

A Gly1127Ser Mutation in an EGF-Like Domain of the Fibrillin-1 Gene Is a Risk Factor for Ascending Aortic Aneurysm and Dissection

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Summary

Ascending aortic disease, ranging from mild aortic root enlargement to aneurysm and/or dissection, has been identified in 10 individuals of a kindred, none of whom had classical Marfan syndrome (MFS). Single-strand conformation analysis of the entire fibrillin-1 (FBN1) cDNA of an affected family member revealed a G-to-A transition at nucleotide 3379, predicting a Gly1127Ser substitution. The glycine in this position is highly conserved in EGF-like domains of FBN1 and other proteins. This mutation was present in 9 of 10 affected family members and in 1 young unaffected member but was not found in other unaffected members, in 168 chromosomes from normal controls, and in 188 chromosomes from other individuals with MFS or related phenotypes. FBN1 intragenic marker haplotypes ruled out the possibility that the other allele played a significant role in modulating the phenotype in this family. Pulse-chase studies revealed normal fibrillin synthesis but reduced fibrillin deposition into the extracellular matrix in cultured fibroblasts from a Gly1127Ser carrier. We postulate that the Gly1127Ser FBN1 mutation is responsible for reduced matrix deposition. We suggest that mutations such as this one may disrupt EGF-like domain folding less drastically than do substitutions of cysteine or of other amino acids important for calcium-binding that cause classical MFS. The Gly1127Ser mutation, therefore, produces a mild form of autosomal dominantly inherited weakness of elastic tissue, which predisposes to ascending aortic aneurysm and dissection later in life.

Introduction

Aortic aneurysms are relatively common, and aortic rupture and dissection account for 1%–2% of deaths (Hirst et al. 1958). The most common site is the abdominal aorta, where atherosclerosis and systemic hypertension are the major contributing causes, especially in patients of advanced age. Reports of familial occurrences point to the role of genetic factors for abdominal as well as ascending thoracic aortic aneurysms (Hanley and Jones 1967; Majumder et al. 1991). Systemic disorders of collagen, such as Ehlers-Danlos syndrome type IV (EDS-IV), and of fibrillin (Marfan syndrome [MFS]) are known causes of dominantly inherited aortic disease and are diagnosed by the presence of other connective-tissue abnormalities.

EDS-IV is characterized by thin translucent skin, bruisability, and spontaneous rupture of bowel and large arteries including the aorta. This autosomal dominant condition has been linked to the gene for the collagen type III alpha chain (COL3A1) on chromosome 2q31. More than 20 different mutations in COL3A1 have been identified in families with manifestations of EDS IV (Narcisi et al. 1993).

MFS, an autosomal dominant disorder of connective tissue, with variable manifestations involving primarily the cardiovascular, ocular, and musculoskeletal systems, is linked to markers in or near the fibrillin-1 (FBN1) gene in chromosome band 15q21.1 (Kainulainen et al. 1990; Dietz et al. 1991*b*; Lee et al. 1991; Magenis et al. 1991; Tsipouras et al. 1992). Fibrillin, the 350-kD product of the FBN1 gene, is the main component of connective-tissue microfibrils (Maslen et al. 1991). Mutations in the FBN1 locus are now known to result in a spectrum of clinical features ranging from severe congenital MFS (Dietz et al. 1991*a*; Kainulainen et al. 1994) to classical MFS (Dietz et al. 1992, 1993; Tynan et al. 1993) to autosomal dominant ectopia lentis with skeletal features (Lönnqvist et al. 1994) and milder forms such as the MASS phenotype (Peyeritz and Glesby 1989; Dietz et al. 1993).

Familial forms of ascending aortic dilatation/dissec-

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tion without other connective-tissue abnormalities have rarely been reported (Hanley and Jones 1967; Nicod et al. 1989; Toyama et al. 1989) and have not been elucidated at the molecular level. After the initial discovery of a missense mutation in COL3A1 in an unaffected member of a family that included four individuals affected with aneurysms of various arteries (Kontusaari et al. 1990), it was hypothesized that mutations in this gene could be responsible for a major part of isolated aortic aneurysms (Kuivaniemi et al. 1991). A larger study of 54 probands failed, however, to reveal additional mutations in COL3A1 that cosegregated with the aneurysm phenotype (Tromp et al. 1993), suggesting that the initial family was affected with a variant of EDS-IV.

We have identified a family in which 10 individuals in two generations are affected with varying degrees of ascending-aortic disease, ranging from mild aortic-root enlargement to ascending aortic aneurysm or dissection. No other arteries were affected. The segregation pattern of the aortic disease in this family is consistent with autosomal dominant inheritance. Although isolated minor skeletal and ocular features similar to those seen in MFS were found or reported in some affected individuals, no lens dislocation, pectus deformity, scoliosis, dolichostenomelia, or arachnodactyly was present. As part of a large screening study, we carried out molecular genetic and biochemical analyses of fibrillin. A mutation in the FBN1 gene, Gly1127Ser, was discovered that is likely to be responsible for the aortic dilatation in this family. This study extends the spectrum of clinical phenotypes associated with FBN1 mutations and emphasizes the need for careful clinical evaluation of individuals with a family history of ascending aortic aneurysm despite the absence of diagnostic features for MFS or EDS-IV.

