Am. J. Hum. Genet. 64:993–1001, 1999

Recurrence of Marfan Syndrome as a Result of Parental Germ-Line Mosaicism for an *FBN1* Mutation

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Summary

Mutations in the *FBN1* gene cause Marfan syndrome (MFS), a dominantly inherited connective tissue disease. Almost all the identified *FBN1*mutations have been family specific, and the rate of new mutations is high. We report here a de novo *FBN1*mutation that was identified in two sisters with MFS born to clinically unaffected parents. The paternity and maternity were unequivocally confirmed by genotyping. Although one of the parents had to be an obligatory carrier for the mutation, we could not detect the mutation in the leukocyte DNA of either parent. To identify which parent was a mosaic for the mutation we analyzed several tissues from both parents, with a quantitative and sensitive solid-phase minisequencing method. The mutation was not, however, detectable in any of the analyzed tissues. Although the mutation could not be identified in a sperm sample from the father or in samples of multiple tissue from the mother, we concluded that the mother was the likely mosaic parent and that the mutation must have occurred during the early development of her germ-line cells. Mosaicism confined to germ-line cells has rarely been reported, and this report of mosaicism for the *FBN1* mutation in MFS represents an important case, in light of the evaluation of the recurrence risk in genetic counseling of families with MFS.

Introduction

Marfan syndrome (MFS; MIM 154700) is a connective tissue disorder, which in classic form affects ocular, cardiovascular, and skeletal systems. The diagnosis of MFS is given on the basis of clinical and genetic criteria proposed by an international expert committee (De Paepe et al. 1996). The pleiotropic manifestations are the result of a defect in fibrillin-1, a major component of elastic fiber microfibrils in the connective tissue (Sakai et al. 1986). Fibrillin-1 is encoded by the *FBN1* gene, located on chromosome 15q21.1 (Magenis et al. 1991). MFS is inherited autosomal dominantly, and the majority of the >130 *FBN1* mutations reported have been family specific (Collod-Béroud et al. 1998). The prevalence of MFS is ~1:5,000, and the rate of new mutations is quite high because ~25% of patients with MFS carry de novo mutations (Pyeritz 1993).

The disease phenotypes caused by *FBN1* mutations range from mild skeletal affection to a lethal neonatal form of the disease (Kainulainen et al. 1994; Milewicz et al. 1995).The phenotype also is highly variable among family members carrying the same *FBN1* mutation. In several other dominantly inherited connective tissue diseases, such as osteogenesis imperfecta or Ehlers-Danlos syndrome, variable phenotypes have been shown, in some families, to be caused either by somatic or both somatic and germ-line mosaicism for the disease-causing mutation (Cohn et al. 1990; Edwards et al. 1992; Milewicz et al. 1993). Usually, the mosaic parent has been clinically normal or has had only minor manifestations, and the mosaicism is not revealed until the unexpected recurrence of the disease in the family. Recently, two cases of mosaicism have been reported in congenital contractural arachnodactyly (CCA), an MFS-like connective tissue disorder caused by mutations in the fibrillin-2 gene in chromosome 5 (Wang et al. 1996; Putnam et al. 1997). The first report described a mosaic mother with classic CCA who had a daughter with severe, lethal CCA (Wang et al. 1996). In the latter case, the proband's father was a somatic mosaic for the mutation but had no features of CCA (Putnam et al. 1997).

It is a little surprising, considering the number of de novo *FBN1* mutations verified, that no cases of germline mosaicism have been reported with MFS. One example of an apparently mosaic patient with MFS, a young girl who had Marfanoid features only in the left

Received December 30, 1997; accepted for publication February 1, 1999; electronically published March 9, 1999.

