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Prenatal Diagnosis and a Donor Splice Site Mutation in Fibrillin in a Family with Marfan Syndrome

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Summary

The Marfan syndrome, an autosomal dominant connective tissue disorder, is manifested by abnormalities in the cardiovascular, skeletal, and ocular systems. Recently, fibrillin, an elastin-associated microfibrillar glycoprotein, has been linked to the Marfan syndrome, and fibrillin mutations in affected individuals have been documented. In this study, genetic linkage analysis with fibrillin specific markers was used to establish the prenatal diagnosis in an 11-wk-gestation fetus in a four-generation Marfan kindred. At birth, skeletal changes suggestive of the Marfan syndrome were observed. Reverse transcription–PCR amplification of the fibrillin gene mRNA detected a deletion of 123 bp in one allele in affected relatives. This deletion corresponds to an exon encoding an epidermal growth factor–like motif. Examination of genomic DNA showed a G→C transversion at the +1 consensus donor splice site.

Introduction

It is now well established that the Marfan syndrome, an autosomal dominant disorder manifested primarily by skeletal, ocular, and cardiovascular abnormalities (Godfrey 1993), is caused by defects in fibrillin. Immunochemical studies were the first to suggest this association (Godfrey et al. 1990; Hollister et al. 1990); and the cloning of the gene encoding fibrillin permitted genetic linkage to the Marfan syndrome (Lee et al. 1991*a*). The deduced primary structure of fibrillin is composed of repeated epidermal growth factor (EGF)-like motifs interspersed at varying intervals by 8-cysteine domains of the TGF-β1-binding protein type (Lee et al. 1991*a*). Amino acid substitutions causing the Marfan syndrome have been documented within the EGF-like domains (Dietz et al. 1991, 1992*a*, 1992*b*; Kainulainen et al. 1992). The genomic deletion of three such motifs has also been shown to segregate with the Marfan phenotype in one family (Kainulainen et al. 1992). In addition, an unusual exon-skipping mutation, caused by a premature termination codon, has also been described (Dietz et al. 1993).

Prenatal diagnosis in the Marfan syndrome has previously been attempted using ultrasonographic techniques (Koenigsberg et al. 1981), but predictions based on those results were not better than the a priori risk. The virtual absence of genetic heterogeneity in the Marfan syndrome (Tsipouras et al. 1992) permits the use of molecular-based linkage analysis in prenatal or presymptomatic diagnosis in informative families. Here we report prenatal diagnosis in a Marfan syndrome family by using these molecular methodologies. In addition, the lesion causing the Marfan syndrome in this kindred is described.

Material and Methods

Extraction of Nucleic Acids

DNA.—DNA was extracted from peripheral whole blood by lysing cell membranes in a buffer containing 0.32 M sucrose, 10 mM Tris-HCl (pH 7.5), 5 M $MgCl_2$,

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and 1% Triton X-100. The intact nuclei were recovered by centrifugation, suspended in a detergent solution (50 mM KCl, 10 mM Tris-HCl [pH 8.3], 2.5 mM $MgCl_2$, 0.1 μg gelatin/ml, 0.045% Nonidet P40, 0.45% Tween 20), and incubated with Proteinase K at a final concentration of 0.1 μg/ml at 50°C for 16 h.

Extraction of DNA from the chorionic villus sample (CVS) was carried out by incubating 20 mg of tissue in 200 μl of lysis buffer (10 mM Tris-HCl [pH 8.0], 2 mM EDTA, 10 mM NaCl, 5% SDS, and 200 μg Proteinase K/ml) at 55°C for 16 h. In all extractions, the aqueous DNA was recovered using phenol/chloroform extractions and isopropanol precipitation. The extracted DNA was suspended in a 10 mM Tris-HCl (pH 7.4), 1 mM EDTA (pH 8.0) solution, and concentration and purity were assessed spectrophotometrically.

RNA.—RNA was extracted from dermal fibroblasts of the proband and from cultured CVS cells by using established techniques (Chomczynski and Sacchi 1987). Briefly, cells were lysed in situ in a guanidine thiocyanate buffer containing β-mercaptoethanol. RNA was extracted with phenol/chloroform, precipitated with ethanol, and stored at −80°C prior to use.

cDNA Production

Production of cDNA from RNA was performed using a commercially available kit (Amersham) according to the manufacturer's protocol.