Patients and Methods

Clinical Evaluations and Sample Collection

Informed consent was obtained from all family members, under a research protocol approved by the Human Subjects in Medical Research Committee at Stanford University Hospital. Skin biopsies were performed on II-1 and III-6 (fig. 1), and fibroblast cultures were established. Genomic DNA was extracted from peripheral blood lymphocytes of all individuals in generations II and III, except for II-10, who had died several years earlier. Physical examinations of individuals II-4, III-4, III-5, and III-6 were performed at the Stanford University Center for Marfan Syndrome and Related Connective Tissue Disorders. Height, arm span, upper-to-lower segment ratio, lengths of fingers, hands, and feet, skin texture and extensibility, joint mobility, presence or absence of pectus carinatum or excavatum deformity, pes

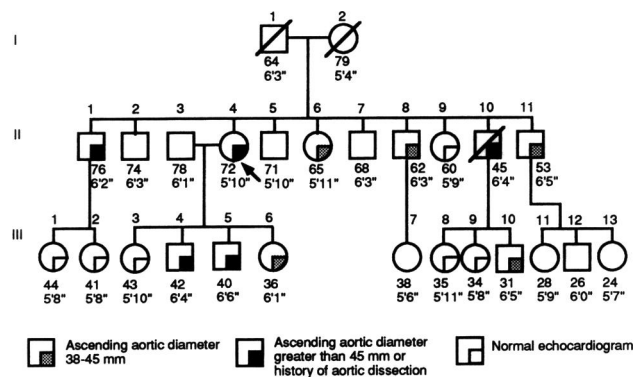


Figure 1 Pedigree of family D, with heights as measured or reported, and ages in 1994 or at time of death. Echocardiographic dimensions of the aortic root and history of aortic aneurysm/dissection are indicated in the lower-right quadrants of each symbol. Individuals indicated by blank symbols were clinically unaffected but did not have echocardiograms.

planus, wrist sign, thumb sign, scoliosis, striae distensae, and venous pattern were recorded. Echocardiographic evaluations including measurement of aortic-root diameters and assessment of valve morphology and function were performed, typically by using M-mode, 2D, and Doppler methods, either at Stanford University Hospital (individuals II-4, III-5, and III-6) or at other institutions (individuals II-6, II-11, III-1, III-2, III-3, III-4, III-8, III-9, and III-10). Chest and abdominal computed-tomography reports were obtained on II-8, and surgical and cardiac catheterization records were reviewed on II-1. Complete ophthalmologic evaluation including assessment of visual acuity, dilated slit-lamp examination for the presence or absence of ectopia lentis, iridodonesis, iris hypoplasia, corneal and anterior chamber abnormalities, and fundus examination was performed on III-4, III-5, III-6. Information on other members of the pedigree, regarding symptoms and diagnoses in the ocular, cardiovascular, and skeletal systems, were obtained by telephone interview, review of medical records, and/or questionnaire.

Mutation Screening of the Fibrillin-1 cDNA and Sequence Analysis

RNA isolation from fibroblasts, reverse transcription, PCR amplification of overlapping fragments of the FBN1 cDNA, single-strand conformation analysis (SSCA), and sequencing were performed as described elsewhere (Tynan et al. 1993). Reagents and procedures used for amplification and sequencing of the Gly1127Ser mutation are as follows. Oligonucleotide primers 348 (5'-GGCACACTGATACTTCCCT) and 349 (5'-GATATCAATGAGTGCAAGATG) complementary to sequences in exons 25 and 28 of the FBN1 gene, were used to amplify a 462-bp cDNA fragment containing

codon 1127 in exon 27. Primers 424 (5'-GACGAAGG-CTATGAAAGTG) and 348 complementary to sequences in exons 26 and 28 were used to amplify an ~1 kb genomic DNA fragment. The PCR products were subcloned using the TA Cloning System (Invitrogen) and were sequenced from both strands.

Allele-Specific Oligonucleotide (ASO) Hybridization Detection of the Gly1127Ser Mutation

Genomic PCR products generated using primers 424 and 348, containing codon 1127, were denatured and applied to Hybond-N membrane by using a vacuum slot blotter as described elsewhere (Tynan et al. 1993). Oligonucleotides complementary to the two alleles were synthesized (normal Gly1127, 5'-GGCAAACAC-CACCTCGG; and mutant Ser1127, 5'-CCGAGG-TAGTGTGGCC). Oligonucleotides were end-labeled with ³²P and annealed to the Hybond-N membranes at 50°C. The filters were washed in 2 × SSPE (0.3 M NaCl, 20 mM NaH₂PO₄, 2 mM sodium EDTA, pH 7.4), 0.1% SDS, for 10 min each at room temperature and at 52°C prior to exposure to X-OMAT AR (Eastman-Kodak) film for 1–8 h.

Studies of Fibrillin Synthesis, Secretion, and Deposition

Quantitative pulse-chase studies of ³⁵S-cysteine-labeled fibrillin were performed on two separate aliquots of skin fibroblast cultures from individuals II-1 and III-6, as described elsewhere (Aoyama et al. 1993). Intensities of fibrillin bands were quantitated by densitometry of autoradiographs or directly by using a phosphor-imager and associated software (Molecular Dynamics).

