth-factor (EGF)–like motifs, 43 of which are of the calcium-binding (cb) type (cb-EGF).[6] Fibrillin-1 monomers associate to form com-

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plex extracellular macroaggregates, termed "microfibrils," which are important for the integrity and homeostasis of both elastic and nonelastic tissues.[7–8] The protein also contains seven eight-cysteine motifs, which bear homology to motifs found in the latent transforming-growth-factor beta–binding proteins (TGFβ-BPs), and a proline-rich region. The relationship between *FBN1* and TGFβ signaling has been underscored by the identification of mutations in *TGFBR2* (MIM 190182) in patients with MFS and Marfan-like conditions[9] and in pathological studies in knockin and knockout mice.[10] Indeed, fibrillins and TGFβ-BPs constitute a family of structurally related and interacting proteins. In MFS mouse models, deficiency of fibrillin-1 alters matrix sequestration of the large latent complex of TGFβ, rendering the cytokine more prone for activation.[10] Recently, a specific fibrillin-1 sequence encoded by exons 44–49 has been shown to regulate the bioavailability of endogenous TGFβ1.[11]

Two major mutation categories—premature termination codons (PTCs) and inframe mutations—have been reported in the *FBN1* gene.[12] A total of 559 pathogenic mutations were reported in the last update of the Universal Marfan Database (UMD)–*FBN1,*[12] and most of these are unique to individual families. Two-thirds are missense mutations, the majority of which are cysteine substitutions.

Besides classic MFS, mutations in *FBN1* have been associated with a broad spectrum of phenotypes, including neonatal MFS,[13] isolated ectopia lentis[14] (MIM 129600), isolated ascending aortic aneurysm and dissection,[15] isolated skeletal features,[16,17] and Weill-Marchesani syndrome[18] (MIM 608328). So far, genotype-phenotype correlations in *FBN1* have been weak except for the cluster of mutations in exons 24–32 reported in neonatal MFS.[13,19–22]

Indeed, previous reported studies investigating genotype-phenotype correlations were performed with a maximum of 101 patients.[23–27] Those authors compared patients with mutations leading to a PTC versus patients with missense mutations, as well as subjects with missense mutations involving a cysteine versus individuals with other missense mutations. Moreover, they focused on major cardiac, ocular, and skeletal involvement. Differences between the groups, with regard to patients' age at follow-up, were not taken into account.

Large sets of both clinical and molecular data are needed to study (1) the association between *FBN1* mutation type and severity of the disease and (2) the specificity of organ involvement in relation to a mutation type. The international UMD-*FBN1,* set up in 1995, provides these data for 1,013 probands with known *FBN1* mutations who were recruited from specialized MFS clinics all over the world. We report the results of the statistical analysis of these data. These results provide possible clues into pathophysiological processes.

Patients, Material, and Methods

Patients

A total of 1,191 probands who had received a diagnosis of MFS or another type I fibrillinopathy were identified between 1995 and 2005 via the framework of the UMD-*FBN1*[12] and participating centers. The inclusion criteria in our study were (1) the presence of a heterozygous pathogenic *FBN1* gene mutation and (2) the availability of clinical information.

Overall, 178 patients (15%) had to be excluded from the study (no clinical data available for 129; insufficient data about cardiac, ocular, or skeletal involvement for 44; two different mutations on the same allele for 4; and compound heterozygosity for *FBN1* mutations for 1). The 1,013 patients included in our study originated from 38 countries on five continents. The majority (72%) were white Europeans or were of European ancestry, 14% were from North and South America, 8% were from Oceania, 4% were from Asia, and 2% were from Africa. Table 1 summarizes the participation of the different laboratories in the study. Patient age at inclusion ranged from birth to 72 years. The clinical data were collected mainly from standardized questionnaires sent to the referring physician, and a minority were from previous publications that reported sufficient clinical data. The clinical information included a range of qualitative and quantitative clinical parameters, including age at diagnosis of MFS or another type I fibrillinopathy and the presence or absence of clinical features including cardiac, ophthalmologic, skeletal, dermal, pulmonary, and dural manifestations. The age at diagnosis and at surgery for aortic dilatation, ectopia lentis, and scoliosis was also noted. All questionnaires were collected by one individual (L.F.) to rule out duplication of patients in the study. To avoid bias as a result of familial clustering, affected family members of a proband were not included in the analysis.

The pathogenic nature of a putative mutation was assessed using recognized criteria. In brief, all nonsense mutations, all deletions or insertions (in or out of frame) were considered pathogenic; for all splice mutations, the wild-type and mutant strength values of the splice sites were compared using genetic algorithms,[28–30] and only mutations displaying significant deviation from the norm were included. Missense mutations were considered pathogenic when at least one of the following features was found: (1) de novo missense mutation, (2) missense mutation substituting or creating a cysteine, (3) missense mutation involving a consensus cb residue,[21] (4) substitution of glycines implicated in correct domain-domain packing,[31] and (5) intrafamilial segregation of a missense mutation involving a conserved

Table 1. Number of Patients Recruited to the Study and Their Laboratory of Origin

Origin	No. of Patients (Laboratories)
Belgium	167 (1)
United Kingdom	166 (7)
Germany	156 (4)
France	154 (3)
United States	146 (8)
Italy	89 (2)
Australia	80 (1)
Asia	22 (6)
Other European countries	22 (3)
Other North and South American countries	11 (2)
Total	1,013 (37)

Table 2. Frequency of Clinical Features in the Different Systems Involved in MFS and Type I Fibrillinopathies ($N = 1,013$)