Southern Blot

Southern analysis was carried out using well-established methods (Sambrook et al. 1989). Briefly, 10 μg of genomic DNA was digested with restriction endonuclease *Taq*I (Promega), was separated by agarose gel electrophoresis, and was transferred to a nylon membrane (Oncor). The fibrillin-specific probe MF-13 (Lee et al. 1991*a*) was random primed using a commercial kit (Amersham). After hybridization, the membrane was washed at high stringency and exposed to X-ray film at −70°C with intensifying screens.

VNTR (TAAAA)n

Genotypes of the fibrillin (TAAAA)n VNTR marker were determined using the oligonucleotide primers and conditions described elsewhere (Lee et al. 1991*a*).

PCR and Nucleotide Sequencing

Reverse transcription (RT)–PCR.—Amplification of the proband's fibroblast and fetal CVS cDNA, in the region of the deletion, was performed using the oligonucleotide primers forward, 5′ GGA GGT TTT GAA TGC ACC TGC 3′ and reverse, 5′ GTA GCA CCT CTG TGA AGC AG3′ at the following conditions: denaturing at 94°C for 1.5 min., annealing 60° for 2.5 min., and extension 72° for 2.5 min. for 30 cycles.

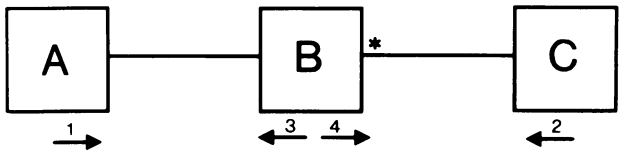

Figure 1 Illustration of primary structure of the fibrillin gene in the region of the deleted exon (B). Genomic PCR with primers 1 and 2 showed only one fragment, suggesting that the mutation was not due to genomic deletion. Amplification and sequencing with primers 1 and 3 showed no alteration of the consensus splice sites for the intron between exons A and B. Amplification and sequencing with primers 4 and 2 showed a mutation in the consensus splice donor site (indicated by an asterisk [*]). Primer sequences are primer 1, 5′ GGA GGT TTT GAA TGC ACC TGC 3′; primer 2, 5′ CCC TCT TCA CAC TCA TCC TCA 3′; primer 3, 5′ TCG GAA GGC ACA GAG CAG AG 3′; and primer 4, 5′ TGG GAT ATG TGC TCA GAG AAG A 3′.

Genomic PCR.—Amplification of genomic DNA from affected, unaffected, and control individuals was performed as illustrated in figure 1. The oligonucleotide primers used to amplify the intron harboring the mutation—forward, 5′ TGG GAT ATG TGC TCA GAG AAG A 3′; and reverse, 5′ CCC TCT TCA CAC TCA TCC TCA 3′—were used at the following conditions: denaturing at 94°C for 1.5 min, annealing at 62°C for 2.5 min, and extension at 72°C for 3.0 min, for 30 cycles.

Amplified material was cloned prior to sequencing, using TA cloning vectors from a commercial source (Novagen, Madison) according to manufacturer's instructions. Briefly, approximately 3–5:1 mass ratio of gel-purified PCR product to vector was ligated at 16°C for 16 h. The ligated product was used for transformation of competent cells (Nova Blue; Novagen). Clones containing inserts (white colonies) were expanded, and plasmid DNA was extracted for sequencing. Sequencing of plasmid DNA from TA-cloned PCR products was performed using Sequenase version 2.0 (U.S. Biochemical) according to the manufacturer's protocol. Universal M13 forward and reverse primers were used for all sequencing reactions.

Results

Clinical Report

The proband, a 19-year-old woman with Marfan syndrome, was referred to the University of Wisconsin