Analysis of FBN1 Intragenic Dinucleotide-Repeat Polymorphisms

Short tandem-repeat markers mts-1, mts-2, mts-3, and mts-4 were amplified by using a ³²P-gamma-ATP end-labeled primer, electrophoresed on 6% polyacrylamide denaturing gels and were visualized by autoradiography as described elsewhere (Pereira et al. 1994). mts-4 was also amplified, by using unlabeled primers and alpha-³⁵S-dATP incorporation (Weber and May 1989).

Results

Clinical Histories and Evaluations

The proband in this family (individual II-4) was referred because of an aortic root aneurysm, aortic-valve regurgitation, and mitral valve prolapse. Her height was 174.5 cm, arm span was 174.2 cm, weight was 61 kg, and upper-to-lower segment ratio was 0.89 (–1 to –2 SD; patients with MFS usually have ratios <0.85). She did not have arachnodactyly. Third fingers measured 8 cm bilaterally, and palms measured 11.5 cm (both 75th

percentile). She had a somewhat elongated face and moderate dental crowding. No pectus or spinal deformity was present. Shoe size was 11AAA. Pedal arches were pronated, and hammer toes were present. She had soft skin texture but no striae and no joint hypermobility. Eye findings included myopia of –3.75 diopters, a history of eye surgery for low-tension glaucoma resulting in a traumatic cataract, but no lens dislocation. At cataract surgery at age 69 years, the zonules were noted to be fragile, necessitating lens implantation into the anterior chamber. Cardiac catheterization revealed dilatation of the sinuses of Valsalva to a diameter of 66–70 mm (40 mm/m² body surface area). Aortic diameter was normal distal to the sinotubular ridge. She underwent aortic valve and root replacement at Stanford University Hospital. Pathologic examination revealed that her aortic valve had three cusps and was normal in appearance. Histologic changes of the aortic tissue included small accumulations of acidic mucopolysaccharides and separation but not fragmentation of elastic fibers.

Both parents (I-1) and (I-2) had immigrated from Holland. I-1 was 6'3" tall and had varicose veins. He died at age 64 years of a sudden, short illness that was characterized by severe abdominal pain and was thought to be related to cholelithiasis. No autopsy was performed. I-2 had undergone a thyroidectomy and died at age 79 years of breast cancer. She was 5'4" tall.

II-1 was noted at age 65 years to have a wide-appearing aortic arch on chest x-ray, and cardiac catheterization revealed an aortic dissection with an entry site just proximal to the origin of the innominate artery and with extension into the ascending aorta and aortic arch. On surgery he was found to have chronic type A aortic dissection, normal aortic valve, and normal sinuses of Valsalva. A Dacron tube graft extending from the supra-coronary area of the ascending aorta to the transverse arch was placed. Blood pressure had occasionally been noted to be mildly elevated but had not required treatment. At age 74 years he was in stable condition, 6'2" tall, weighed 210 pounds, and reported, by questionnaire, absence of any skeletal and ocular features associated with MFS.

II-2 was 6'3" tall and weighed 180 pounds at age 73 years. By questionnaire, he denied myopia, lens dislocation, aortic aneurysm, scoliosis, pectus deformity, and arachnodactyly. He has not had an echocardiogram but was treated for a myocardial infarction at age 71 years. II-5 is reported to be 5'10" tall and has had an extra kidney removed. No further clinical information is available.

II-6 was 5'11" tall, weighed 142 pounds, and reported that she has had myopia since childhood and developed cataracts in adulthood. An echocardiogram at age 65 years revealed an aortic-root diameter of 38 mm by 2D

examination and 35 mm by M-mode examination (21 mm/m²). Mild mitral regurgitation, mitral valve thickening, left ventricular hypertrophy, and moderate tricuspid regurgitation without evident pulmonary hypertension were reported. She denied scoliosis but reported joint hyperextensibility. She reported long arms and difficulty finding clothing with long enough sleeves. II-7 is 6'3" tall and has had a history of thrombophlebitis.

II-8 was 6'3" tall and weighed 200 pounds. He reported that he began wearing eyeglasses at age 16 years. A chest CT scan in 1988 documented both mild dilatation of the ascending aorta that measured ~40 mm just above the aortic annulus and a stable abdominal aortic aneurysm.

II-9 was 5'9" tall and weighed 148 pounds. She had no vision problems until she developed presbyopia at age 50 years. An echocardiogram performed at age 54 years reportedly revealed no aortic aneurysm but a "leaking valve." She also reported having long arms and "weak ankles and wrists."

II-10 had died suddenly at age 45 years. An autopsy revealed a ruptured type A aortic dissection with cardiac tamponade. An intimal tear was noted 2 cm distal to the coronary ostia, and the dissection extended distally to both common iliac arteries. The cardiac valves were described as normal with no evidence of mitral valve prolapse. Atherosclerosis was average for age. The physical features noted at autopsy were not felt to be suggestive of MFS, and arachnodactyly and dislocated lenses were reported to be absent. II-10 had been 6'4" tall, weighed 230 pounds, had myopia, and started diuretic therapy for hypertension (150/100) 4 mo prior to his death.