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0002-9297/99/6404-0011$02.00

Table 1

Clinical Features of the Family Members, According to the Diagnostic Criteria for MFS

Feature/Finding	Patient 1	Patient 2	Mother	Father
Age (years)	9	6.4	37	36
Height (cm/SD)	149.8/+2.9	131.4/+2.2	161.5/−.8	182.0/+.8
Span/height	1.02	1	1	1.02
Sitting height (%)	50	51.6	51.5	51.4
Skeletal system:				
Mild pectus excavatum	+	+	−	−
Wrist and thumb sign	+	−	−	−
Pes planus	+	−	−	−
Joint hypermobility	+	−	−	−
Highly arched palate	+	+	−	−
Ocular system:				
Ectopia lentis[a]	+	+	−	−
Myopia	+	+	−	−
Cardiovascular system:				
Dilatation of ascending aorta[a]	+	+	−	−
Mitral valve prolapse	+	−	−	−
Family/genetic history:				
Affected 1st-degree relative[a]	+	+	+	+
FBN1 mutation[a]	+	+	−	−

NOTE.—Table modified from the report by de Paepe et al. (1996). A plus sign (+) denotes feature present; A minus sign (−) denotes feature absent.

[a] Major criteria for MFS.

side of her body, has been described by Burgio et al. (1988). The mutation of the patient has not been reported, but immunofluorescence analysis with a fibrillin 1–specific antibody showed clearly reduced staining of microfibrillar fibers in the fibroblast cultures from the affected side of the body versus only somewhat diminished staining on the "unaffected" side (Godfrey et al. 1990). Another possible example of somatic and germline mosaicism for an *FBN1* mutation, although not discussed by Hewett et al. (1994) in their report, may be the case of a mother with asymmetrical features of MFS. She had a daughter who was affected with a severe phenotype actually suggestive of Shprintzen-Goldberg syndrome (Sood et al. 1996). Because the daughter had died in 1963, the clinical information was not sufficient to confirm the diagnosis. This same *FBN1* mutation was described later in a patient with clinically evident Shprintzen-Goldberg syndrome (Sood et al. 1996), a syndrome of craniosynostosis with neurodevelopmental abnormalities and features of MFS (Shprintzen and Goldberg 1982). Finally, a report has just been published that describes a de novo *FBN1* mutation in a patient with classic MFS and in the patient's mother, who was only mildly affected and proved to be a somatic mosaic for the mutation (Montgomery et al. 1998).

Here we report the same missense mutation in the *FBN1* gene in two sisters with classic MFS who were born to clinically unaffected parents. The origin of the mutation was studied by analysis of DNA derived from different tissue samples of the parents, with a quantitative solid-phase minisequencing method (Syvänen et al. 1993). The analysis suggests that the mutational event must have occurred in the mother's germ line during her early fetal development.

Subjects and Methods

Clinical Summary

Patient 1 is a 9-year-old girl who was referred for diagnostic evaluation at the age of 5 years 9 mo. Physical examination showed features typical for MFS including myopia, dolichostenomelia, high arched palate, muscular hypotonia, and joint hypermobility. Cardiac ultrasound showed dilatation of the ascending aorta at the level of the sinus of Valsalva (25–26 mm, +3.0 SD) and a mitral valve prolapse. Ophthalmologic examination showed bilateral lens dislocation. She fulfilled the diagnostic criteria for MFS (De Paepe et al. 1996). Patient 2 is the sister of patient 1 and is aged 6 years 5 mo. She was evaluated for MFS first at age 4 years because of her sister's diagnosis. Careful clinical examination confirmed the diagnosis of MFS. Lens dislocation was bilateral, and aortic dilatation of +2.0 SD at the level of the sinus of Valsalva was present. The relevant findings of MFS of the two patients are presented in table 1. The family history was negative for MFS. Careful clinical examination, including ophthalmologic evaluation and cardiac ultrasound study of the parents, did not show any signs suggestive of MFS (table 1). The mother had

her third pregnancy during the present studies. The pedigree of the family is shown in figure 1*A*.