System and Clinical Feature(s)	No. of Events	No. of Available Data	Percentage
Skeletal:			
Arachnodactyly	751	969[a]	78
Dolichostenomelia	522	947[a]	55
Joint laxity	600	956[a]	63
Scoliosis	508	965[a]	53
Pectus deformity[b]	570	962[a]	59
Limited elbow extension	153	974[a]	16
Protrusio acetabulae	69	298[a]	23
Facial dysmorphism	443	913[a]	49
High-arched palate	639	932[a]	69
Dental malocclusion	372	843[a]	44
Pes planus	402	864[a]	47
Orthopedic surgery	113	983[a]	12
Major skeletal involvement	327	1,013	32
Minor skeletal involvement	564	1,013	56
Ocular:			
Ectopia lentis	542	1,013	54
Myopia	453	865	52
Cataract	39	983	4
Retinal detachment	65	980	7
Glaucoma	19	905	2
Surgery for ectopia lentis	122	910	13
Other eye surgeries	43	905	5
Major eye involvement	542	1,013	54
Cardiac:			
Dilatation of the ascending aorta	775	1,013	77
Dissection of the ascending aorta	145	1,013	14
Dilatation or dissection of the descending or abdominal aorta before age 50 years	66	1,013	7
Mitral valve prolapse	533	983	54
Mitral regurgitation	313	959	33
Aortic insufficiency	205	975	21
Aortic surgery	282	1011	28
Isolated valvular surgery	45	1004	4
Major cardiac involvement	776	1,013	77
Minor cardiac involvement	108	1,013	11
Skin:			
Striae	444	945	47
Herniae	96	988	10
Minor skin involvement	480	1,013	47
Lung:			
Pneumothorax	73	1,002	7
Minor lung involvement	73	1,013	7
CNS:			
Dural ectasia	154	292	53
Major CNS involvement	154	1,013	15

[a] Nineteen patients were classified as having minor, major, or neither minor nor major skeletal features, but details about their skeletal manifestations were not available.

[b] Includes pectus excavatum, 246 (26%) of 962; pectus carinatum, 288 (30%) of 962; and undefined pectus malformation.

amino acid. For 38 mutations not displaying one of the above features, additional data provided by SIFT,[32,33] BLOSUM-62,[34] and biochemical values (Kristine Yu's Web site) were gathered and analyzed using a new UMD tool (M. Frédéric, C. Boileau, D. Hamroun, M. Lalande, M. Claustres, C. Béroud, G. Collod-Béroud, unpublished data).

Involvement of Different Organ Systems

The proportion of each specific clinical feature or system involved was compared in the different groups of mutation types or locations. The following were each considered as a system: the skeleton, the eye, the heart, the skin, and the dura. The clinical features of each system are listed in table 2. No attempts were made to incorporate dilatation of the pulmonary artery, calcification of the mitral valve annulus, apical blebs, flat cornea and iris, or ciliary muscle hypoplasia in the analyses, since those phenotypes were rarely evaluated. Similarly, the axial globe length had rarely been measured, and the definition of myopia varied widely from center to center. For this reason, myopia of any degree was included. In consequence, the presence or absence of minor oph-

thalmological involvement could not be assessed. The ages at diagnosis and at surgery were collected for scoliosis, ectopia lentis, and aortic dilatation or dissection. The probability of surgery for ectopia lentis was studied only in the group of patients with ectopia lentis. Similarly, the probability of aortic dissection and surgery for aortic dilatation or dissection was studied only in the group of patients with aortic dilatation. The number of systems involved was also assessed according to the Ghent nosology.[4] Patients were classified as having MFS if the diagnostic Ghent nosology criteria were met, including the presence of an *FBN1* mutation as a major feature and, in a second step, excluding the presence of an *FBN1* mutation as a major feature. All other patients were considered as displaying a type I fibrillinopathy. For the purpose of this study and in the absence of consensus diagnostic features, patients were classified as having neonatal MFS when severe features of MFS, including severe valvular insufficiencies, were present before age 4 wk.

Mutation Screening

Mutation screening, with the consent of the patient or a guardian, was performed in the 38 different laboratories by use of SSCP analysis, denaturing high-performance liquid chromatography, heteroduplex analysis, long-range RT-PCR, or direct sequencing of RNA extracted from cell lines or of genomic DNA from peripheral-blood samples. PTC mutations were classified as those that would be likely to produce no or a truncated FBN1 protein (frameshifts, stop codons, and out-of-frame splice mutations), whereas inframe mutations were classified as missense mutations, inframe deletions/duplications, or inframe splice mutations. Twenty-nine splice mutations could not be classified and were therefore excluded from the analyses that compared types of mutations.

Statistical Analysis

The frequency of many features of MFS increases with age. Since the patients had different lengths of follow-up, χ^2 tests are not appropriate for comparing clinical features between groups. Thus, we used a time-to-event analysis technique to estimate a reliable cumulative probability of observing the different manifestations of MFS. This technique could be applied for the following events: diagnosis of type I fibrillinopathy and diagnosis of scoliosis, ectopia lentis, and/or aortic dilatation or dissection, as well as surgery for these different manifestations, for which the ages at diagnosis were systematically collected. In all time-to-event analyses, the baseline date (time zero) was the date of birth. The time-to-event diagnosis was defined as the interval between the baseline date and the date of first observation of the event. Subjects who did not manifest the studied event during the follow-up course were censored at their last follow-up. Subjects for whom the age at diagnosis of a specific manifestation was not available were excluded from these analyses (a maximum of 4% of patients). The number of observations for each clinical feature is indicated in table 2. The Kaplan-Meier method[35] was used to estimate the cumulative probabilities of clinical manifestations of the disease at ages 10, 25, and 40 years, to describe the diagnosis of clinical features over time. Clinical differences among the different mutation groups (different locations or types of mutation) were tested using the nonparametric log-rank test. Overall survival was also described and compared, using the same method, according to the type/location of the mutation. To underline the importance of taking into account the time to diagnosis of clinical features, we compared the results obtained for aortic dilatation using a χ^2 test and the time-to-event technique.