II-11 was 6'5" tall. He was reported to have moderate myopia and no lens dislocation. Two small retinal tears were treated with laser in 1992. A 2D M mode Doppler echocardiogram at age 52 years revealed aortic root dilatation (diameter 42 mm) and mild aortic regurgitation. He denied skeletal features of MFS but reported a history of thrombophlebitis.

III-1 and III-2, daughters of II-1, were both 5'8" tall, weighed 150 pounds, and had normal echocardiograms at ages 43 and 40 years, respectively, with aortic-root diameters of 29 and 28 mm, respectively, and absence of mitral valve prolapse. III-1 has been wearing eyeglasses, for myopia, since age 13 years, whereas III-2 reported normal vision. III-3, a daughter of the proband, was 5'10" tall and had normal skeletal and eye findings as reported by questionnaire. An echocardiogram performed as part of this study revealed a normal aortic diameter.

The proband's oldest son (III-4) had an aortic root diameter of 50 mm at age 41 years and had holosystolic mitral valve prolapse. Height was 192.5 cm, and arm span was 192.3 cm. Upper-to-lower segment ratio was

0.94 (0 to -1 SD). The skin was soft, with a venous chest pattern. Scars were normal, and no striae distensae or skin hyperextensibility was present. Hands were proportionally large, with the third finger measuring 9.3 cm and a total hand length of 21.3 cm. Thumb and wrist signs were negative. He had orthodontic treatment because of dental crowding and had a long narrow face with a high, narrow palate. A mild pectus excavatum was present. Scoliosis was not present. Shoe size was 13AAA. Pedal arches were pronated, and hammer toes were present. Ophthalmologic evaluation revealed an uncorrected visual acuity of 20/20 and no iridodonesis or lens dislocation. Subtle ocular features of MFS, however, such as diffuse iris stromal thinning, blunted crypts and furrows, and poor dilation to maximal cycloplegics, were noted.

His brother (III-5) had a maximal aortic root diameter of 44 mm at age 38 years, with an incidental finding of a left atrial myxoma, which was surgically removed. At age 40 years, maximal aortic root diameter was measured at 47-48 mm, with no aortic regurgitation and normal mitral valve. Height was 198.4 cm, with an arm span of 198.6 cm and a weight of 225 pounds. Upper-to-lower segment ratio was 0.92 (-1 SD). Skin texture was soft, with a venous chest pattern but without striae, abnormal scars, or skin hyperextensibility. His third finger was 9.3 cm, and total hand length was 21.3 cm, without joint hypermobility. Thumb and wrist signs were negative. Shoe size was 14 narrow. Pedal arches were pronated.

His sister (III-6) had a mildly dilated aortic root with diameters of 22 mm at the annulus, 40 mm at 1 cm above the annulus, and 41 mm maximum dilatation. Mitral valve prolapse was present by 2D and M-mode criteria. Transverse and abdominal aortic diameters were normal. Physical examination of III-6 revealed a height of 184 cm, weight of 75 kg, arm span of 186.7 cm, and upper-to-lower segment ratio of 0.86; the third finger measured 9.4 cm, and the palm measured 10.7 cm. Thumb and wrist signs were negative. Her face appeared somewhat long and narrow, and her palate was slightly high. Teeth were not crowded. She had a minor asymmetry of the anterior chest wall. No scoliosis, sternal deformity, joint hyperextensibility, or contractures were present. Shoe size was 12½. Detailed ophthalmologic evaluation revealed no lens dislocation, no glaucoma, normal iris, and normal vision. Type III procollagen synthesis, secretion, and efficiency of conversion to type III collagen in cultured fibroblasts from III-6 were normal. The electrophoretic mobility of the type III collagen chains was also similar to those in the control cells (studies performed in the laboratory of Dr. Peter Byers, University of Washington).

III-7 was 165.9 cm tall, weighed 66 kg, and denied vision or heart problems, scoliosis, or other bone or joint abnormalities. He has not had an echocardiogram.

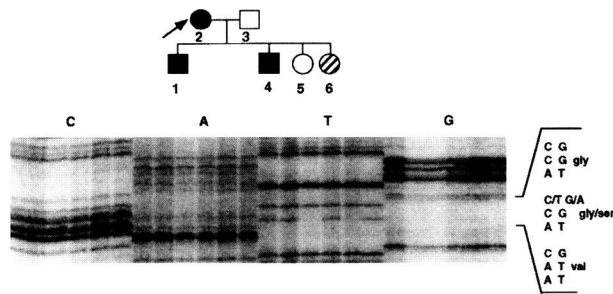


Figure 2 Partial sequence of exon 27 of the FBN1 gene. C, A, T, and G reactions from the proband (arrow; II-4 in fig. 1) and from her husband and children are loaded side by side in the order 1 to 6. Black symbols denote aortic aneurysm; the hatched symbol denotes aortic diameter of 40–47 mm; and white symbols denote normal aortic diameter. PCR-amplified genomic DNA, sequenced from the antisense strand, revealed a C-to-T substitution in lanes 1, 2, 4 and 6, which corresponds to a G-to-A mutation in the sense strand, predicting a substitution of serine for glycine at codon 1127 (Gly1127Ser).

III-8 was 5'11" tall, weighed 155 pounds, and had a "negative evaluation for MFS" at a local hospital when she was pregnant with her first child. She reported having had myopia since age 19 years, as well as long arms and fingers. Echocardiogram at age 34 years revealed an aortic root diameter of 31.5 mm and normal aortic, mitral, and pulmonic valves.