Samples

Peripheral blood samples were collected from the mother, father, and patient 2. Skin biopsies were obtained from the mother, father, and patient 1. One sperm sample was obtained from the father, and buccal, hair root, and ovarian samples were obtained from the mother. The buccal samples were obtained by scraping the mucosa of both cheeks with a cotton swab. Ten hairs with an intact root, plucked from the head, were used per each DNA extraction. The paraffin-embedded ovarian sample of the mother had been taken during an operation for endometriosis prior to the conception of her first child, patient 1. Chorionic villus sampling of the mother was performed during week 11 of gestation of her third pregnancy under ultrasound guidance. All the samples were taken according to the Helsinki Declaration.

DNA and RNA Extraction

DNA was extracted from peripheral blood leukocytes and the chorionic villus sample (CVS) by standard methods (Sambrook et al. 1989). We extracted DNA from the buccal cells, hair root cells, and semen using the Chelex-resin procedure (Walsh et al. 1991), and 5–20 μl of the supernatant was used per PCR amplification. From the paraffin block of ovarian tissue, several pieces were removed with a scalpel, and DNA was extracted separately from each tissue piece with the method described by Shibata et al. (1988). Ten microliters of the lysate was used per PCR amplification.

Fibroblast cultures derived from the skin biopsy specimens were grown in Dulbecco's minimal essential medium (Gibco BRL, Life Technologies) containing 10%

