Am. J. Hum. Genet. 55:1083–1091, 1994

A Compound-Heterozygous Marfan Patient: Two Defective Fibrillin Alleles Result in a Lethal Phenotype

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Summary

We describe here the identification of defined mutations in both alleles of the fibrillin gene (FBN1) in a compound-heterozygote Marfan syndrome (MFS) child who had a very severe form of MFS resulting in death from cardiac failure at the age of 4 mo. The nonconsanguineous parents were both affected with MFS. The father's heterozygous point mutation has earlier been reported to result in W217G substitution, the mother was here shown to carry a heterozygous point mutation resulting in G2627R substitution, and the child had inherited both these mutations. The mutant FBN1 alleles were demonstrated to be transcribed with equal efficiency compared with the normal alleles, but metabolic labeling of fibroblast cultures from the child and both parents showed reduced biosynthesis and secretion of profibrillin. Also, the respective amounts of fibrillin in cell-culture media and extracellular-matrix extracts were markedly diminished, particularly in the cell cultures from father and child. In addition, immunofluorescence analysis of the cell cultures of all three family members revealed a drastically reduced amount of microfibrils, and virtually no visible fibrils could be seen in the case of the compound-heterozygote child. These findings demonstrate incomplete dominance of fibrillin mutations and underline the fatal consequences of the complete absence of normal fibrillin molecules in the microfibrils.

Introduction

A recent series of studies have demonstrated that different mutations in the chromosome 15 gene (FBN1) coding for fibrillin (Fib15) cause Marfan syndrome (MFS), a dominantly inherited connective-tissue disorder with manifestations in the cardiovascular, skeletal, and ocular systems (Dietz et al. 1991, 1992, 1993; Kainulainen et al. 1992, 1994; Godfrey et al. 1993; Hewett et al. 1993; Tynan et al. 1993). A large spectrum of clinical phenotypes is seen among different families, varying from a very mild form of the disease to the extremely severe neonatal type of MFS, which leads to death in early infancy (Pyeritz 1993).

Fib15 is a large cysteine-rich glycoprotein component of extracellular microfibrils (Sakai et al. 1991). The major building modules of Fib15 are 47 epidermal growth-factor precursor (EGF)–like motifs interspersed with transforming growth-factor-β1 binding protein (TGF-β1-bp)–like motifs. Forty-three of the 47 EGF-like motifs are shown to contain the proposed consensus sequence for calcium-binding (cbEGF), and it has actually been shown that Fib15 molecules bind calcium, a phenomenon that, together with the intrachain disulfide-bond formation of these motifs, may be essential for the stability of the linear conformation of the Fib15 polypeptide (Corson et al. 1993). It has also been suggested that the cbEGF-like motifs serve to mediate protein-protein interactions between Fib15 and ligands important to microfibril assembly and that the TGF-β1-bp–like domains in Fib15 play a role in interchain disulfide-bridge formation between individual Fib15 molecules (Corson et al. 1993; Pereira et al. 1993).

Here we report the molecular background of an interesting MFS case: an infant with a severe form of the disorder finally leading to death at the age of 4 mo. He represents an exceptional case of MFS, since both his parents were diagnosed as having a moderate form of this disease. We have earlier defined a heterozygous point mutation in the father (Kainulainen et al. 1994), and here we describe a heterozygous point mutation in the mother and demonstrate that the infant had inherited both differently mutated FBN1 alleles from his affected parents. Biochemical studies of the fibroblast cultures of family members pointed to a severely disturbed assembly of microfibrils.

Subjects and Methods

The Family

The clinical findings of this family have been reported elsewhere (Schollin et al. 1988). In short, the proband was the second child of his parents. At birth he had multiple skinfolds all over the body, and at 1 wk of age the ophthalmologic examination revealed bilateral lens luxation (Dr. Jens Schollin, personal communication). The baby had long extremities, and he died at the age of 4 mo, because

Received March 1, 1994; accepted for publication August 17, 1994.

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0002-9297/94/5506-0003$02.00

of congestive heart failure. The father had previously been operated on for bilateral lens luxation. He was 195 cm tall, with long extremities; he suffered from arachnodactyly and pectus carinatum; and echocardiography revealed a slight dilatation of the aortic root. The paternal grandfather was 195 cm tall, and he had lens luxation and arachnodactyly. The mother of the proband was diagnosed with MFS as a teenager. She had a tall stature, 185 cm, and arachnodactyly, and she suffered from myopia. No signs of cardiac disease could be detected by echocardiography performed when she a teenager, but she has been unwilling to be examined since. The maternal grandmother died at the age of 51 years, of a ruptured aortic aneurysm, and her brother had cardiac manifestations of MFS (Dr. Jens Schollin, personal communication). There were no samples or detailed clinical information available from the grandparents or other relatives, and all the family members have refused to be examined further.