For the other features (skeletal features other than scoliosis, skin, lung, and dural involvement) for which the age at diagnosis was not collected, age at last follow-up was the only information available about the time of observation of clinical features. To indirectly take into account the patient's length of follow-up even in this situation, we adjusted all comparisons of MFS manifestation proportions for the age at last follow-up, categorized into 10-year age groups. These adjusted comparisons were performed using the Mantel-Haenszel (MH) test.[36] Since this test is appropriate only if the relationship between the mutation type and the clinical manifestation is similar in the different strata of age at last follow-up, we checked the homogeneity between strata using the Breslow-Day χ^2 test of homogeneity.[37] If an interaction was observed, results were presented for each category of age at last follow-up. In both analyses, if no information was available for a patient's given clinical feature, he or she was excluded from the analysis of that specific lesion.

To study the effect of mutation types, we compared (1) patients with a PTC with patients with an inframe mutation, (2) patients with a particular subtype of mutation (nonsense, frameshift, splicing, missense, or inframe deletions/insertions) 2 by 2, and (3) patients with missense mutations eliminating or creating a cysteine. There was no recurrent mutation frequent enough to allow a correlation study regarding the other *FBN1* mutations. To study the consequences of mutations in different structural and functional domains, we compared (1) patients with a mutation located in an EGF domain (cb and non-cb) with those with a mutation located in a TGFβ-BP domain; (2) patients with a mutation at the 5′ end of the *FBN1* gene (exon 1–21, inclusive) with those with a mutation at the 3′ end of the gene (exon 43–65, inclusive), to take into account the regions involved in the processing of the protein; (3) patients with a mutation located within the so-called neonatal region (exons 24–32) with those with a mutation located in other exons; and (4) patients with a mutation located within the TGFβ1 regulating sequence (exons 44–49) with those with a mutation located in other exons. When locations of mutations were compared, studies were performed with all types of mutations and with missense mutations alone, to exclude a bias due to different types of mutations. Mutations located in exons 24–32 were compared with mutations located elsewhere, with and without the inclusion of neonatal MFS.

SAS software version 9.2 and Stata software version 8 were used for all statistical analyses. Only P values <.001 were considered significant, since multiple tests were performed.

Results

Mutations

A total of 803 pathogenic mutations were found in 1,013 probands (including 114 recurrent mutations in 324 probands). The distribution of mutations is presented in figure 1. Of the missense mutations, 61% (348 of 573) involved a cysteine (284 replacing and 64 creating a cysteine). Sixty-eight percent (665 of 984) were inframe, whereas 32% (319 of 984) generated a PTC (29 splicing mutations were not classified, since the consequence at the mRNA level could not be determined unambiguously). The majority of mutations were located in an EGF domain

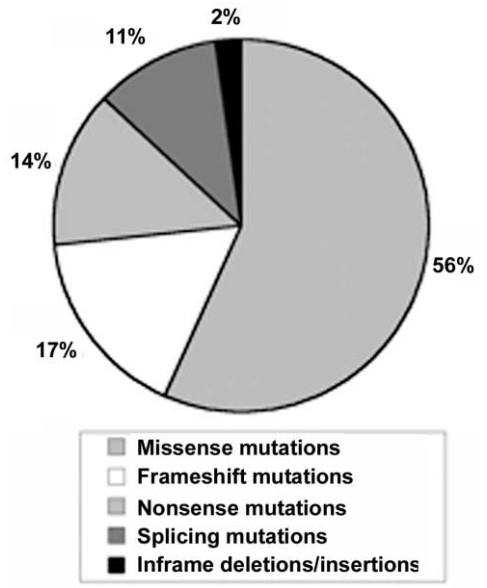


Figure 1. Types of *FBN1* mutations included in the study. Of 1,013, 573 (56%) could be classified as missense mutations, 170 (17%) as frameshift mutations, 137 (14%) as nonsense mutations, 110 (11%) as splicing mutations, and 23 (2%) as inframe deletions or insertions.

(74% [747 of 1,013])—701 of which were located in a cbEGF domain—and 15% (153 of 1,013) were located in a TGFβ-BP domain. Twenty-nine percent (293 of 1,013) of mutations were found in the 5′ region of the gene, and 379 (37%) were found in the 3′ region of the gene. Twenty percent (198 of 1,013) of mutations were located in exons 24–32. Ten percent (102 of 1,013) of mutations were located in exons 44–49. No major differences in mutation-type categories were detected between laboratories.

Phenotype in the Overall Patient Cohort

Fifty-four percent of patients were males and 46% were females. A family history of MFS was found in 52% of cases. The median age at last follow-up was 29 years (interquartile range [IQR] 15–40 years), including 322 patients aged <18 years (32%). The median age at diagnosis of type I fibrillinopathy was 20 years (IQR 9–34 years). Five percent of patients (n = 48) had a neonatal presentation. Overall, at the time of analysis, 61 (6%) had died, 31 (51%) in the context of neonatal MFS, 19 of aortic dissection, 10 during or after aortic surgery, and 1 from a cause unrelated to MFS.

The frequency of manifestations of each organ system in the full cohort of patients is listed in table 2. In particular, of the 1,013 patients, 145 (14%) had dissection of the ascending aorta, 43 (4%) had dissection of the descending aorta, and 30 (3%) had dissection of the abdominal aorta. Protrusio acetabulae and dural ectasia—although they are included in the Ghent nosology—were rarely looked for in our patients (n = 298 and n = 292, respectively). The majority (89%) of patients could be classified, according to Ghent nosology, as having MFS at their last follow-up, when the presence of an *FBN1* mutation was considered a major feature (72% when the presence of an *FBN1* mutation was not considered a major feature). Phenotypic differences depending on the sex of the proband were studied for all clinical parameters. Significant differences were found only for the cumulative probability of aortic surgery for patients with aortic dilatation. Indeed, 46% of males had surgery for aortic dilatation before or at age 40 years (99.9% CI 38%–55%) compared with 34% of females (99.9% CI 26%–50%) (P = .0002). A marginally significant result was found for the cumulative probability of ascending aortic dilatation, with a probability of 80% before or at age 40 years in males (99.9% CI 5%–84%) compared with 70% in females (99.9% CI 64%–76%) (P = .0036).