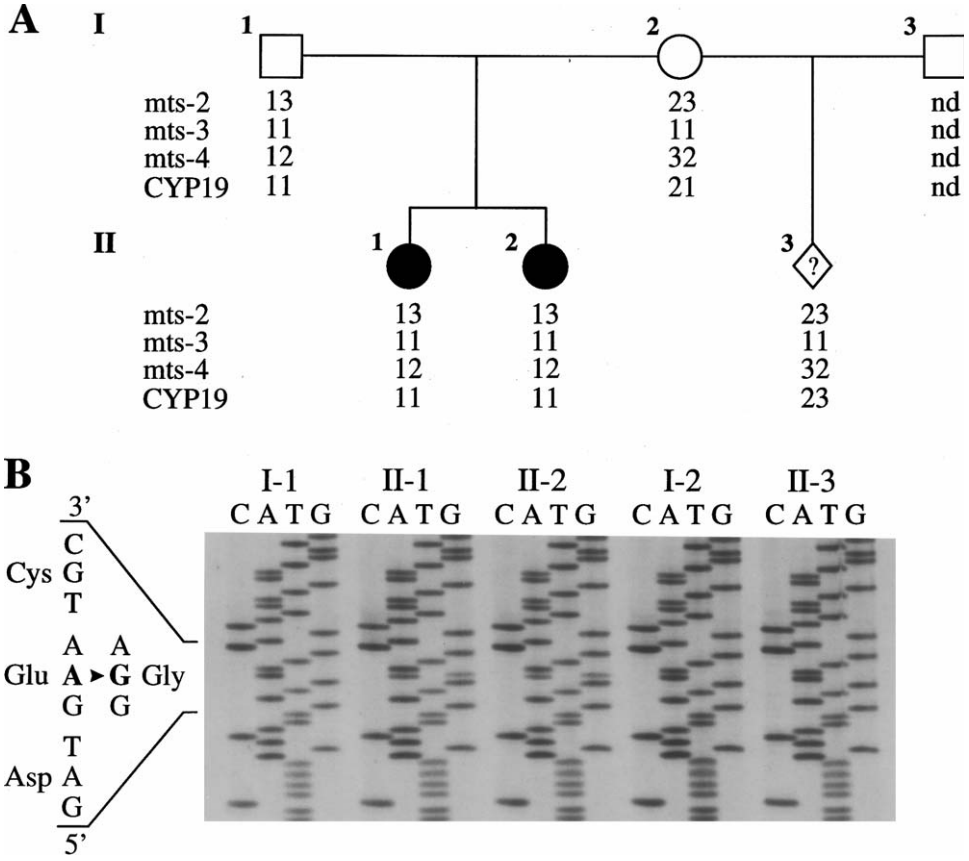

Figure 1 *A,* Pedigree of the family with a mosaic mutation. The haplotypes formed by microsatellite markers are shown. Mts-2, -3, and -4 are multiallelic intragenic *FBN1* polymorphisms, and CYP19 is a multiallelic polymorphism located in the immediate vicinity of *FBN1* (Pereira et al. 1994; Rantamäki et al. 1994). "nd" denotes not determined. Blackened symbols denote affected individuals. The diamond-shaped symbol denotes a fetus. *B,* Nucleotide and amino acid sequence of exon 29 in the region of the mutation 3599A→G. Genomic DNA was sequenced from the father (I-1), mother (I-2), patient 1 (II-1), patient 2 (II-2), and the fetus (II-3). The heterozygous mutation is seen only in the patients' samples.

FCS (Gibco BRL) and antibiotics. DNA was extracted from confluent cells in a 260-ml flask (Nunc), at the third to fifth passage of cell culture, by standard methods (Sambrook et al. 1989). We extracted total RNA from the fibroblast cultures, using the guanidinium isothiocyanate/cesium–chloride method (Chirgwin et al. 1979).

SSCP Analysis and Sequencing

We prepared cDNA from the total RNA by using reverse transcription with *FBN1*-specific primers. A set of 17 PCR primer pairs was used to amplify the complete *FBN1* cDNA in overlapping fragments, and the fragments were analyzed by SSCP as described by Kainulainen et al. (1992, 1994). A PCR product obtained by use of the primers flanking exons 29–34 (2*a* and 2*b* in Kainulainen et al. 1992) that produced a shift in SSCP was sequenced by use of the solid-phase sequencing method (Syvänen et al. 1989).

PCR

Twenty nanograms of genomic DNA was amplified in a 50-μl reaction, with one biotinylated primer at a concentration of 0.2 μM, one unbiotinylated primer at a 1 μM concentration, 0.2 mM of dNTPs, and 1 U Dynazyme DNA polymerase in its buffer. The primers were located in the introns flanking exon 29 and had the following sequences: primer A, 5′-CAGACATCCAAACCATATCAG-3′ (nucleotide position −49 to −29), and primer A-bio, 5′-AACCTACTGAGAGATTCAACAT-3′ (5′-end biotinylated; nucleotide position +41 to +20). For the hair root, buccal, and ovarian lysates, a nested PCR was done with one primer from the intron preceding exon 29; primer B, 5′-CATCCAAACCATATCAGAAGGTG-3′ (nucleotide position −45 to −23), and another, exonic primer, B-bio, 5′-CGGTGCATGATCTCTGGTCAG-3′ (5′-end biotinylated; nucleotides 3712–3692 according to the sequence in Pereira et al. 1993) (fig. 2). One microliter of a 1/100 diluted product from the first PCR was added to each nested PCR reaction. PCR was initiated by a hot start at 95°C, and the cycling parameters were 1 min at 95°C, 1 min at 57°C (primers A and A-bio) or 58°C (primers B and B-bio), and 1 min at 72°C, for 30–32 cycles. The quantity and specificity of the PCR products were assessed by 1% agarose gel electrophoresis. Control samples without DNA were included in the first PCR and in the nested PCRs.

Quantification by Solid-Phase Minisequencing

The relative amount of the alleles in different tissue samples was measured by use of the solid-phase mini–sequencing method (Syvänen et al. 1993). Ten microliters of the PCR product was captured in a streptavidin-coated microtiter well (Labsystems). After denaturation with 50 mM NaOH, a single-nucleotide-primer extension reaction was performed for 10 min at 50°C with 10 pmol minisequencing primer (5′-TCATTTCTTTTAGACATTGATG-3′); 0.1 μl of either ^{3}H-dATP, corresponding to the normal allele (81 Ci/mmol; Amersham), or ^{3}H-dGTP, corresponding to the mutant allele (31 Ci/mmol; Amersham); and 0.1 U DNA polymerase (Dynazyme II DNA Polymerase, Finnzymes) in 50 μl of its buffer. The primer was released with 50 mM NaOH, and the incorporated radioactivity was measured in a liquid scintillation counter (Rackbeta 1209, Wallac). Two parallel minisequencing reactions for both nucleotides were done for each PCR product. Figure 2 illustrates the strategy for the minisequencing method.