Extraction of DNA and RNA, and Synthesis of cDNA

All the samples in this study were taken in accordance with the Helsinki declaration. Skin fibroblasts were cultured as described elsewhere (Kainulainen et al. 1992), and total RNA was isolated from the cultured fibroblasts by the guanidium isothiocyanate–CsCl method (Chirgwin et al. 1979). One microgram of total RNA was reverse transcribed with 16 units of AMV reverse transcriptase (Promega), and cDNA was synthesized using FBN1-specific primers (Kainulainen et al. 1992, 1994). Genomic DNA was extracted from frozen peripheral blood, in accordance with the method described by Vandenplas et al. (1984). The control pool of 180 control individuals' DNA was produced as described by Syvänen et al. (1993).

PCR and SSCP Analysis

The 8.6-kb coding region of FBN1 cDNA was screened for mutations by PCR and SSCP analysis. The primers and conditions for PCR amplification and SSCP analysis have been described elsewhere (Kainulainen et al. 1992, 1994).

Solid-Phase Sequencing

PCR was performed using primers, one of which was 5′ biotinylated. The sequencing primers and the biotinylated PCR primers are listed in table 1. Forty microliters of the PCR product was added to 5 μl of a 5% (w/v) suspension of avidin-coated polystyrene particles (FLUORICON; Baxter) and was allowed to bind for 15 min at room temperature. The particles were washed once with 200 μl of TENT buffer (40 mM Tris-HCl pH 8.8, 1 mM EDTA, 50 mM NaCl, and 0.1% Tween 20), and the bound PCR product was denatured by incubating the particles for 5 min in 50 mM NaOH. The particles were washed twice as before, were suspended in water, and were used in dideoxy-termination sequencing reactions as a single-stranded template. The sequencing reactions were performed using a commercially available kit (USB) and in accordance with the manufacturer's protocol.

Determination of the Steady-State Level of FBN1 mRNAs

The solid-phase minisequencing method was used to determine the steady-state level of mRNA transcribed from the different FBN1 alleles, relative to the amount of genomic DNA (Ikonen et al. 1992). The PCR and detection primers used in minisequencing tests are given in table 1, and a schematic presentation of the method is shown in figure 1. One microgram of total RNA was reverse transcribed, and a 2-μl aliquot of the reverse-transcribed reaction and 25 ng of genomic DNA were amplified separately by PCR with primers flanking the mutated region (table 1). The biotinylated PCR products were bound to avidin-coated microtitration wells by passive adsorption, and the immobilized product was denatured by treating the wells with 50 mM NaOH. The wells were washed thoroughly to remove all free nucleotides. The primer-annealing and -extension reactions were carried out simultaneously by incubating the wells at 50°C for 10 min in the reaction mixture: 10 pmol of the detection primer, 0.5 μCi of ^{3}H-dNTP, and 0.25 U of *Taq* DNA polymerase in PCR buffer (20 mM Tris-HCl pH 8.8, 1.5 mM $MgCl_2$, 15 mM $(NH_4)_2SO_4$, 0.01 % gelatin, and 0.1 % Tween 20). The extended primer was released with 50 mM NaOH, and the eluted radioactivity was measured in a liquid scintillation counter. The ratio (R) between the incorporated [^{3}H]dATP, corresponding to the mutant allele, and the incorporated [^{3}H]dGTP, corresponding to the normal allele, was calculated (Syvänen et al. 1990).

Pulse-Chase Studies

Cell cultures of both parents and of the compound-heterozygous infant were examined in parallel with cell cultures from two normal adults and from a normal 2-mo-old baby. On day 1, 100,000 cells were seeded in 15-mm dishes (four-well dishes, 1.8 cm^2; Nunclon) in cell culture medium (MEM; Gibco) containing 10% FCS, 5 mM glutamine, and antibiotics. On day 3, the cultures were washed and incubated for 30 min in MEM without Met, Cys, and FCS (MEMdep; Northumbria) and finally were pulsed for 30 min with 50 μCi of TranSlabel [^{35}S]Met/Cys (ICN) in 200 μl of MEMdep. The medium was removed, and the cells were washed twice with complete MEM and were chased with 200 μl of MEMdep supplemented with 10 mM Met and 1 mM Cys. The chase times were 0, 1, and 2 h. At each of these time points, the media were transferred to 200-μl of double-strength sample buffer (Laemmli 1970) supplemented with 0.5 M urea and 10% mercaptoethanol. The cells were washed three times with ice-cold Hank's balanced salt solution (HBSS) lacking Ca/Mg and were lysed for 20 min on ice in 200 μl of 10 mM Hepes pH 7.4 containing 1% NP-40 and 1 mM each of PMSF and NEM. The cell lysates were transferred into Laemmli buffer as