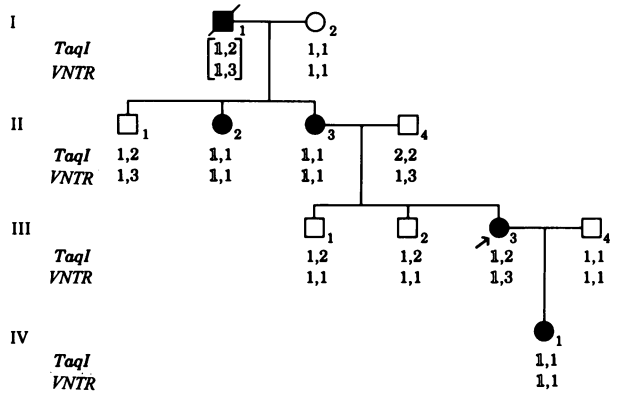

Figure 2 Summary of *Taq*I RFLP and VNTR analysis demonstrating segregation of the *Taq*I 1 and VNTR 1 haplotype (open numerals) in all affected individuals. The proband's grandfather (I.1) was not analyzed; therefore, the haplotypes have been inferred from his children. The proband's daughter (IV.1) was ascertained prenatally. Arrow indicates the proband.

prenatal genetics service during the 8th wk of her first pregnancy (fig. 2, III.3). By history, she had arachnodactyly at birth, and at the age of 4 years she was noted to be tall and thin, with pectus excavatum, scoliosis, marked joint instability, and dolichostenomelia. Urine and plasma amino acid analyses were normal. She developed a murmur at age 5 years; cardiac evaluation, including echocardiography, showed mitral valve prolapse and regurgitation, and a dilated aortic root. Left ectopia lentis was also identified at that time, and the diagnosis of Marfan syndrome was established. She has since developed severe myopia. Harrington rods were placed at age 13 years because of increasing thoracic scoliosis. She had an extremely narrow palatal arch which required extensive maxillofacial surgery. Her mitral valve prolapse persisted, but regurgitation gradually resolved. At the time of referral, her echocardiogram showed moderate mitral valve prolapse without regurgitation, normal left ventricular size and function, and an aortic root diameter of 32 mm. Physical examination showed a height of 182 cm, an arm span of 186 cm, and an upper/lower segment ratio of .67. She had mild flexion contractures of all of her fingers but normally formed auricles. The remainder of the exam confirmed the previously noted findings. Monthly echocardiograms throughout the remainder of pregnancy showed no increase in the aortic root diameter.

At the age of 33 years, the mother (fig. 2, II.3) of the proband was identified as having Marfan syndrome shortly after the diagnosis was established in the proband. She had a history of two spontaneous pneumothoraces during adolescence. She was found to have tall stature, myopia, a narrow palatal arch, pectus excavatum, scoliosis, arachnodactyly, dolichostenomelia, and joint laxity manifested with dislocations. Cardiac evaluation showed mitral valve prolapse without regurgitation or aortic root dilatation. Physical examination at 47 years of age showed a height of 177 cm, an arm span of 190 cm, and an upper/lower segment ratio of .83 and midfacial hypoplasia. She had no flexion contractures of the digits, and the remainder of the examination confirmed the findings noted above.

A maternal aunt (fig. 2, II.2) is also known, by history, to have tall stature, high myopia, pectus excavatum, mitral valve prolapse, severe scoliosis, and joint laxity. The maternal grandfather (fig. 2, I.1) was 6 ft 5 inches tall and died at age 34 years as the result of a sudden cardiac event. By history, he had pectus excavatum, pes planus, joint instability, an enlarged heart, and high myopia. The brothers, maternal uncle, and great aunt and uncle of the proband appear to be unaffected. The proband's husband, father, and one brother were examined and had no clinical evidence of Marfan syndrome.

The female infant (fig. 2, IV.1) was born vaginally after a 37-wk gestation, weighing 2.69 kg, and with a length of 47.8 cm and a head circumference (OFC) of 35 cm. There were no perinatal or neonatal complications. An ophthalmological examination at age 6 wk showed no evidence of ectopia lentis. Physical examination at 9 wk of age showed a length of 59.3 cm (75th percentile), weight of 4.66 kg, OFC of 39 cm, and an arm span of 59.8 cm. Although her arm span did not significantly exceed her length, her upper/lower segment ratio was low at 1.1. She had normal midfacial development and a normal palatal arch. She also had a mild pectus excavatum which became more pronounced when she cried and which, by history, was more noticeable when she had nasopharyngeal congestion during a recent cold. Her cardiovascular examination was normal. She had hyperextensible fingers and hips. Her hands were 7.3 cm long (12.3% of her total body length), but she did not have significant arachnodactyly.