Paternity and Maternity Testing

The paternity and maternity, of the tissue samples, as well as the identity, were confirmed by genotyping with the highly polymorphic microsatellite markers D3S1359, HumTH01, HumTPO, HumFES, HumvWA, and MCT118. Testing was done in the routine paternity testing laboratory of the National Public Health Institute in Helsinki (Budowle et al. 1991; Rostedt et al. 1996).

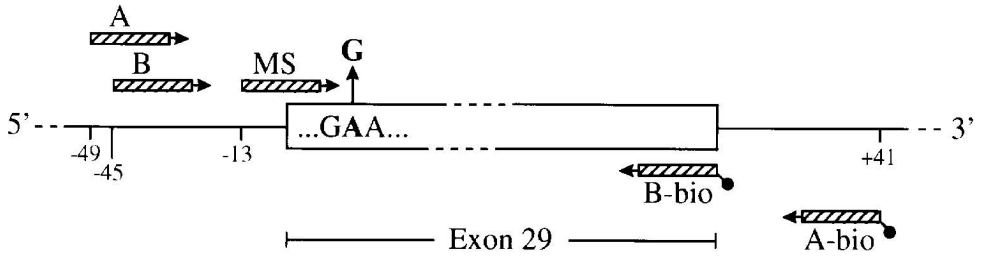

Figure 2 Schematic representation of the minisequencing strategy and location of the PCR and minisequencing primers. A and A-bio were used in the first PCR, B and B-bio in the nested PCR, and MS in the minisequencing assay. The nucleotide numbers defining the positions of the primers in the introns are given. "Bio" denotes a biotin residue.

Genotyping with Intragenic FBN1 *Markers*

Three multiallelic intragenic *FBN1* polymorphisms, mts-2, mts-3, and mts-4, and a multiallelic polymorphism in the vicinity of the *FBN1* gene, CYP19, were used to determine the *FBN1* haplotypes in the family (Pereira et al. 1994; Rantamäki et al. 1994).

Results

Identification of the FBN1 *Mutation*

cDNA derived from the fibroblasts of patient 1 was screened for *FBN1* mutations by SSCP analysis. On the basis of a shift in SSCP, the respective fragment was sequenced, and a nucleotide change of A→G was identified at position 3599 in exon 29 of the *FBN1* cDNA (3599A→G). The corresponding amino acid change is glycine for a glutamic acid at position 1200 of the fibrillin-1 polypeptide (E1200G). The mutation was confirmed by sequencing genomic fibroblast DNA with intronic primers flanking exon 29 (fig. 1*B*). Sequence analysis of genomic DNA extracted from peripheral leukocytes of the patient's younger sister (patient 2) showed the same mutation. However, when the leukocyte DNA from both the unaffected parents was sequenced, only the normal allele (A) was identified at position 3599 (fig. 1*B*). The paternity and maternity were unequivocally confirmed by use of six highly informative polymorphic microsatellite markers, by power of evidence values of 99.98% for both sisters (Budowle et al. 1991; Rostedt et al. 1996). The 3599A→G mutation was not identified in DNA samples from 40 unrelated patients with MFS nor from a pool of DNA from 90 control individuals (Syvänen et al. 1993).

To determine the phase of inheritance of the *FBN1* alleles, the intragenic *FBN1* markers, mts-2, mts-3, and mts-4, in addition to CYP19, a highly polymorphic marker in the vicinity of the *FBN1* gene, were used to define the haplotypes in genomic DNA samples (Pereira et al. 1994; Rantamäki et al. 1994). The haplotypes formed by these four markers indicate that the patients had inherited the same *FBN1* allele from their father and the same *FBN1* allele from their mother. Thus, the parental origin of the mutation was not obvious (fig. 1*A*).