Table 1

Primers Used in PCR, Sequencing, and Minisequencing

Mutation	Primer Sequence—5′ to 3′	5′ Nucleotide
	PCR Primers	
W217G	GCAACCAGATGTGCCAGGGA	572
	BioGGAATGAAGCCACGGCGGCA	710
G2627R	GAAAACGAATGCCTCAGTGCTC	7822
	BioCATTCATTGATGTCTTGGCATCC	7955
	Sequencing Primers	
W217G	GCAACCAGATGTGCCAGGGA	572
G2627R	GAAAACGAATGCCTCAGTGCTC	7822
	Minisequencing Primers	
W217G	GTGCCACAGTCGGCCGAGCC	629
G2627R	GAAAACGAATGCCTCAGCGCTC	7859

described above. The remainder in the dish—by definition, extracellular matrix (ECM)—was washed three times with HBSS and was extracted with 440 μl of normal strength sample buffer (0.5 M urea and 10% mercaptoethanol) for 10 min at room temperature. The samples (440 μl for each compartment) were boiled for 3 min and were centrifuged, and 30 μl of each were used for 4%/7%-step gel SDS-PAGE as described by Raghunath et al. (1993). Fluorographic bands representing vimentin and an anonymous intracellular nonsecretory protein ("protein X") migrating above profibrillin (proFib) harvested from the cell lysates were scanned densitometrically (McGookey Milewicz et al. 1992). All samples were electrophoresed a second time, with the loads (30 μl maximum) corrected for differences of the amount of radioactivity in the band representing the protein X. The fluorograms of the second analysis are shown in figure 2. The respective gel exposure times were 4 d for cell lysates, 5 d for culture media, and 6 d for ECM extracts.

As a rough approximation, to monitor the efficiency of cell lysis and a potential spillover of intracellular material into matrix extracts, a 2-h chase experiment without metabolic labeling was performed, and the activity of lactate dehydrogenase (LDH) was measured (Deutsche Gesellschaft für Klinische Chemie 1972) in the ultrasonic cell homogenates, in cell lysates, and in ultrasonic homogenates of the remainder ECM. In the pulse-chase experiments, radioactivity incorporated into proFib/Fib15 was compared with the representatives of cellular mass—vimentin and protein X—after quantitation by scanning densitometry of fluorograms. The results obtained with the patients' cell cultures were compared with the averaged results from the three controls (densitometric data not shown).

Immunofluorescence Assay

Approximately 10^6 cells/35-mm dish (Nunc) were grown, on glass coverslips for 48–72 h, to hyperconfluency and then were fixed in methanol for 10 min at −20°C and were air-dried. The monolayers were rehydrated, and unspecific binding sites were blocked by incubating the fixed cells for 30 min in 1% BSA/PBS solution containing 10% normal rabbit serum. The blocking was drained off and replaced by monoclonal antibody (Mab201) against Fib15 diluted to 1:100. Incubation was for 2 h at room temperature. A TRITC-conjugated rabbit anti-mouse antibody (DAKO), diluted to 1:40 in PBS and applied for 30 min at room temperature, was used to detect the bound specific antibody. Photographs were taken on Kodak TMax 400 black-and-white film, with a Zeiss Axiophot photomicroscope with a fixed exposure time of 10 s.

Results

Mutation Analysis

Total RNA extracted from the fibroblast cultures of each family member was reverse transcribed with FBN1 cDNA-specific primers (Kainulainen et al. 1992, 1994). SSCP analysis of 18 overlapping PCR fragments covering a total of 8.6 kb of coding region of FBN1 cDNA revealed two sets of aberrantly migrating fragments (shifts). The mutation of the father has elsewhere been described by us (Kainulainen et al. 1994). A shift was identified in the mother's cDNA sample amplified with primers 7a and 7b, and the child's cDNA sample revealed two SSCP shifts, corresponding to those observed from his parents' samples (for use of primers in SSCP analysis, see Kainulainen et al. 1992, 1994).