Types of Mutations

Missense mutations substituting or creating a cysteine versus other missense mutations.—The probability of ectopia lentis was significantly higher with missense mutations involving a cysteine when compared with other missense mutations (log-rank test P < .0001) (fig. 2). The cumulative probability of ectopia lentis diagnosed before or at age 25 years was 59% (99.9% CI 50%–68%) for patients with missense mutations involving a cysteine compared with 32% (99.9% CI 22%–44%) for patients with other missense mutations. Consequently, the percentage of patients with positive Ghent criteria was higher in the group of patients with missense mutations involving a cysteine when compared with other missense mutations (76% vs. 63% when the presence of an *FBN1* mutation was not considered as a major feature; MH test P = .0003).

PTC versus inframe mutations.—Patients with a PTC mutation more frequently had major skeletal involvement (40% vs. 28%; MH test P = .0008) with a higher proportion of arachnodactyly, dolichostenomelia, joint hyperlaxity, pectus deformity, high-arched palate, and pes planus. Moreover, a higher frequency of striae distensae (64% vs. 40%; MH test P < .0001) was observed in patients with a PTC mutation (fig. 3). The cumulative probability of a diagnosis of ascending aortic dilatation before or at age 40 years was 77% (99.9% CI 8%–85%) for patients with PTC mutations compared with 74% (99.9% CI 67%–80%) for patients with an inframe mutation (log-rank test P = .7791). Conversely, the cumulative probability of a diagnosis of ectopia lentis and ophthalmologic surgery was significantly lower for patients with PTC mutations compared with patients with an inframe mutation (log-rank test P < .0001 and P = .0001, respectively) (table 3 and figure 2). These results became insignificant for the age at diagnosis of ectopia lentis and ophthalmologic surgery when patients with PTC mutations were compared with patients with missense mutations not involv-

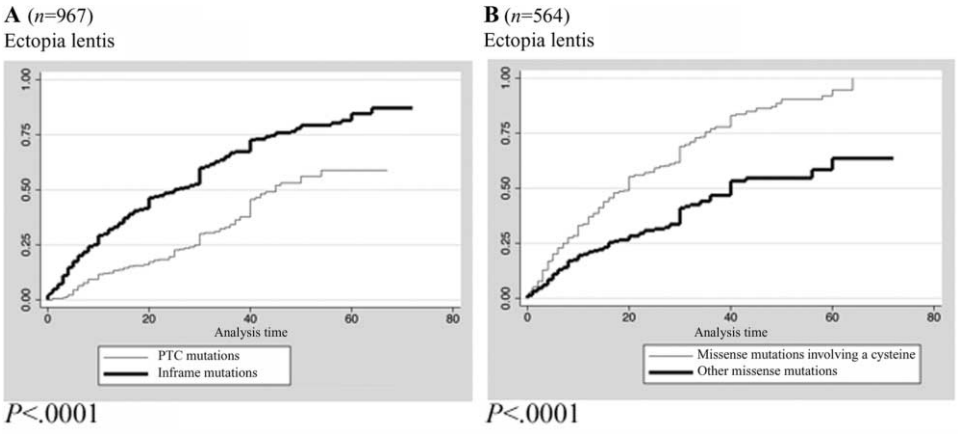


Figure 2. Kaplan-Meier analyses for the probability of ectopia lentis diagnoses for patients with different types of mutations. *A,* Probability of ectopia lentis in PTC versus inframe mutations. The cumulative probability of diagnosis of ectopia lentis before or at age 25 years was 23% (99.9% CI 15%–32%) for patients with PTC mutations (*thin line*) compared with 50% (99.9% CI 43%–57%) for patients with inframe mutations (*thick line*) (log-rank test $P < .0001$). *B,* Probability of ectopia lentis for patients with missense mutation involving a cysteine versus other missense mutations. The cumulative probability of diagnosis of ectopia lentis before or at age 25 years was 59% (99.9% CI 50%–68%) for patients with missense mutations involving a cysteine (*thin line*) compared with 32% (99.9% CI 22%–44%) for patients with other missense mutations (*thick line*) (log-rank test $P < .0001$).

ing a cysteine (log-rank test $P = .0424$ and $P = .1020$, respectively).

Other mutation subtypes.—As expected for the results of PTC mutations versus inframe mutations, a lower probability of ectopia lentis was found when nonsense or frameshift mutations were compared with missense mutations (log-rank test $P < .0001$). No significant difference was found for any manifestation when patients with nonsense mutations were compared with those with frameshift mutations or when patients with missense mutations were compared with those with splicing mutations. A higher probability of ascending aortic dilatation (log-rank test $P < .0001$) and mitral valve prolapse (log-rank test $P = .0007$) and a higher frequency of arachnodactyly and joint laxity (MH test $P = .0002$ and $P = .0006$, respectively) were found in patients with a mutation eliminating a cysteine than in patients with a mutation creating a cysteine.