III-9 was 5'8 1/4" tall, weighed 160 pounds, and had an arm span of 5'9 1/2" and an upper-to-lower segment ratio of 0.98. She has worn eyeglasses for myopia and astigmatism since age 7 years but denied lens dislocation, retinal detachment, or skeletal characteristics. Echocardiogram at age 28 years was normal, with an aortic root diameter of 25 mm.

III-10 was 6'5" tall and weighed 245 pounds. He has worn eyeglasses, for myopia, since age 14 years. He denied ectopia lentis, retinal detachment, scoliosis, pectus deformity, pneumothorax, and long arms, legs or fingers. An echocardiogram at age 31 years revealed an aortic root diameter of 38 mm and no mitral valve prolapse. III-11 was 5'9" tall, weighed 142 pounds, denied vision problems and skeletal features, and has not had an echocardiogram or cardiac symptoms. She reported easy bruising.

III-12 was 6' tall and weighed 180 pounds, and III-13 is 5'7" tall, weighs 145 pounds. Both have had myopia since age 14 years, have not had an echocardiogram, and denied skeletal features.

SSCA and Sequencing: Identification of the Gly1127Ser Mutation

SSCA of the entire FBN1 cDNA from individual III-6 revealed a single abnormality that involved the PCR

product corresponding to exons 25–28 (data not shown). Sequencing revealed a G-to-A transition at nt 3379 in exon 27. This base-pair change predicts the substitution of serine for glycine at codon 1127. The sequence change was confirmed on genomic DNA from the proband (II-4) and three of her four children (fig. 2).

Screening by ASO Hybridization Assay: Gly1127Ser Mutation Unique to This Family

Genomic DNA from 23 members of this family, as well as from 64 unrelated individuals affected with MFS, 30 individuals affected with MFS-related phenotypes, and 84 normal controls was tested for the presence of the G3379A nucleotide change, by means of the ASO hybridization assay. Ten family members, nine of whom have aortic dilatation, aortic dissection, or an aortic diameter at the upper limits of normal, were found to be heterozygous for the G3379A transversion (figs. 1 and 3). Although no sample was available for testing, individual II-10 was included as heterozygous, since two of his children were found to carry this mutation. Thirteen family members were homozygous for the normal sequence, as were all the individuals with MFS or related phenotypes and unaffected controls. One family member, II-1, was identified who had a history of chronic aortic dissection but lacked the G3379A change, as determined by the analysis of nucleic acids isolated from blood leukocytes and fibroblast cultures. The ASO hybridization studies have recently been confirmed by SSCA of genomic PCR products from all members of this family. The mutation generates a diagnostic SSCA pattern (not shown).

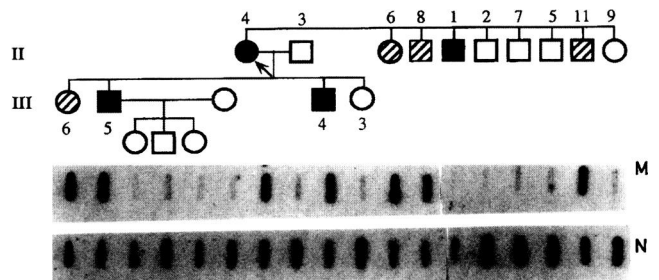


Figure 3 ASO hybridization to detect the Gly1127Ser mutation. Individuals are numbered as in fig. 1, and the shading indicates the status of the ascending aorta (black symbols denote aneurysm or dissection; hatched symbols denote aortic diameter of 38–47 mm; and white symbols denote either a normal aorta or that aortic diameter is unavailable). PCR products including codon 1127 from genomic DNA of individuals II-4, II-6, II-8, II-11, III-6, III-5, and III-4 hybridize with both the mutant and normal ASOs, indicating Gly/Ser heterozygosity. PCR products from the remaining individuals hybridize only with the normal sequence oligonucleotide, indicating homozygosity for the normal (Gly) allele. Hybridization to PCR products from II-1 is weak in this experiment. He had a homozygous normal pattern in other ASO experiments and by sequencing.

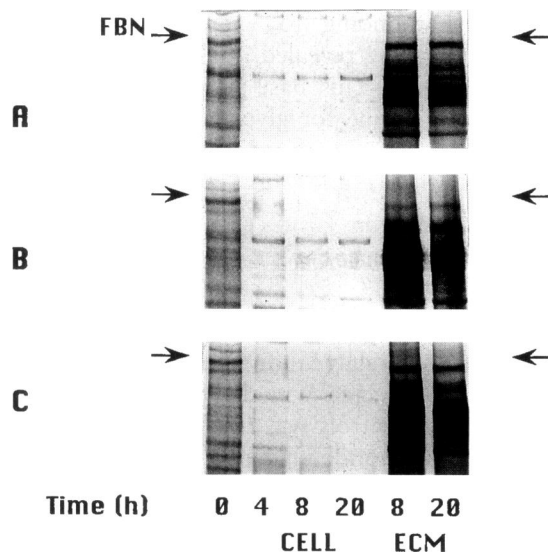


Figure 4 Fibrillin synthesis, secretion, and deposition studies in cultured skin fibroblasts from (A) an unaffected control, (B) III-6 (FB848) with the Gly1127Ser mutation, and (C) II-1 with aortic aneurysm in the absence of the mutation. Cells were pulse-labeled for 30 min with ^{35}S -cysteine and incubated with unlabeled cysteine for 4, 8, or 20 h. Labeled proteins from cell lysates (CELL) and insoluble extracellular matrix (ECM) obtained at the time points indicated were separated by gel electrophoresis. The 350-kD fibrillin band (FBN) is indicated by arrows. Comparison of intensities of fibrillin bands in the three samples reveals equal synthesis (0 time point) but reduced deposition (ECM) at 8 and 20 h in FB848 (B) vs. control (A). The sample in C shows deposition in the normal range.