Heterozygous base changes in these cDNA regions were detected by solid-phase sequencing. A T-to-G transversion in the FBN1 cDNA of the father resulted in the substitution of glycine for tryptophan (W217G). A G-to-A transition in the FBN1 cDNA of the mother caused a substitu-

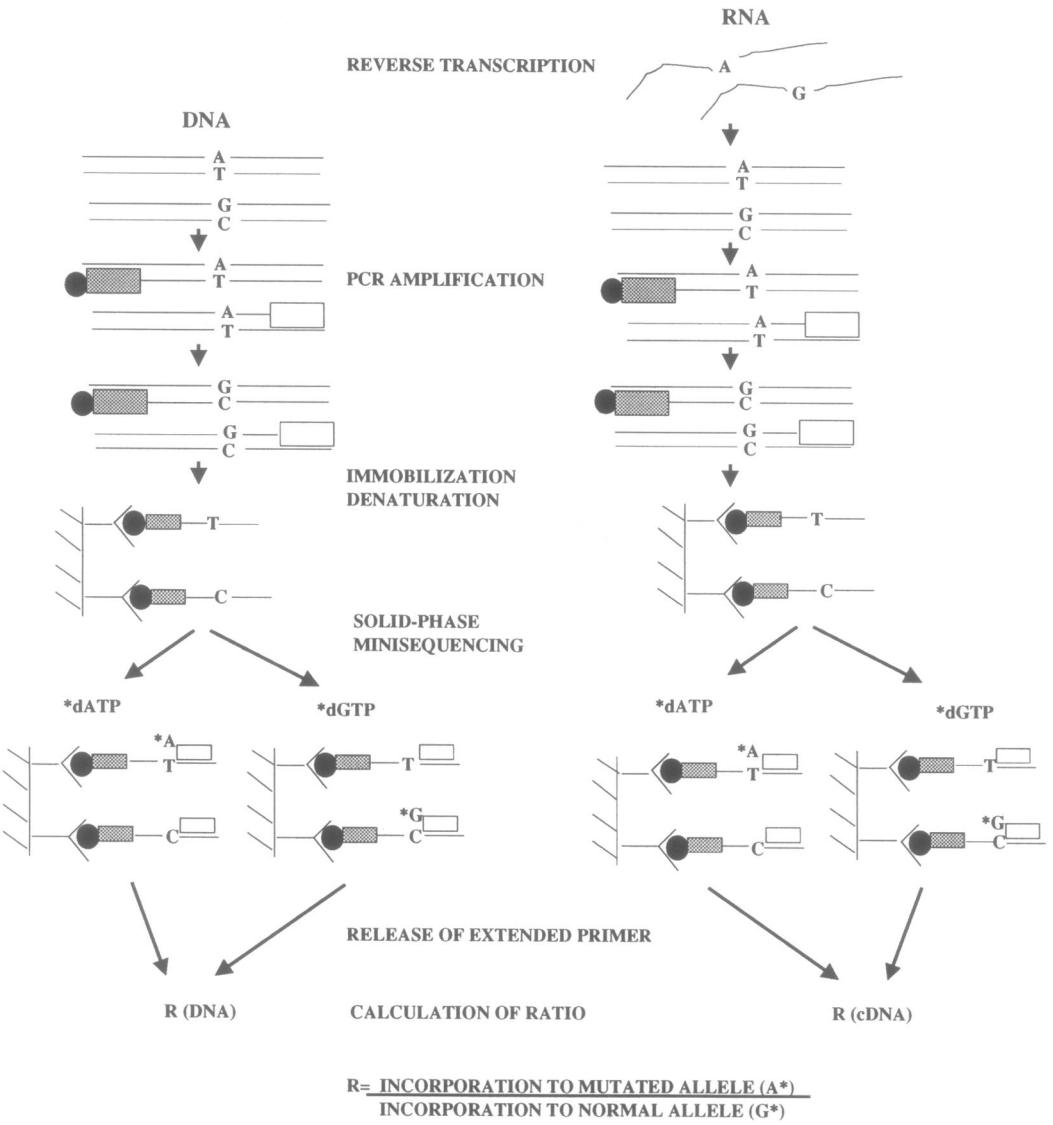

Figure 1 Description of the minisequencing test in determination of the ratio of FBN1 transcripts. A cross-hatched segment with a black circle denotes a 5′-biotinylated primer; and an asterisk indicates the labeled nucleotide.

tion of arginine for glycine (G2627R). Sequence analysis of the corresponding regions from the genomic DNA of the infant revealed that he had inherited his parents' defective FBN1 alleles (fig. 3). The minisequencing method was used to screen for both the mutations in a pool of 180 control individuals' DNA samples, as well as in the DNA samples of 60 unrelated MFS patients, and neither of the mutations could be identified in the controls or in any other MFS patient.

Determination of the Steady-State Level of FBN1 mRNAs

The R value obtained with the cDNA in the minisequencing test, which reflects the ratio between mutant and normal mRNA molecules present in the sample, was compared with the R value obtained from the genomic DNA in which the alleles are present in a 1:1 ratio. The R values obtained with the cDNA from the child's cells and with the cDNA with the parents' cells were similar to those obtained with the genomic DNA samples (table 2). It could thus be concluded that neither of the mutations significantly affected the steady-state level of the FBN1 transcripts.