Location of Mutations

Exons 24–32 versus other exons.—A neonatal onset of the disease was found in 22% ($n = 42$) of patients with a mutation in exons 24–32, compared with 0.6% ($n = 4$) of patients with a mutation in other exons (χ^2 test $P < .0001$). When patients with mutations within exons 24–32 were compared with patients with mutations in the other exons, significant differences were found for joint limitations (34% vs. 11%; MH test $P < .0001$), scoliosis, ectopia lentis, ascending aortic dilatation, aortic surgery, mitral valve abnormalities (mitral valve prolapse, mitral regurgitation, and/or mitral surgery), younger age at diagnosis of type I fibrillinopathy, and a shorter overall survival (log-rank test $P < .001$) (table 3 and fig. 4). Indeed, 76% of patients with mutations in exons 24–32 were alive at age 40 years (99.9% CI 61%–87%) compared with 98% (99.9% CI 93%–99%) of patients with mutations located in other exons (log-rank test $P < .0001$). Moreover, the cumulative probability of ascending aortic dilatation diagnosed before or at age 40 years was 87% (99.9% CI 77%–95%) for patients with a mutation in exons 24–32 compared with 72% (99.9% CI 67%–78%) for patients with a mutation in other exons (log-rank test $P < .0001$), and the cumulative probability of aortic surgery before or at age 40 years was 55% (99.9% CI 35%–77%) for patients with a mutation in exons 24–32 compared with 38% (99.9% CI 30%–48%) for patients with a mutation in other exons (log-rank test $P < .0001$). Apart from ectopia lentis, the results were similar when patients with a neonatal onset were excluded. Results were also similar when all types of mutations were included in the analysis and when only missense mutations (except for ectopia lentis) or only missense mutations involving a cysteine were included.

The distribution of types of mutations was significantly different in exons 24–32 from the distribution in the other exons, with an overrepresentation of missense mutations and an underrepresentation of nonsense mutations (table 4) (Fischer test $P = .0002$). PTC mutations within exons 24–32 were rarely associated with a neonatal MFS presentation (2 [5%] of 43) compared with inframe mutations within this region (41 [95%] of 43). A higher frequency of neonatal presentations was found for mutations in exon 25 when compared with mutations distributed in exons 24–32 (χ^2 test $P < .0001$).

Exons 44–49 versus other exons.—No significant difference was found for any clinical parameter for patients with a mutation located in the specific sequence that regulates

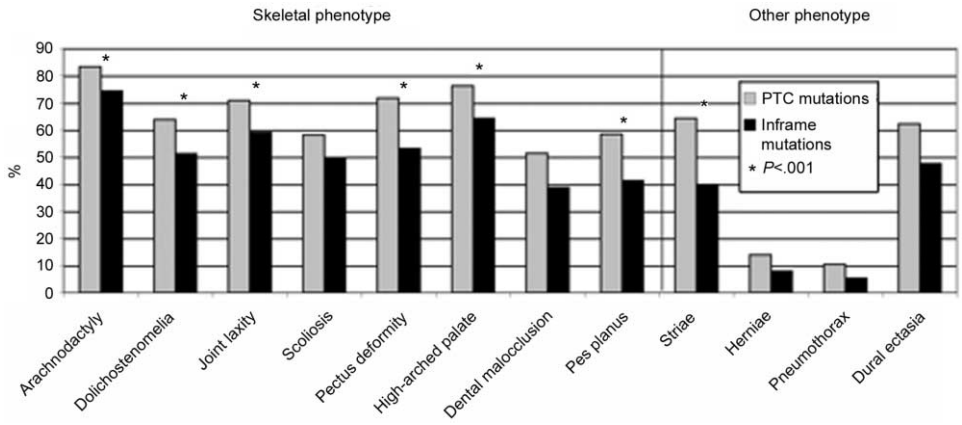


Figure 3. Frequency of skeletal, skin, pulmonary, and dural phenotypes in study participants with PTC mutations (*gray bars*), compared with those with inframe mutations (*black bars*). An asterisk (*) indicates that differences between groups were statistically significant (MH test $P < .001$).

the bioavailability of endogenous TGFβ1, compared with those with a mutation located elsewhere.

EGF/TGF β-BP domains.—No significant difference was found for any clinical parameter for patients with a mutation located in an EGF domain compared with those with a mutation in a TGFβ-BP domain, nor between patients with a mutation in a cb-EGF domain and those with a mutation in a non–cb-EGF domain. Similar results were found when all types of mutations or only missense mutations were included in the analysis.

5′ versus 3′ mutations.—Patients with a mutation located in the 5′ region of the gene had a higher probability of ectopia lentis (log-rank test $P = .001$). This result was highly significant when only missense mutations were included in the analysis (log-rank test $P < .0001$), whereas all the other results remained nonsignificant.

Discussion

FBN1 mutations have been associated with a broad spectrum of phenotypes now often called "type I fibrillinopathies," ranging from single connective-tissue manifestations, such as isolated ectopia lentis, to MFS and lethal neonatal MFS. Every patient with an *FBN1* mutation is at risk for developing severe cardiovascular, skeletal, and ophthalmologic complications (L. Faivre, G. Collod-Beroud, B. Loeys, A. Child, C. Binquet, E. Gautier, B. Callewaert, E. Arbustini, K. Mayer, M. Arslan-Kirchner, A. Kiotsekoglou, P. Comeglio, N. Marziliano, D. Halliday, C. Beroud, C. Bonithon-Kopp, M. Claustres, H. Plauchu, P. N. Robinson, L. Adès, J. De Backer, P. Coucke, U. Francke, A. De Paepe, C. Boileau, G. Jondeau, unpublished data). Here, we aimed at identifying the type or location of a given *FBN1* mutation that could be associated with the presence of a clinical feature, severity, and/or age at onset. Although no specific manifestation or set of features is pathognomonic for a particular subtype of *FBN1* mutation, the occurrence of specific organ involvement differed significantly in some instances.

The mechanism by which mutations in *FBN1* result in disease is unclear, since the biochemical pathway of fibrillin-1 assembly into microfibrils is still poorly understood and since the role of fibrillin-1 in TGFβ signaling has only recently emerged. A dominant negative model was first proposed,[38–39] in which the mutant monomer disrupts assembly of normal fibrillin-1 into microfibrils or is itself misincorporated into the microfibril. Recent studies have given evidence of a critical contribution of haploinsufficiency in the pathogenesis of MFS.[40] Different effects on trafficking have also been demonstrated, with some mutations acting as dominant negative and others as haploinsufficient.[41] Here, our data suggest that both genetic mechanisms are involved and that their tissue distribution may differ.