Segregation

Immunochemical analysis with antibodies to fibrillin of skin and dermal fibroblast cultures of the proband showed significantly reduced staining of elastin-associated microfibrils at the dermal/epidermal junction, poorly coated elastin profiles in the dermis, and decreased apparent accumulation of immunostainable

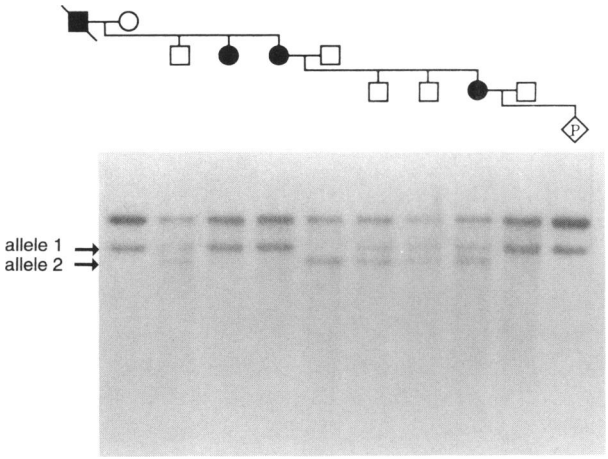

Figure 3 Southern analysis of *Taq*I RFLP of fibrillin gene on chromosome 15, demonstrating informative segregation of the Marfan phenotype with fibrillin, which allowed prenatal diagnosis of the proband's fetus (P). The uppermost band is invariant when genomic DNA, cut with the restriction enzyme *Taq*I, is hybridized with a fibrillin cDNA probe (Lee et al. 1991*a*). Since this is a dimorphism, up to two variable bands can be seen below the invariant band. These have been labeled "allele 1" and "allele 2."

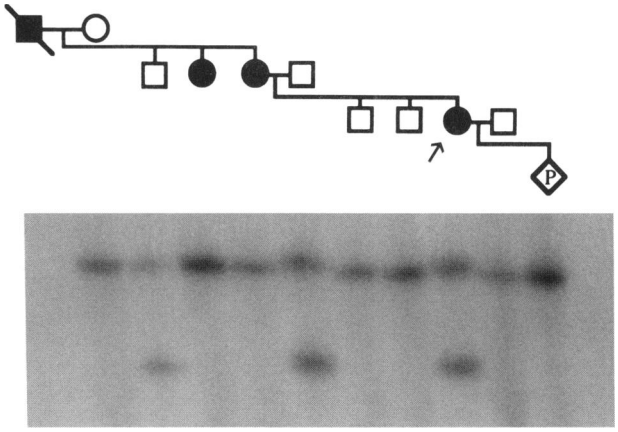

Figure 4 VNTR (TAAAA)n of the fibrillin gene on chromosome 15, demonstrating segregation with the Marfan phenotype and assignment of a positive prenatal diagnosis to the proband's fetus (P). Arrow indicates the proband.

fibers in hyperconfluent fibroblast cultures. These findings are consistent with our previous findings for Marfan syndrome (Godfrey et al. 1990; Hollister et al. 1990).

Prenatal diagnosis of the proband's fetus was accomplished using two different fibrillin polymorphic markers. Analysis of the *Taq*I RFLP (fig. 3) showed segregation of the Marfan phenotype with allele 1. Since the fetus was a 1,1 homozygote, it had inherited the maternal allele linked to the Marfan phenotype. Similarly, VNTR analysis (fig. 4) confirmed the inheritance of the linked allele, since the proband is also heterozygous for this marker. A summary of the linkage data is shown in figure 2. The genotype of the proband's grandfather (I.1) has been inferred from his children and spouse (I.2). The proband's daughter (IV.1), found prenatally to have inherited the affected allele, has already shown some of the skeletal changes associated with the Marfan phenotype.

Mutation Analysis

Our mutation-screening approach uses PCR amplification of the fibrillin cDNA and analysis of the amplified products by single-strand conformation polymorphism (SSCP) or denaturing gradient-gel electrophoresis (DGGE). Overlapping 250–500 bp regions of the fibrillin cDNA are amplified to ascertain possible single-base changes. In order to identify the molecular lesion in this kindred, the above strategy was implemented. The initial 11 sets of primers yielded a single fragment when the proband cDNA was amplified, and no abnormally migrating bands were observed in SSCP and DGGE analyses of those amplifications.