Pulse-Chase Studies of Fibroblast Cultures

On the basis of results of scanning of the radioactive signals in pulse-chase experiment, the cells from the cell cultures of all the affected family members appeared to synthesize one-third less proFib than did the controls. Further, no intracellular retention of proFib could be observed in these cells after 2-h chase, in comparison with the control cells (fig. 2, *upper panel*). When the release of Fib15 into the chase medium was monitored, proFib and

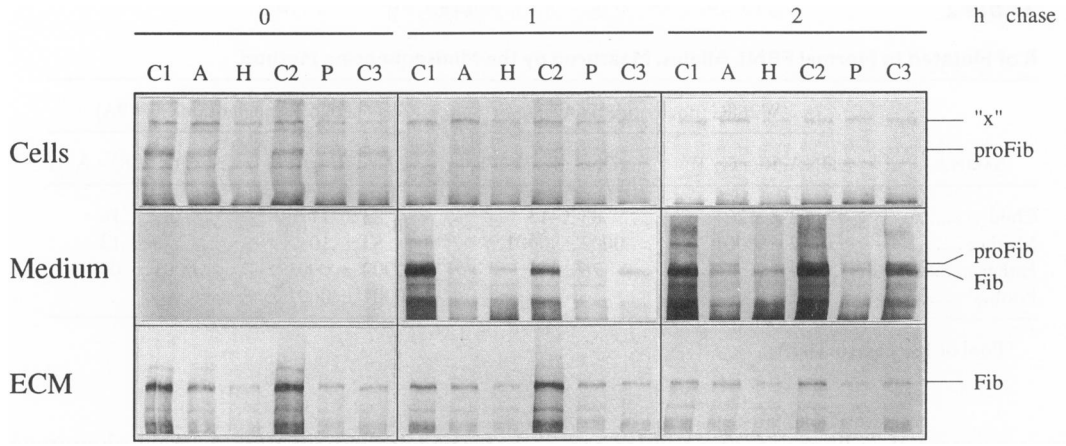

Figure 2 SDS-PAGE analysis of Fib15 (Fib) and its precursor, proFib, produced by the fibroblast cultures of the controls (lanes C1, lanes C2, and lanes C3) and the cell cultures of three individuals with MFS: a mother (lanes A), a father (lanes H), and their compound-heterozygous child (lanes P). Only the truncated 4% layers of the 4%/7%-step gels are shown. *Cells,* Results of early chase times reveal presence of proFib in cell lysates of the controls, the parents, and their child. ProFib is chased off all cell cultures, without evidence of delayed secretion or intracellular retention of proFib in the cell cultures of any of the MFS patients. However, the intracellular amounts of proFib in all the patient's cells appear markedly reduced. "X" indicates an anonymous protein (McGookey Milewicz et al. 1992) serving as an additional intracellular marker. *Medium,* Presence of Fib15 and its precursor proFib in the culture medium, at maximal intensity after 2 h of chase. The amount of Fib15 released by all three cell cultures of MFS patients is markedly reduced, but least in the maternal cells. *ECM,* Matrix extracts of the father (lanes H) and the child (lanes P), which contain markedly reduced amounts of Fib15 at all chase times. The cell culture from the mother (lanes A) incorporated half as much Fib15 as did the cell cultures from controls, but still more than those of the father and the child together. The presence of labeled proteins after 0 h of chase—and the simultaneous absence of proFib/Fib15 in culture media—suggests a substantial spillover of intracellular proteins at this time point.

its processed form, Fib, were observed in the control cell-culture media after 1-h chase, and the electrophoretic bands showed maximal intensity at 2-h chase. At this chase time point, medium of the cell cultures from the three family members affected with MFS showed strikingly reduced amounts of proFib/Fib, on the basis of the densitometric scanning of the fluorograms—66% (mother), 16% (father), and 25% (child)—in comparison with the averaged results of the cell cultures from three controls (fig. 2, *center panel*). Analogously, at this chase point also, ECM extracts showed an apparent reduction of Fib15 to 50% (mother), 25% (father), and 21% (child), compared with levels in the controls (fig. 2, *lower panel*).