Fibrillin Protein Studies: Normal Synthesis and Reduced Deposition

Fibrillin protein synthesis and deposition were studied by pulse-chase analysis after cellular proteins were labeled with radioactive cysteine. Initial studies of cultured skin fibroblasts of individual III-6 (FB848) indicated normal levels of fibrillin synthesis (92% of control values) but decreased fibrillin deposition into the extracellular matrix (43% of control values at 20 h after the 30-min pulse) (Aoyama et al., in press). On repeat analysis of another cell aliquot, synthesis was 95%, and deposition was 23% of control values (fig. 4). In both studies, secretion of labeled fibrillin from the cells into the culture medium was normal. In order to determine whether the observed fibrillin abnormality was associated with the Gly1127Ser mutation, a skin biopsy was obtained from individual II-1, who had chronic aortic dissection in the absence of the mutation. Fibrillin synthesis and deposition studies performed on his cultured fibroblasts gave normal results (84% synthesis and 92.5% deposition) (fig. 4).

Typing of FBN1 Intragenic Polymorphisms: Exclusion of Contribution of the Other Allele to Aortic Dilatation Phenotype

Results of typing the dinucleotide markers mts-1, mts-2, mts-3, and mts-4, as well as the Gly or Ser alleles at

1127, are diagrammed in figure 5. Marker mts-4 was informative in generation II and served to identify the FBN1 alleles derived from the non-Gly1127Ser-carrying parent. Phase and generation I haplotypes (A–D) were inferred. The B haplotype, which includes the Gly1127Ser mutation, segregated closely with the aortic dilatation phenotype in this family.

Discussion

We have identified a kindred in which 10 individuals in two generations are affected with abnormalities of the ascending aorta, ranging from mild root dilatation to aortic aneurysm or dissection. There was no history of involvement of other arteries, and no features of EDS-IV, other than smooth thin skin, were present. Biochemical studies of type III collagen, performed by Dr. Peter Byers, definitively ruled out EDS-IV as an underlying cause. Likewise, none of the affected members of this family have classical MFS. Diagnostic criteria include, in the absence of an unequivocally affected first-degree relative, involvement of the skeleton and at least two other systems (ocular, cardiovascular, pulmonary, skin and integument, and CNS) and at least one major manifestation (ectopia lentis, dilatation of the ascending aorta, aortic dissection, or dural ectasia) (Beighton et al. 1988). The affected family members who have been examined have, in addition to either aortic dilatation or aortic dissection, skeletal features that include tall stature with upper-to-lower segment ratios below the mean but within the normal range (<0.85) and proportionally large hands. It should be noted, however, that tall stature is present in nearly all family members, regardless of aortic status or of heterozygosity for the FBN1 mutation. High palate, long narrow face and dental crowding, narrow feet, and hammertoes were occasionally seen, and mild scoliosis and mild pectus deformity were noted in one case each. No dural ectasia, hernias, or pneumothorax have been detected or reported by questionnaire. Only nonspecific, subtle, or incidentally discovered ocular features were present, including myopia, retinal tears (in one case), iris hypoplasia and weak dilator muscle (in one case), and “fragile” zonules discovered in one case, during cataract surgery, in the absence of ectopia lentis. Interestingly, aortic dissection with hypoplasia of iris stroma and dilator muscle has previously been reported in a family without features of MFS (Bixler and Antley 1976). While the combined clinical data on our extended family suggest the presence of a systemic connective-tissue disorder that overlaps with MFS, such a diagnosis would not be considered for most of the mutation carriers with ascending aortic aneurysm if they were to present in isolation.

Mutation screening of the entire FBN1 gene in this family has identified a single nucleotide substitution in