RT-PCR of RNA from the proband's dermal fibroblasts, with the 12th-pair primers tested (see above), showed the appearance of two fragments when a region

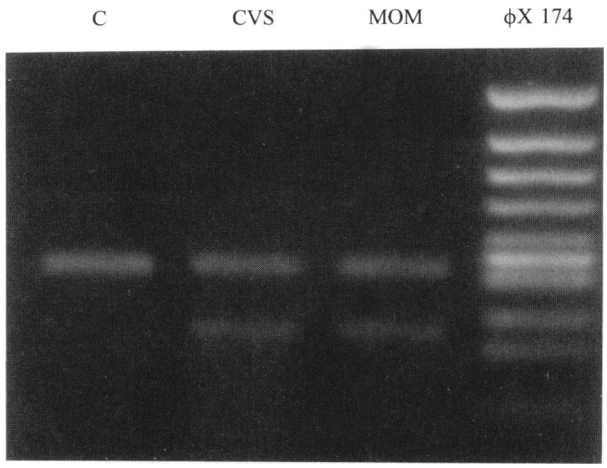

Figure 5 RT-PCR results demonstrating the presence of one fragment in control (lane C) and two fragments in the fibroblasts from the mother (lane MOM) and from fetal CVS cells. The marker lane is ϕX 174 cut with *Hin*cII. Both upper and lower bands from MOM and CVS were cloned and sequenced.

of approximately 250 bp of cDNA was amplified (fig. 5). In addition, cDNA derived from CVS cells produced the same two-band pattern (fig. 5). Control fibroblasts (fig. 5) and CVS cells (data not shown) demonstrated only one fragment. Both upper and lower bands were used for cloning and sequencing.

Sequencing of the cloned fragments from the proband demonstrated a deletion of 123 bp of the coding region in the lower band, when compared with the upper band or control individual (fig. 6). The deleted region begins at position 6664 and corresponds to an entire EGF-like coding exon (sequence provided by Dr. F. Ramirez).

Genomic PCR failed to demonstrate a genomic deletion in affected individuals when compared with controls. Therefore, amplification and sequencing of the introns surrounding the deleted exon, in cDNA analysis, was pursued. Figure 1 illustrates the primers used in the ascertainment of the molecular lesion at the gene level.

No abnormalities were found when the consensus splice sites of the 5′ intron were sequenced after cloning of products amplified with primers 1 and 3 (data not shown). Sequencing of clones from amplification with primers 4 and 2 (fig. 1) demonstrated two changes in the consensus donor splice site of the 3′ intron in three of six clones of the proband and in two other affected members. Neither change was observed in a control individual or in two unaffected relatives. Figure 7 illustrates the exon/intron boundary, demonstrating two changes in the donor consensus splice site. The invariant +1 position G is changed in the mutant allele to a C. In addition, the +3 position of the same allele is a T, while the corresponding base in the normal allele and in a control sample is a G. These same two changes in the same allele were observed in three affected members of this kindred, but not in two unaffected relatives. It is unclear whether the second change is a neutral polymorphism, although both changes were seen only in the abnormal allele. On the basis of our current understanding of splicing mutations, we concluded that the +1 change is responsible for the skipping of this EGF-like coding exon, while the other substitution is likely to represent a neutral polymorphism.

In order to test that possibility, we designed an antisense intronic oligonucleotide primer (5′ TTC CCA GCC TTC TCC TAC TAA 3′) about 100 bp from the donor splice site. The above primer was used with an exonic sense oligonucleotide (fig. 1, primer 4) to generate a product of about 150 bp. Amplified products were restricted with *Hin*fI, since the change of G to T at the +3 position eliminates a *Hin*fI restriction site. Restriction mapping analysis of 50 unrelated chromosomes failed to show any individual with a possible +3 position change.

Discussion

The availability of markers for numerous inherited disorders has enabled the prenatal or presymptomatic diagnosis in families (Evans et al. 1989; Maddalena et al. 1992). The recent discovery of the gene encoding fibrillin and the proof that fibrillin mutations cause the Marfan syndrome and its autosomal dominant inheritance with virtually complete penetrance have permitted prenatal and presymptomatic diagnosis for this disorder as well. The desire for prenatal diagnosis in the Marfan syndrome antedates the discovery of the "Marfan gene" by more than a decade. Koenigsberg and coworkers (1981) used ultrasonic techniques, but the results were not better than the a priori risk of inheriting the disorder.