The Origin of the Mutation

DNA extracted from the leukocytes and fibroblasts of both parents, from the sperm of the father and from hair root and buccal cell samples of the mother, were further analyzed to define the origin of the *FBN1* mutation. In addition, a paraffin block of ovarian tissue from the mother was available for sampling. The identity of the tissues was verified by use of the same panel of microsatellite markers as that used for parental testing. The amount of mutant alleles in different tissues was measured by use of the solid-phase–minisequencing method, which allows the detection of a mutant allele present in <1% of a sample (Syvänen et al. 1992).

The result of the minisequencing assay is expressed as the ratio between the labeled dNTPs incorporated in the two reactions detecting the mutant allele (G) and the normal allele (A). Leukocyte DNA from a control (0% mutant) and a patient (50% mutant) and mixtures of the patient and control DNA (0.8%–25% mutant) were analyzed to construct a standard curve for quantitative analysis of the mutation in the tissue samples (table 2). The G/A ratios obtained in the series of quantification standards show that there is a linear relationship between the amount of mutant DNA and the G/A ratios and that ~1% of mutant *FBN1* DNA is detectable when compared with the G/A ratio of the control sample. The analysis of the tissue samples is summarized in table 3. In the heterozygous patient samples, G/A ratios of .3–.4 were obtained, whereas the control samples gave G/A ratios of .01. As can be seen from the G/A ratios obtained in the tissue samples from the parents of the patients, none of the tissue samples analyzed contained a detectable amount of mutant allele (table 3). The absence of the mutant allele in the father's sperm sample excludes the presence of mutation in his germ cells. Unfortunately, the ovarian tissue of the mother was badly damaged, and no oocytes could be seen by the hematoxylin/eosin staining. Thus, the DNA extracted from the block apparently represented DNA from ovarian tissue cells and not the DNA of the germ cells. Our conclusion, on the basis of the sensitive and quantitative analysis of representative tissues from both parents, is that most likely the mother is a mosaic in her germ line only for the

Table 2

Series of Minisequencing Standards for Quantification of the Mutant *FBN1* Allele

% Mutant DNA	Incorporated Radioactivity [a] (cpm)		G/A Ratio (range)[a]
	^{3}H-dGTP	^{3}H-dATP	
50 (patient 2)	869	2,823	.307 (.299–.314)
25	502	4,708	.107 (.106–.108)
13	288	5,307	.053 (.048–.058)
6.3	175	5,153	.034 (.032–.036)
3.1	136	5,713	.025 (.019–.031)
1.6	86	5,075	.017 (.013–.020)
0.8	66	5,308	.012 (.011–.013)
0 (Control)	84	7,302	.012 (.011–.012)
PCR-H_2O	25	27	...

[a] Mean values and variation range of duplicate minisequencing assays from the same PCR product.

Table 3

Quantitative Determination of the Mutant *FBN1* Allele in Tissue Samples of the Family Members

Origin of the DNA and Family Member	G/A Ratio[a]
Leukocytes:	
Patient 2	.307 ± .011
Father	.010 ± .002
Mother	.007 ± .002
Control	.012 ± .001
Fibroblasts:	
Patient 1	.462 ± .084
Father	.011 ± .002
Mother	.009 ± .003
Control	.011 ± .002
Semen:	
Father	.009 ± .003
Hair bulb:	
Mother	.009 ± .003
Oral epithelium:	.007 ± .001
Mother	
Ovarian tissue:	
Mother	.007 ± .002
CVS:	
Fetus	.007 ± .003

NOTE.—Mutant *FBN1* allele determined by use of solid-phase minisequencing.

[a] Mean values and SDs of G/A ratios from at least four parallel minisequencing assays.

mutation and carries the mutation in an unknown fraction of her oocytes.