To monitor the potential spillover of intracellular material into ECM, the LDH activity of ECM extracts was measured. Fifty percent of LDH activity was recovered in the cell lysates, compared with what was recovered in the cell homogenates. The ECM homogenates contained 8% of LDH, compared with the cell lysates. As also discussed recently, these findings indicate that cell lysis is incomplete

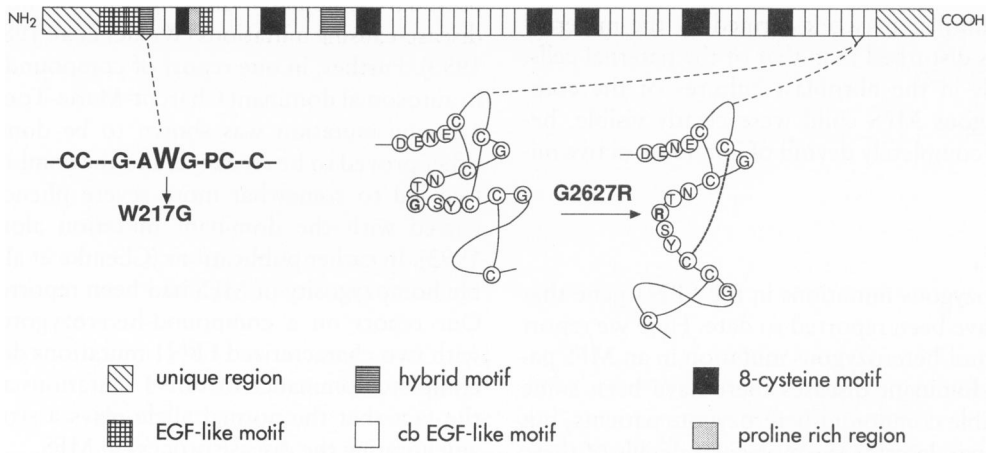

Figure 3 Schematic presentation of proFib. The mutation W217G substitutes a glycine for conserved tryptophan in one of the hybrid motifs of proFib. The mutation G2627R substitutes arginine for highly conserved glycine in the penultimate calcium-binding EGF-like motif of proFib.

Table 2

R of Mutated to Normal FBN1 Alleles, Measured by the Minisequencing Method

	W268G (nucleotide T649G)		G2627R (nucleotide G7879A)	
SAMPLE	DNA: R = G/T	cDNA: R = G/T	DNA: R = A/G	cDNA: R = A/G
Child	.63 ± .12	.63 ± .13	.83 ± .11	.83 ± .16
Mother	.0002 ± .0001	.0002 ± .0001	.83 ± .10	.76 ± .13
Father	.64 ± .15	.76 ± .15	.0002 ± .0001	.0001 ± .0001
$Pool_{180}$[a]	.0001 ± .0001	...	.0001 ± .0001	...

[a] Pool of 180 control DNAs.

and that there is a potential spillover of intracellular proteins into material harvested from the ECM (Milewicz and Duvic 1994). However, LDH being cytosolic may not be entirely representative of the degree of extraction of secretory proteins from organelles such as the endoplasmic reticulum or Golgi apparatus during cell lysis followed by the harsher matrix-extraction procedure. Only the data obtained from the latest chase time point were quantitated, at which point no appreciable amounts of proFib were apparent in the cell lysates, since a spillover from protein of the secretory pathway would be expected to be substantial at the zero time point of chase (see fig. 2).

Morphological Analysis of Microfibrils Deposited in Fibroblast Cultures

Immunocytochemistry of fibroblast cultures with Fib15 antibodies revealed microfibrils giving a brilliant linear immunofluorescence in the control cells: the microfibrils were accumulated on the normal hyperconfluent fibroblast cultures, as a dense and finely woven meshwork (fig. 4). In contrast, the fibroblast cultures of the affected parents appeared to be only sparsely covered with microfibrils, the intensity of fluorescence appearing very much reduced and the staining pattern appearing weak; also, the microfibrils participating in this network were thinner (fig. 4). However, the microfibrillar network formed by the maternal cells seemed less disturbed than that of the paternal cells. Finally, the cells in the fibroblast cultures of the compound-heterozygous MFS child were clearly visible, because they were completely devoid of immunoreactive microfibrils.

Discussion

Several heterozygous mutations in the FBN1 gene that result in MFS have been reported to date. Here we report the first compound-heterozygous mutation in an MFS patient. In other dominant diseases there have been some reports on possible compound-heterozygote patients, but only a few of them describe the molecular details of these cases (Eikenboom et al. 1993; Roa et al. 1993;). To our knowledge this is also the first description of a compound-heterozygote patient with a lethal phenotype in a dominant disease where consequences of specified mutations in both alleles have also been analyzed on the mRNA and polypeptide levels.

The two mutations were found as a compound-heterozygous genotype in a severely affected MFS infant whose phenotype closely resembles that of neonatal MFS infants (Buntinx et al. 1991; Raghunath et al. 1993; Milewicz and Duvic 1994). However, his clinical findings did not include several features reported to be typical of this sporadic, lethal form of MFS, such as joint contractures and crumpled ears (Schollin et al. 1988). He also represents an exceptional case, as he is the child of unrelated parents who both suffer from MFS and were shown to carry one different defective FBN1 allele. We suggest that the severe phenotype of the child is not due to commonly found intrafamilial variation in MFS but, rather, represents the consequences of two defective FBN1 alleles, which both encode an abnormal polypeptide chain.