The first striking result of this study is the strong correlation found between ectopia lentis and the presence of a mutation affecting a cysteine residue, confirming earlier conclusions on a smaller sample.[24–27,42] It is noteworthy that phenotypes strongly overlapping with type I fibrillinopathies are associated with mutations in *TGFBR1/2* and thus altered TGFβ signaling. However, the main feature of these phenotypes, as compared with type I fibrillinopathies, is the almost consistent absence of ocular involvement.[43] Thus, it could be speculated that the functional aspect of fibrillin-1 that is altered in patients with ectopia lentis is not involved in TGFβ signaling but is a structural function in the extracellular matrix. Our data suggest that correct cysteine localization and disulfide bonding play an important role in the structural integrity of the suspensory ligaments of the lens, itself relying on the structural function of fibrillin-1 in this organ.[44] Also, in the subgroup of patients with a mutation affecting a cysteine residue, we found a significantly higher probability of ascending aortic dilatation and mitral valve pro-

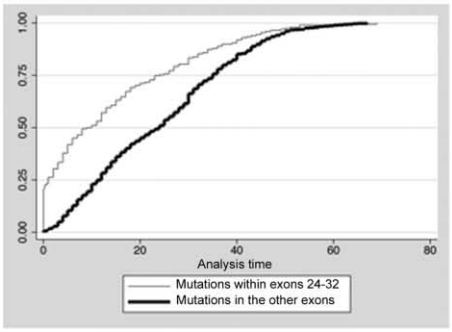
Table 3. Comparison of Probabilities of Clinical-Feature Diagnosis at a Specific Age for Patients with MFS and Other Type I Fibrillinopathies, According to the Type or Location of *FBN1* Mutations

Clinical Feature	*n*	Frequency (%)	Probabilities of Clinical Features Diagnosis (%) at Age 10	25	40	*n*	Frequency (%)	Probabilities of Clinical Features Diagnosis (%) at Age 10	25	40	*P*[a]
	PTC Mutations					Inframe Mutations					
Age at diagnosis of type I fibrillinopathy	319	100.0	20.4	51.7	85.2	665	100.0	32.3	61.2	86.9	.0103
Ascending aortic dilatation	259	81.2	10.6	38.7	76.9	491	73.8	20.2	43.5	73.8	.7791
Aortic dissection in the population presenting with ascending aortic dilatation	65	25.1	.0	3.6	31.1	78	15.9	.2	5.1	22.9	.2014
Aortic surgery in the population presenting with ascending aortic dilatation	102	39.4	.0	9.4	42.0	147	29.9	1.8	12.2	40.4	.5301
Survival	303	95.0	99.7	98.8	95.1	43	6.5	95.5	94.4	93.2	.2311
	Missense Mutations Involving a Cysteine					Other Missense Mutations					
Age at diagnosis of type I fibrillinopathy	348	100.0	32.8	62.6	89.1	225	100.0	28.9	57.8	82.2	.0983
Ascending aortic dilatation	262	75.3	21.3	46.3	75.6	158	70.2	17.6	37.4	69.4	.0797
Aortic dissection in the population presenting with ascending aortic dilatation	41	15.6	.0	4.6	29.9	25	15.8	.7	5.2	10.8	.4249
Aortic surgery in the population presenting with ascending aortic dilatation	80	30.5	1.3	14.0	47.4	49	31.0	2.7	10.4	29.0	.2712
Survival	330	94.8	96.5	96.1	94.5	14	6.2	96.0	93.5	93.5	.6785
	Exons 24–32					Other Exons					
Age at diagnosis of type I fibrillinopathy	198	100.0	51.0	75.8	91.9	815	100.0	23.1	53.7	85.2	<.0001
Ascending aortic dilatation	164	82.8	42.5	65.7	87.3	609	74.7	11.3	36.4	72.4	<.0001
Aortic dissection in the population presenting with ascending aortic dilatation	21	12.8	.7	5.8	28.5	124	20.4	.2	4.3	25.7	.3064
Aortic surgery in the population presenting with ascending aortic dilatation	52	31.7	4.7	17.5	55.2	201	33.0	.5	9.9	38.4	<.0001
Survival	159	80.3	84.5	81.1	76.1	22	2.7	99.6	99.1	97.5	<.0001

NOTE.—All ages are in years. Results are Kaplan-Meier estimates.

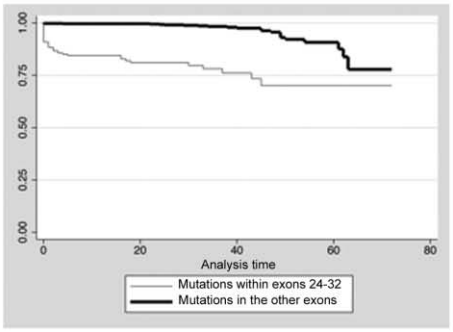
[a] Log-rank test *P* values were for PTC mutations versus inframe mutations, for missense mutations involving a cysteine versus other missense mutations, or for mutations within exons 24–32 versus mutations in other exons.