The attitude toward the availability of prenatal analysis in single-gene disorders has been well studied for cystic fibrosis. An average of 70% of people responding to questionnaires indicated a desire for prenatal diagnosis (Botkin and Alemagno 1992; Decruyenaere et al. 1992; Denayer et al. 1992; Watson et al. 1992; Wertz et al. 1992). Of all populations studied, few disapproved of these analyses (Miller and Schwartz 1992). These studies are indicative of the desire for people to become psychologically prepared about the birth of a child with a genetic disorder, since only a minority would consider termination of the pregnancy. These questionnaire studies have been done to a limited degree in the Marfan syndrome. In one large family, 78% of affected individuals would choose prenatal diagnosis if it were available (Bridges et al. 1992). In a separate study, 96% of respondents would use prenatal diagnosis, if available, versus 3% who would not (Child et al. 1992). It is important to note that these analyses are not only useful for prenatal diagnosis but also may have a much broader and significant impact on presymptomatic diagnosis. In fact, in the Marfan family surveyed by Bridges et al. (1992), 96% would have chosen a definitive screening test if available.

There are many families with the Marfan syndrome, in which some relatives may have few minor clinical manifestations yet may be considered affected primarily because of a positive family history. The ability to determine, at the molecular level, whether one has inherited the defective allele will allow for proper follow-

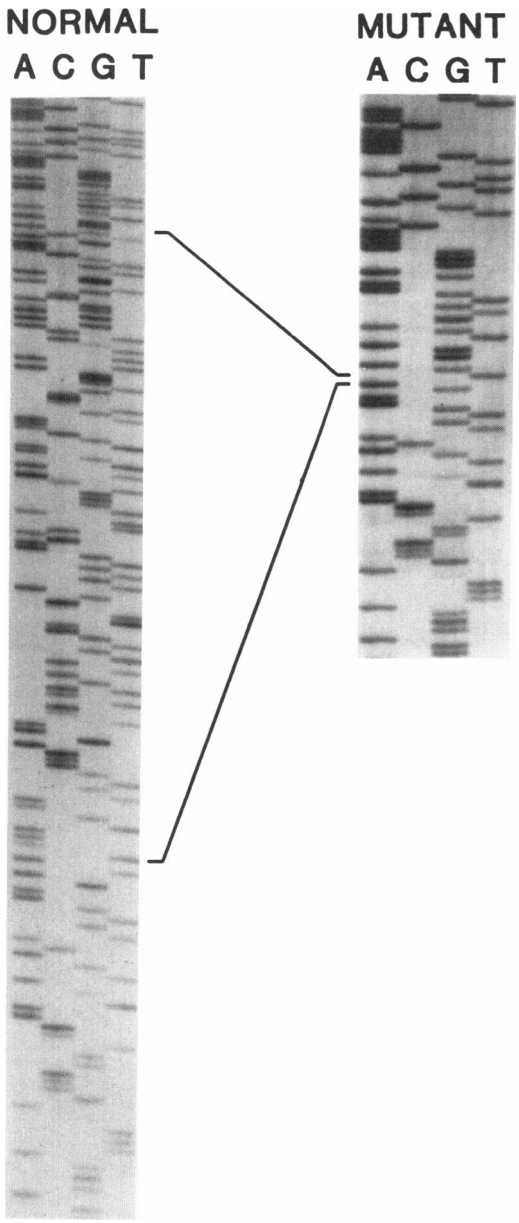

Figure 6 Sequence of cloned cDNA of both the upper (*normal*) and lower (*mutant*) bands (see fig. 5) from the mother, showing a deletion of 123 bp, which corresponds to an entire EGF-like exon. The deletion begins at position 6664 (position numbers are from the entire coding region, provided by Dr. F. Ramirez) and extends 123 bp. This exon corresponds to the 35th of 44 EGF-like exons in the fibrillin gene on chromosome 15.

up and counseling of that individual. Of equal importance, the determination that a relative has not inherited the defective allele will permit the individual to live a more normal life and be spared the expense of sophisticated annual medical tests.