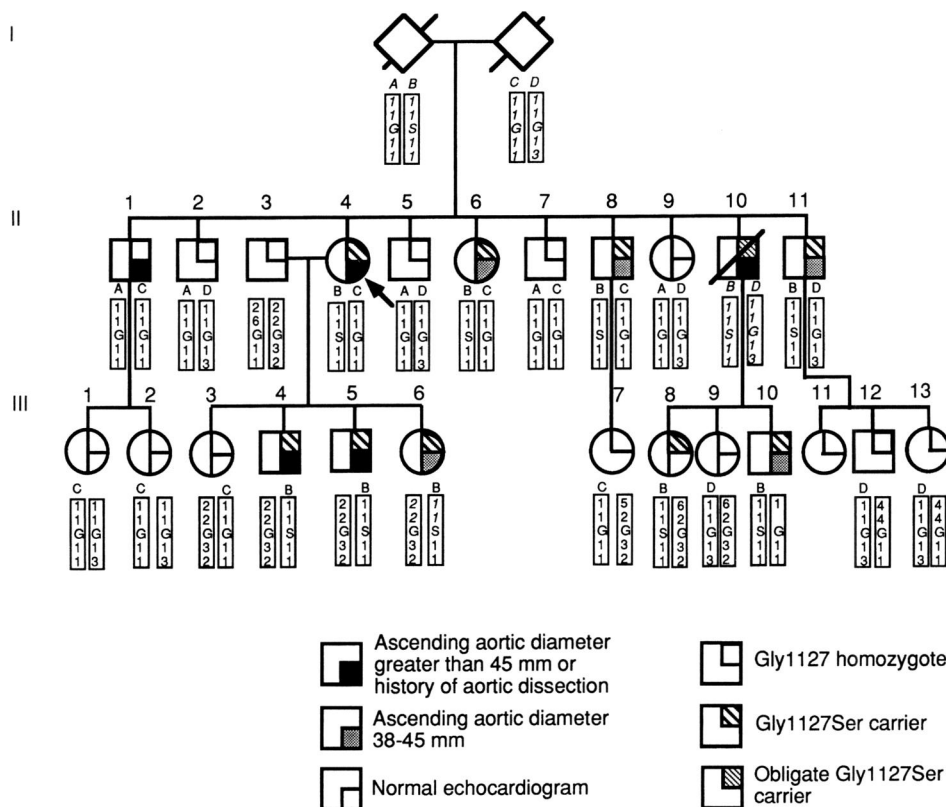


Figure 5 Segregation of Gly1127Ser mutation and FBN1 haplotypes. As in fig. 1, aortic phenotypes are indicated in the lower-right quadrants of the symbols. Genotypes at codon 1127 are shown in the upper-right quadrants of the symbols. II-10 is an obligate Gly1127Ser heterozygote (*thin-hatched shading*), since two (III-8 and III-10) of his three children have inherited a Gly1127Ser allele. The order of markers from top to bottom is mts-1, mts-2, G/S at 1127, mts-3, and mts-4. The normal Gly1127 allele is indicated by a “G,” and the mutant Ser1127 allele is indicated by an “S.” Inferred genotypes and haplotypes (A, B, C, or D) are in italics. It is apparent that the B haplotype containing the mutant S allele is concordant with aortic dilatation/dissection, with the exception of II-1 and III-8. For explanation, see the text.

exon 27, which generates a serine codon at position 1127 instead of the usual glycine codon (Gly1127Ser). This mutation was detected in 9 family members, with a 10th, who had died from a ruptured ascending aortic aneurysm, identified as an obligate carrier. Six of the nine living mutation carriers have either aortic root dimensions greater than the upper limits of normal or a history of aneurysm with aortic root replacement; two have aortic diameters of 38 mm, at the upper limit of normal; and one (III-8), with an aortic root size of 31 mm at age 34 years, may be presymptomatic. We have also identified the mutation in children of generation IV (not shown), who will be followed closely.

Analysis of fibrillin synthesis and deposition in cultured fibroblasts indicates that the Gly1127Ser mutation is associated with alterations at the biochemical level. A normal level of fibrillin synthesis but reduced deposition into the extracellular matrix was observed in cultured fibroblasts from a carrier of the Gly1127Ser mutation who also has mild aortic enlargement. This pattern of fibrillin abnormalities was seen in about half of 55 indi-

viduals with classical MFS (the other half having reduced synthesis in addition to reduced deposition) but not in normal controls (Aoyama et al. 1994). The argument that the reduced deposition is due to the Gly1127Ser mutation is supported by our studies of II-1, who at age 65 years had a chronic type I aortic dissection but does not carry the Gly1127Ser mutation. His skin fibroblasts had a normal fibrillin biochemical phenotype. The aneurysm in II-1 was unusual in that it apparently spared the sinuses of Valsalva, a region of the aorta that is typically affected both in MFS (Pyeritz and McKusick 1979) and in this family. It is therefore likely that his chronic aortic dissection is etiologically distinct from the aortic disease in the other affected family members, who are each heterozygous for the Gly1127Ser mutation. For example, atherosclerosis plays an increasing role in the causation of aortic aneurysm, with advancing age (Eisenberg et al. 1993).

Four polymorphic markers spanning the FBN1 gene were typed to assess a possible contribution of a specific non-Gly1127Ser allele to the phenotype in this family.

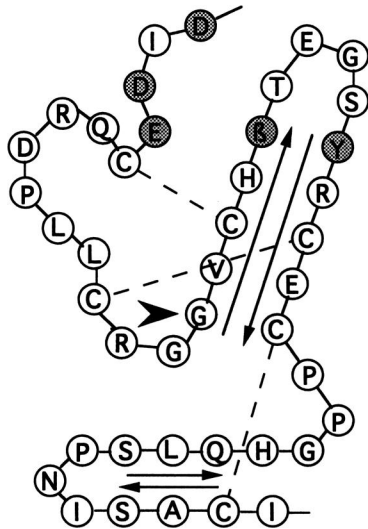


Figure 6 Model of the EGF-like domain encoded by FBN1 exon 27, with location of disulfide bonds (*broken lines*) and amino acids implicated in calcium binding (*shaded*). The arrowhead marks the location of the mutated glycine residue at the entrance to the antiparallel beta-strand structure (*arrows*).