A (*n*=1,013)
Age at diagnosis of type I fibrillinopathy



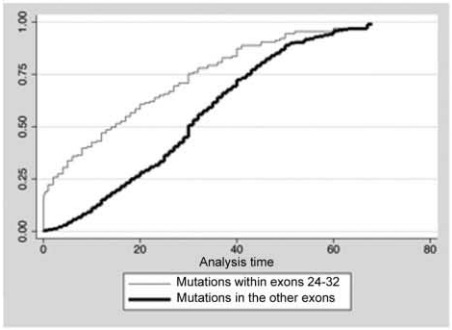
P<.0001

B (*n*=1,013)
Survival



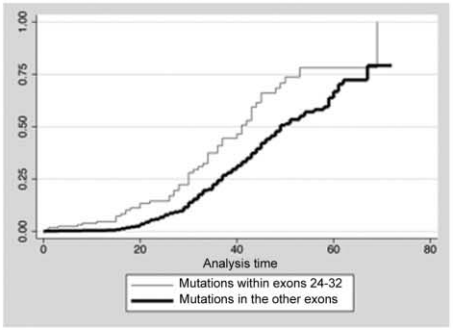
P<.0001

C (*n*=1,011)
Ascending aortic dilatation



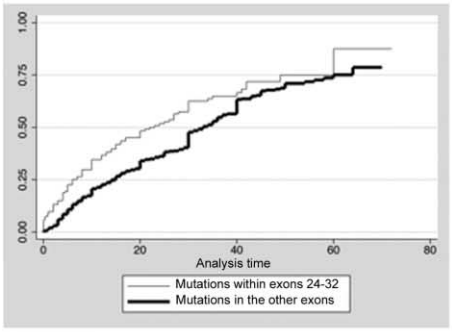
P<.0001

D (*n*=773)
Ascending aortic surgery



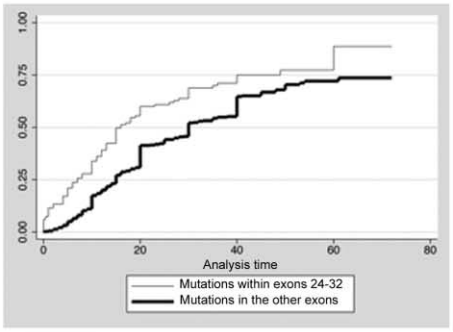
P<.0001

E (*n*=996)
Ectopia lentis



P<.0001

F (*n*=954)
Scoliosis



P<.0001

lapse and a higher frequency of arachnodactyly and joint laxity, when comparing patients with a mutation eliminating a cysteine with those with a mutation creating a cysteine. Therefore, it seems that the disappearance of a conserved cysteine residue implicated in a disulfide bond leads to a more severe disorganization of a given module than does the introduction of a new and supernumerary cysteine residue.

The second striking result of our study is the strong correlation between *FBN1* PTC mutation and severe skeletal and skin phenotypes. Contrary to what is described in the preceding paragraph, mutations in *TGFBR1/2* are associated with skeletal and sometimes skin alterations highly comparable to those found in patients with *FBN1*. Thus, it is expected that a function or pathway common to fibrillin-1 and the TGFβ type 1/2 receptors is altered in these patients. It could be speculated that haploinsufficiency for fibrillin-1 in bone and skin has a stronger effect on the TGFβ signaling function of the protein than on its structural function—and thus that, in bone growth, fibrillin-1 acts as a mediator of TGFβ signaling. The different correlations found for skeletal and skin manifestations on the one hand versus the ocular system on the other hand might then be explained by differences in the composition and function of fibrillin-rich microfibrils in different organs and further underscore the complexity of the composition of microfibrils and their interactions within tissues. Patients with a mutation in exons 24–32 had a more severe and complete phenotype, including younger age at diagnosis and higher probability of scoliosis, ectopia lentis, ascending aortic dilatation, aortic surgery, mitral valve abnormalities, and shorter survival. However, patients with aortic dilatation and a mutation in exons 24–32 did not present a higher probability of aortic dissection than did patients with aortic dilatation and a mutation located elsewhere. These data can be explained in part by a higher probability of aortic surgery in such patients. It can also be postulated that, because of the general severity of the phenotype, type I fibrillinopathy was diagnosed before the occurrence of aortic dissection in patients with a mutation in exons 24–32. Since the majority of these results were replicated even when neonatal MFS cases were excluded, we conclude that patients with a mutation in exons 24–32 have a poorer prognosis, with earlier onset of morbidity than in patients with a mutation located elsewhere. The presence of a mutation in this region appears to be the best indicator of early-onset aortic risk. More than a "neonatal region," it should be considered a "severe region." This result can be explained by the role of these exons in the central stretch of contiguous EGF-like domains and their importance for alignment and stability of the 10-nm microfibrils in the extracellular matrix.[45] The distribution of the mutation types in exons 24–32 is different from the distribution found in other exons of the gene. Mutations leading to PTC are significantly underrepresented, contrasting with an overrepresentation of inframe mutations. In particular, nonsense mutations have never to our knowledge been described in association with neonatal MFS, and this observation may be of importance

Table 4. Types of Mutations Found in Exons 24–32, Compared with Mutations in the Other Exons

Mutation	No. (%) of Mutations in Exons 24–32 (n = 198)	No. (%) of Mutations in Other Exons (n = 815)
Nonsense	13 (6.6)	124 (15.2)
Frameshift	27 (13.6)	143 (17.5)
Splicing	15 (7.6)	95 (11.7)
Missense	139 (70.2[a])	434 (53.3[b])
Inframe deletion/insertion	4 (2.0)	19 (2.3)

NOTE.—Global Fischer exact test for differences was used, according to the location of mutations. P = .0002.

[a] 54% involving a cysteine.

[b] 63% involving a cysteine.