Prenatal Diagnosis

The mother became pregnant by her new spouse, and prenatal diagnosis was performed from a CVS obtained in week 11 of gestation. Genomic DNA extracted from the CVS was amplified by PCR, and the A3599G mutation was analyzed both by solid-phase sequencing and minisequencing. The *FBN1* haplotypes obtained from the CVS were determined by use of one extragenic and three intragenic polymorphic markers. The A3599G mutation was not identified in the CVS DNA, and, moreover, the *FBN1* haplotype obtained from the CVS was different from the haplotypes of patients 1 and 2, indicating that the fetus had inherited a different maternal *FBN1* allele from the patients.

Discussion

In the present study we describe the occurrence of the same *FBN1* mutation in two sisters, with classic MFS, born to healthy parents who proved to be noncarriers of the mutation, in their leukocyte DNA. Possible explanations for recurrence of the disease in a family could be nonpenetrance of the disease, recessive or non-Mendelian inheritance, wrong paternity, or presence of a hot spot for mutations. Finally, either of the parents may be a germ-line mosaic for the disease-causing mutation. Because MFS is dominantly inherited, the penetrance is considered to be 100%, the majority of patients have unique mutations, and no hot-spot region for the mutations has been identified, these explanations for the event could be excluded in our case (Pyeritz 1993). Both maternity and paternity were unequivocally confirmed; thus, it seems most likely that one of the parents was mosaic in the germ line for the identified mutation.

The assumption that the identified nucleotide change 3599A→G is a true MFS-causing mutation is supported by the location of the resulting amino acid change E1200G. Fibrillin-1 polypeptide contains several repeating domains, of which the calcium-binding epidermal growth factor–like domain is the most common. These domains have a consensus sequence, D/N-x-D/N-Q/E-x_m-D/N-x_n-Y/F, that is known to be associated with calcium binding (Handford et al. 1991). The mutation E1200G changes the glutamic acid of this consensus sequence to glycine. This glutamic acid has been shown to participate in direct liganding of Ca^{2+} (Handford et al. 1991). Therefore, its change to glycine evidently results in reduced affinity for Ca^{2+} and thus destabilizes this particular domain, resulting in defective fibrillin-1 molecules. At least 10 missense mutations affecting the calcium-binding consensus sequence of fibrillin-1 have been described in patients with MFS (Collod-Béroud et al. 1998). Interestingly, the mutation E1200G is located in the so-called neonatal region, encoded by exons 24–32 of *FBN1*. This name originated from the clustering of mutations causing lethal neonatal MFS to this region (Kainulainen et al. 1994; Putnam et al. 1996). All the missense mutations causing a very severe MFS phenotype have been concentrated in the narrow region of exons 24–27, and missense mutations found in patients with classic MFS have been located in exons 28–32 (Rantamäki et al. 1997). Therefore, the region encoded by exons 24–27 seems to be functionally more essential in the fibrillin-1 polypeptide than the region encoded by exons 28–32. Consequently, the classic phenotype in our patients with a missense mutation in exon 29 cannot be considered as an exceptional finding.

The aims of our study were to determine which parent had transmitted the E1200G mutation to the two sisters and whether the parent carried the mutation only in the germ-line cells or also in somatic cells. The simplest way to identify the carrier parent would have been via genotyping, but the genotyping with polymorphic *FBN1* markers showed only that both patients had inherited a common maternal and a common paternal allele. If the mutation had caused a size difference in *FBN1* it would have been useful in carrier detection. Furthermore, we failed to identify any additional polymorphisms near the mutation that would have helped to distinguish paternal

and maternal alleles. In a recent report by Yates et al. (1997) of apparent isolated maternal germ-line mosaicism in tuberous sclerosis, the authors were able to identify the carrier parent by using a polymorphic restriction-enzyme–digestion site. Affected siblings were heterozygous for restriction site present/restriction site absent polymorphism. By restriction digestion of a PCR product containing the mutation, it could be demonstrated that patients had inherited the mutation from their mother. Unfortunately, there are no such restriction-site polymorphisms identified in *FBN1*. The only polymorphism near the mutation 3599A→G is mts-3, for which the patients were homozygous.