Almost all autosomal dominant human disorders emerge to more severe phenotype in homozygotes than in heterozygotes. Only in the case of Huntington disease and in an autosomal dominant neoplasia syndrome, MENI, is the phenotype of the heterozygotes and homozygotes reported to be similar, indicating complete dominance of the disease-causing mutations (Wexler et al. 1987; Brandi et al. 1993). Further, in one report of compound heterozygotes in autosomal dominant Charcot-Marie-Tooth disease type 1A, one mutation was shown to be dominant and the other proved to be recessive; and the combination of these two led to somewhat more severe phenotype than occurred with the dominant mutation alone (Roa et al. 1993). In earlier publications (Chemke et al. 1984), probable homozygosity in MFS had been reported to be lethal. Our report on a compound-heterozygote MFS patient with two characterized FBN1 mutations demonstrates incomplete dominance of FBN1 mutations and emphasizes the fact that the normal allele plays a significant role in ameliorating the disease process in MFS.

The W217G mutation of the father occurred in the so-called hybrid motif at exon 6 of FBN1 gene, where a tryp-

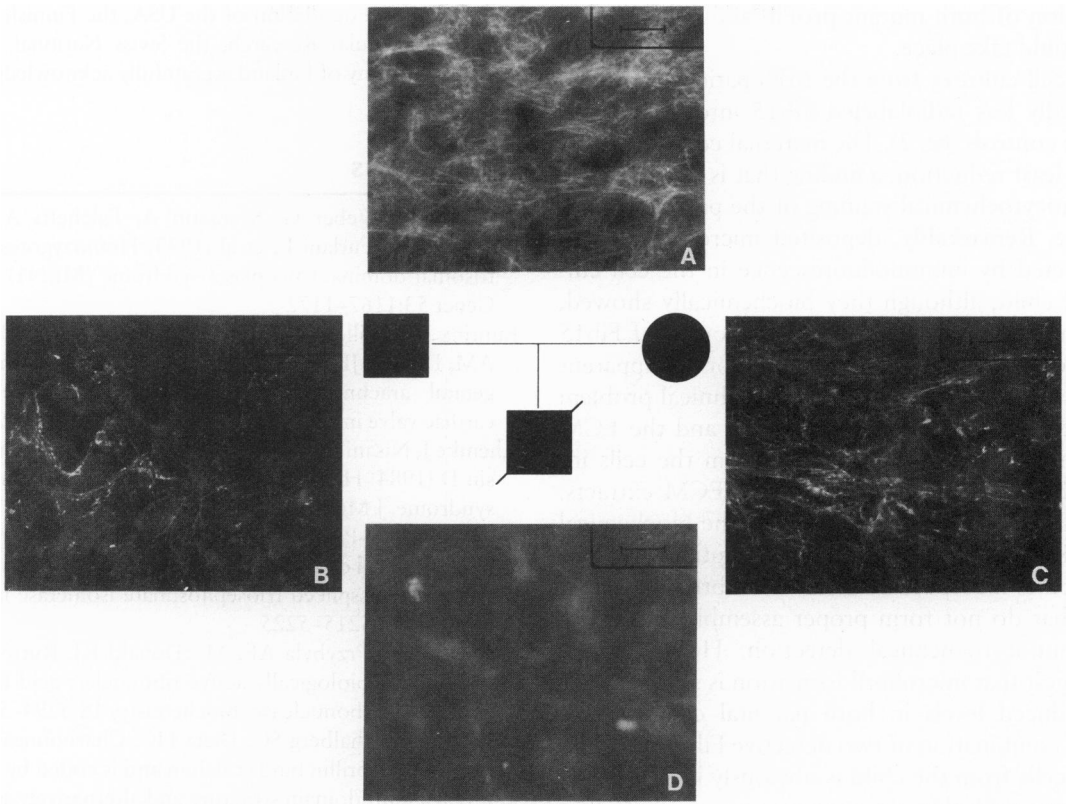

Figure 4 Immunofluorescence pictures of the fibroblast cultures of the control (*A*), the father (*B*), the mother (*C*), and the compound-heterozygous child (*D*).

tophan is conserved through all the TGF-β1-bp-like and hybrid motifs of the Fib15. This mutation has been discussed by us elsewhere (Kainulainen et al. 1994). The G2627R mutation of the mother substitutes an arginine residue for a glycine residue in the cbEGF-like motif at exon 63 of the FBN1 gene. The glycine residue at this position is conserved through all the cbEGF-like motifs. In fact, nuclear-magnetic-resonance studies of EGF-like motifs have revealed that this particular glycine contains one of the two backbone carbonyls that serve as ligands in the binding of calcium ions (Selander-Sunnerhagen et al. 1992). The crucial role of this specific glycine strongly suggests that this mutation would disturb the secondary structure of the cbEGF-like motif.