Figure 4. Kaplan-Meier analyses for the probability of MFS clinical-features diagnosis for patients with different locations of mutations. *A,* Age at diagnosis of type I fibrillinopathy with a mutation in exons 24–32 versus in other exons. Fifty percent of patients with a mutation in exons 24–32 (*thin line*) received a diagnosis at age 9 years (IQR 1–24 years) versus age 24 years (IQR 12–35 years) of patients with a mutation in other exons (*thick line*) (log-rank test $P < .0001$). *B,* Survival of patients with mutations in exons 24–32 versus in other exons. Seventy-six percent of patients with mutations within exons 24–32 (*thin line*) were alive at age 40 years (99.9%CI 61%–87% years) compared with 98% (99.9% CI 93%–99%) of patients with mutations located in other exons (*thick line*) (log-rank test $P < .0001$). *C,* Probability of diagnosing a dilatation of the ascending aorta for patients with mutations in exons 24–32 versus in other exons. The cumulative probability of diagnosis of ascending aortic dilatation before or at age 40 years was 87% (99.9% CI 77%–95%) for patients with mutations in exons 24–32 (*thin line*) compared with 72% (99.9% CI 67%–78%) for patients with mutations in other exons (*thick line*) (log-rank test $P < .0001$). *D,* Probability of aortic surgery for patients with mutations in exons 24–32 versus in other exons. The cumulative probability of aortic surgery before or at age 40 years was 55% (99.9% CI 35%–77%) for patients with mutations in exons 24–32 (*thin line*) compared with 38% (99.9% CI 30%–48%) for patients with mutations in other exons (*thick line*) (log-rank test $P < .0001$). *E,* Probability of ectopia lentis for patients with mutations in exons 24–32 versus in other exons. The cumulative probability of ectopia lentis diagnosis before or at age 25 years was 53% (99.9% CI 39%–67%) for patients with mutations in exons 24–32 (*thin line*) compared with 38% (99.9% CI 33%–44%) for patients with mutations in other exons (*thick line*) (log-rank test $P = .0003$). *F,* Probability of scoliosis for patients with mutations in exons 24–32 versus in other exons. The cumulative probability of scoliosis diagnosis before or at age 25 years was 61% (99.9% CI 47%–75%) for patients with mutations in exons 24–32 (*thin line*) compared with 44% (99.9% CI 38%–51%) for patients with mutations in other exons (*thick line*) (log-rank test $P < .0001$).

in elucidating the pathogenetic role of the exon 24–32 region.

In this study, we sought to avoid the main bias inherent in our study design. Clinical/molecular correlations are complicated by a wide age range in individuals. In younger patients, the clinical phenotype and symptoms may not be fully developed. Indeed, incorrect significant results can be obtained when χ^2 tests are used, and the use of the Kaplan-Meier approach allowed us to take into account the heterogeneity of the length of follow-up among groups, as well as the young median age of the patients in the cohort. Since age at lesion onset cannot be assessed for MFS, notably for aortic dilatation, we used the ages at which each main clinical manifestation was discovered. We considered only probands, to minimize the possible influence of early medical interventions in relatives (earlier monitoring and earlier preventive therapy with β-blockers) and to avoid overrepresentation of a mutation. Finally, we also excluded patients (4%) for whom no information about one of the major systems of MFS (cardiac, ocular, and skeletal system) was available. The low number of excluded patients had no significant impact on our results but provided a homogeneous study population, whatever the clinical feature investigated. However, it should be noted that the majority of patients were of European origin, thus the conclusions may not be totally applicable to all ethnic groups. Another aspect of the proband collection is the high frequency of de novo mutations. It is now well documented that the yield in mutation screening is highest in probands displaying an MFS phenotype diagnosed using the Ghent nosology.[24] Furthermore, molecular confirmation of an apparent de novo event is important for genetic counseling in at-risk relatives. Therefore, the important number of de novo mutations found in the probands does not reveal a higher-than-reported mutation rate but reflects practices and screening results from the contributing centers worldwide.

A few studies have addressed the question of a genotype-phenotype correlation in *FBN1* carriers. Only five included a sufficient number of patients (101, 93, 57, 81 and 76 patients)[23–27] to draw conclusions. A simple χ^2 approach was used, and probands as well as their affected family members were considered in two of the five series. Authors mainly compared the different phenotypes related to PTC mutations with those of cysteine substitutions. A significantly higher frequency of ectopia lentis associated with cysteine substitutions when compared with PTC mutations was a consistent finding. A tendency toward a more severe skeletal phenotype[23–24] and a more severe cardiac phenotype[23,27] in the PTC group was discussed, but with inconsistency in significant results. This could be explained partly by the small sample sizes of the populations studied. The protein phenotypes were studied only by Schrijver et al.[23] A preponderance of probands with PTC mutations had markedly reduced extracellular fibrillin deposition with reduced synthesis, whereas individuals with cysteine substitutions had normal levels of fibrillin synthesis and markedly reduced matrix deposition. Genotype-phenotype correlations in type I fibrillinopathies have also been complicated by clinical heterogeneity among individuals with the same mutation, within and among families.[46–48] The type or location of a mutation alone cannot explain these variations. The existence of genetic or environmental modifiers, as well as the susceptibility of microfibrillar matrices to proteolytic degradation, or intrafamilial variation in *FBN1* expression have been postulated.[40,49]

In conclusion, our results show a strong correlation between ectopia lentis and the presence of a mutation affecting a cysteine residue, whatever its location within the protein. Conversely, PTC mutations are associated with severe skeletal and skin phenotypes. These correlations found between different mutation types and clinical manifestations may indicate different underlying pathophysiologic mechanisms, both genetic (dominant negative vs. haploinsufficiency) and functional (structural function of fibrillin-1 vs. mediator of TGFβ signaling). Finally, we show that the location of a mutation within the exon 24–32 region is associated with a severe prognosis, not only in newborns but at all ages. However, we believe that these results cannot be used for individual prognosis but show that aortic monitoring is warranted in every patient with an *FBN1* mutation.

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Web Resources

The URLs for data presented herein are as follows:

Kristine Yu's Web site, http://cmgm.stanford.edu/biochem218/Projects%202001/Yu.pdf (for paper entitled "Theoretical Determination of Amino Acid Substitution Groups Based on Qualitiative Physicochemical Properties")

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim/ (for MFS, *FBN1, TGFBR2,* isolated ectopia lentis, and Weill-Marchesani syndrome)

UMD, http://www.umd.be/

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