Marker mts-4 was informative in generation II in distinguishing the FBN1 alleles inherited from the parent who did not contribute the Gly1127Ser mutation. As shown in figure 5, either of the mts-4 alleles, 1 or 3, from the non-mutation carrier in generation I were inherited by unaffected, mildly, and severely affected members of generation II. This suggests that both alleles represent normal FBN1 genes that do not contribute to modulation of the phenotype. Under the assumption of no intragenic recombination, the Gly1127Ser-associated haplotype segregated more closely than any other with the aortic dilatation phenotype in this family, making it unlikely that an unidentified FBN1 mutation was present in the non-Gly1127Ser-carrying alleles of any affected individuals.

The Gly1127Ser mutation is located within 1 of the 44 EGF-like domains of FBN1. These domains are present in a variety of proteins and are thought to be important for protein folding and calcium binding (Corson et al. 1993). Substitutions of one of the six conserved cysteine residues of EGF-like domains are the most common missense mutations in MFS (Dietz et al. 1993; Tynan et al. 1993; Kainulainen et al. 1994). Other amino acid substitutions are rare in MFS patients and tend to involve conserved amino acids required for calcium binding (Dietz et al. 1993; Kainulainen et al. 1994). No glycine substitution has previously been reported. Unlike other glycine residues, the mutated glycine at this particular position (fig. 6) is highly conserved in EGF domains of FBN1 and of proteins from many different organisms, presumably because it is located at the turn adjacent to

the predicted antiparallel beta-sheet structure. Interestingly, a glycine-to-serine mutation in the same position in an EGF-like domain of the factor IX gene causes hemophilia B by reducing the biological activity of factor IX to 10% of normal (Denton et al. 1988).

Given the facts that (1) the ascending aortic dilatation/dissection phenotype in this family cosegregates with the glycine-to-serine substitution in a calcium-binding EGF-like domain of the FBN1 gene and (2) only a few amino acid substitutions not involving cysteine residues have been detected in classic MFS, the possibility exists that such mutations tend to cause a "partial" MFS phenotype, as in this family, or that they may remain asymptomatic, as postulated for the Pro1148Ala substitution described elsewhere (Tynan et al. 1993). Consistent with this hypothesis, a Glu2447Lys mutation was identified in a single family with eye and skeletal findings of MFS sparing the cardiovascular system (Kainulainen et al. 1994; Lönnqvist et al. 1994).

The age at which aortic complications develop in association with this mutation is variable in this family and generally later than in MFS. III-8 still has a normal aortic diameter (31 mm) in the 4th decade; III-4, III-5, III-6, and III-10 have aortic diameters ranging from 50 mm to the upper limit of normal, in their 4th decades; II-10 died of aortic rupture in the 5th decade; II-6 has an aortic diameter at the upper limits of normal, in the 7th decade; II-8 and II-11 have mild or moderate aortic enlargement in their 6th and 7th decades, respectively, and II-4 had severe aortic enlargement necessitating surgery in the 7th decade. While we may conclude that the Gly1127Ser mutation predisposes carriers to develop aortic aneurysm and dissection in mid to late adulthood, we do not have long-term follow-up information to provide individual mutation carriers with a prognosis. Serial echocardiogram evaluations and prophylactic treatment as recommended for MFS are certainly indicated. As we begin to screen the next generation (IV) and to identify young mutation carriers who are completely asymptomatic, individual counseling has to include cardiovascular risk factors of diet and lifestyle, in addition to genotype, in an effort to prevent aortic manifestations and complications later in life.

In the family reported by Nicod et al. (1989), death from aortic dissection occurred at a young age, several living members had aortic root dilatations at the level of the sinuses of Valsalva only, and others had aortic root measurements at the upper limit of normal. As in our family, skeletal findings were minimal. Immunofluorescence analysis with fibrillin antibody on fibroblast cultures from surviving members only (none of whom had dissection) were interpreted as normal in the family reported by Nicod et al. Immunofluorescence studies were not done on the family that we studied, because they are nonquantitative and are difficult to interpret if

abnormalities are more subtle. We have used metabolic labeling and pulse-chase studies to quantitate fibrillin synthesis, which was normal, and deposition into the extracellular matrix, which was reduced. These results are consistent with the single amino acid substitution in exon 27, which is not expected to interfere with synthesis or secretion of a normal size fibrillin molecule. Abnormal folding of the EGF-like domain encoded by exon 27, however, is likely to interfere with microfibril formation and probably leads to the reduced matrix deposition observed.

Our findings in this family are consistent with the emerging recognition that FBN1 alterations produce a spectrum of connective-tissue disorders that extend beyond the classical MFS phenotype, for which the term "fibrillinopathy" has been proposed (Aoyama et al., in press). What proportion of individuals with familial or sporadic ascending aortic dissection or aneurysm will ultimately be found to have FBN1 mutations remains to be seen. If fibrillin protein studies give any indication, the search for mutations may be worthwhile, since we have found reduced fibrillin deposition in 6 of 12 individuals with aortic dilatation/dissection and minimal or no associated skeletal or ocular abnormalities (Aoyama et al., in press).

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