Since both the identified mutations represent point mutations, it would not have been possible to detect the ratio of two allelic transcripts by methods that rely on size analysis. To determine the steady-state level of the allele-specific transcript, we employed the solid-phase minisequencing method, which allows determination of the ratio between two sequences that differ from each other by a single nucleotide and are present as a mixture in a sample (Ikonen et al. 1992). Genomic DNA was used as a standard for the ratio of two alleles. The ratio between mutant and normal transcripts was essentially identical to the ratio of the alleles of the genomic DNA, and thus neither of these mutations was shown to significantly affect the steady-state level of the mRNA transcribed from the different FBN1 alleles. The result was more or less as expected, since neither of these mutations affected the length of the transcript or created a premature termination signal that has been demonstrated to result in incomplete or aberrant transport and subsequent degradation of the mRNA (Urlaub et al. 1989; Cheng et al. 1990).

Mutant and normal Fib15 molecules, differing only by one amino acid, could not have been differentiated in the fibroblast cultures; but the total amount of secreted Fib15 polypeptides could be monitored by metabolic labeling. Findings at the protein level in fibroblast cultures of all the family members showed slight reduction of intracellular amounts of proFib in comparison with those in the controls. No overt intracellular retention of mutant proFib could be detected, in contrast to a recent report showing such a phenomenon in the cells of a neonatal MFS patient (Milewicz and Duvic 1994). Markedly reduced amounts of Fib15 in the culture media of the cells of the family members were observed, which would suggest a rapid intracellular degradation of mutant proFib before secretion, which may not allow the identification of intracellularly accumulated material. Alternatively, very early extracellu-

lar degradation of both mutant proFib and its processed form, Fib, could take place.

All three cell cultures from the MFS patients incorporated markedly less radiolabeled Fib15 into their ECM than did the controls (fig. 2). The maternal cells, however, showed the least reduction, a finding that is in agreement with immunocytochemical staining of the patient's fibroblast culture. Remarkably, deposited microfibrils could not be detected by immunofluorescence in the cell cultures of the child, although they biochemically showed, in culture medium and ECM extracts, amounts of Fib15 similar to those in paternal cells. A reason for this apparent discrepancy might be the yet-unsolved technical problem of the admixture of intracellular proteins and the ECM extracts; it is likely that this spillover from the cells interferes with the data obtained with the ECM extracts. An additional explanation could be that the biochemical analysis of ECM extracts does not differentiate between Fib15 molecules incorporated into microfibrils and Fib15 molecules that do not form proper assemblies and thus escape immunocytochemical detection. However, our findings suggest that microfibril formation is still possible, albeit at reduced levels in both parental cell cultures, whereas the combination of two defective Fib15 polypeptides in the cells from the child is obviously incompatible with proper microfibril formation.

Interestingly, elsewhere we have reported dramatically decreased levels of both mRNA and polypeptide of decorin, a small connective-tissue dermatan sulfate proteoglycan, in the fibroblast cultures of this child, but not in the parents' fibroblast cultures (Pulkkinen et al. 1990). Independent reports by other groups have also shown decreased steady-state transcript levels of decorin in several neonatal MFS cases with recently specified mutations (Superti-Furga et al. 1992; Raghunath et al. 1993; Kainulainen et al. 1994). The mutations identified in these severe cases of MFS would seem to indicate a special cross-talk in the expression of Fib15 and decorin genes. FBN1 mutations are an excellent tool for analyzing the essential functional domains of Fib15 and for determining the role of these domains in interactions between Fib15 and other ECM components. Specifically, the MFS mutations reported here, affecting different domains in distinct positions of Fib15 polypeptide and resulting in the dramatic decrease in the steady-state transcript level of decorin gene, can now be utilized to analyze in detail the molecular interactions between these two ECM components, by using different in vitro expression systems.

Acknowledgments

We thank Dr. L. Sakai for providing us with the Fib15 antibody. We also thank Dr. Jens Schollin, Dr. K. Kainulainen, Dr. K.-H. Gustavson, N. Blak, L. Burger, and S. Staubli for their help and contribution to this work. The financial support of the National Marfan Foundation of the USA, the Finnish Foundation for Cardiovascular Research, the Swiss National Foundation, and the Academy of Finland is gratefully acknowledged.

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