Our evidence for the origin of the mutation in the presented case, however, indicates the likely maternal origin of the disease mutation. Because there was no mutant allele detectable in the sperm sample of the father, he could be excluded as a carrier of the mutation. This is supported by the assumption that, in a case of two/two affected children in a family, the mutation has to be present in a significant number of germ cells (Zlotogora 1998) and would have been detected by our very sensitive detection method. Detailed analysis of multiple maternal somatic tissues gave no evidence for somatic mosaicism. This finding is consistent with the isolated germ-line mosaicism of the mother. In isolated germ-line mosaicism, the new mutation occurs during the early fetal development of an individual, after the segregation of somatic cell and germ-line progenitors, whereas in a case of somatic and germ-line mosaicism mutation occurs earlier, before the separation of cell lineages. The number of cells carrying the mutation is higher the earlier the mutation occurs (Zlotogora 1998). We suggest that, in our case, the mother is an obligate carrier and carries the mutation in an unknown fraction of her oocytes. Unfortunately, this conclusion could not be directly shown because the potential representative of maternal germ cells, the paraffin block of ovarian tissue, contained no histologically identifiable oocytes.

The risk for further affected offspring in a case of germ-line mosaicism is difficult to estimate, because the number of germ cells carrying the mutation is not known. This problem was also discussed in our case, during the prenatal genetic counseling after the mother became pregnant by her new spouse. If the mutation had been present in all the primitive germ cells of the mother, then the subsequent meiotic divisions would have diminished the number of oocytes carrying the mutation to one-half. Since there is the possibility that at least half the oocytes do not carry the mutation 3599A→G in a case of a dominant inheritance pattern, the risk for affected offspring would be $\leqslant 50\%$.

It is difficult to estimate how many of the sporadic patients with MFS actually represent offspring of a mosaic parent, especially if only leukocyte DNA has been analyzed by nonquantitative conventional methods with limited detection sensitivity. The degree of mosaicism may not be apparent in the leukocytes but would become evident if other tissues were studied. Cases of somatic and germ-line mosaicism in which the mutation has been undetectable in leukocyte DNA have been described, in, for example, the latest report of mosaicism in CCA (Putnam et al. 1997). The mutation was identified by restriction-site analysis of PCR products in the hair bulbs and buccal cells of the proband's father but was not detectable from the father's leukocyte DNA.

Although not shown earlier, mosaicism could be a more common phenomenon in MFS than has been realized so far. Somatic mosaicism may even be one modifying factor responsible for the phenotypic variability among unrelated patients with MFS. Most important, the present demonstration of germ-line mosaicism in MFS has implications for genetic counseling. The recurrence risk in the families with "sporadic" patients should, therefore, be estimated to be higher than the new mutation rate alone. Whenever a single child is identified with an *FBN1* mutation and more children are desired, the parents should be tested, and tissues other than leukocytes should be analyzed. Furthermore, prenatal diagnosis should be offered.

Acknowledgments

We thank the family for their help and cooperation, Dr. Antti Sajantila for helpful discussions about techniques, Dr. Teppo Varilo for help with obtaining the samples, Dr. Terttu Toivonen for providing us with the histopathological specimen of the ovary, Prof. Juhani Rapola for performing the microscopic analyses of the ovarian samples, Dr. Nina Aula for help with preparing the manuscript, and Ms. Lea Puhakka and the people in the paternity testing laboratory, for technical assistance. This work was supported by grants from the Oskar Öflund Foundation and the 350th Anniversary Foundation of the University of Helsinki.

Electronic-Database Information

Accession number and URL for data in this article are as follows:

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim (for MFS [MIM 154700])

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