In the case described herein, the family considered the status of the fetus to be a critical, but not the sole, factor in their decision regarding continuation of the pregnancy. After the CVS was performed and the results were known, they made the decision to continue on the basis of other considerations. In the end, then, the results of the evaluation did not affect the outcome of the pregnancy per se. Clearly, however, the knowledge that the child will likely be affected enabled the family to begin facing and accepting this prospect.

These data allowed for the unequivocal prenatal assignment of a defective fibrillin allele, by linkage analysis, to an 11-wk-old fetus, constituting prenatal diagnosis of the Marfan syndrome, thus predicting clinical status. In the context of the family history, her skeletal disproportion, joint hypermobility, and pectus excavatum seemed sufficient for clinical confirmation of the diagnosis of Marfan syndrome. Identifying this infant as affected, prior to the development of more obvious phenotypic manifestations, provides a unique opportunity to prospectively follow the natural progression of this disorder.

Since most reported molecular defects have been point mutations, overlapping oligonucleotide primers are used to amplify varying segments of fibrillin cDNA prior to analysis for single-base changes, with techniques such as SSCP or DGGE. In one of the amplified products, we observed two fragments for this proband,

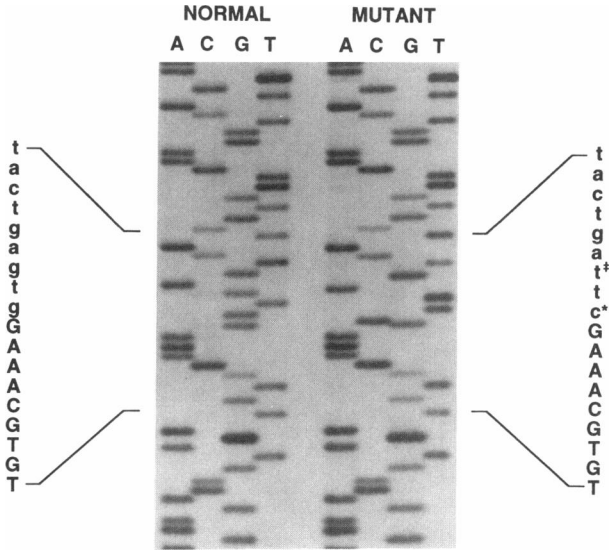

Figure 7 Sequence of cloned genomic material at the exon/intron junction of the misspliced exon demonstrating two changes in the mutant allele. The +1 position (indicated by an asterisk [*]) in the mutant allele is a C rather than a G, and the +3 position (indicated by a double dagger [‡]) in the same allele is a T rather than a G.

but only one for other individuals with Marfan syndrome or normal controls. The same observation was made in material obtained from CVS cells (fig. 5). It is of interest that, although fibrillin mRNA is present in CVS cells in amounts detectable not only by RT-PCR (fig. 5) but also by northern hybridization (data not shown), no immunostainable fibrils were seen in CVS cell cultures. This was true of both the Marfan CVS cells, as well as those of a gestational age–matched control. This suggests that fibril and matrix formation in vitro probably requires the presence of additional factor(s), which may include other microfibrillar proteins, to form a complete fibril.

In order to ascertain whether this finding represented the outcome of a mutation, we cloned and sequenced both fragments amplified from cDNA from the proband's fibroblasts and the fetal CVS cells. Sequence analysis demonstrated a G→C transversion at the +1 donor splice position in one allele (fig. 7). This position has been shown to be invariant in practically all examined eukaryotic genes (Padgett et al. 1986; Shapiro and Senapathy 1987; Jacob and Gallinaro 1989). Examples of mutations at this position causing exon skipping have been documented in several diseases, including another connective tissue disorder, Ehlers-Danlos syndrome (Cole et al. 1990; Kuivaniemi et al. 1990; Weil et al. 1990; Vasan et al. 1991; Pope et al. 1992), as well as lipoprotein lipase deficiency (Gotoda et al. 1991*a*, 1991*b*; Chimienti et al. 1992), Tay-Sachs disease (Arpaia et al. 1988; Landels et al. 1992), aspartylglycosaminuria (Mononen et al. 1992), and APO C-II deficiency (Fojo et al. 1988). Mutations at the +2, +3, and +5 positions of the donor splice site consensus sequence have also been observed (Atweh et al. 1987; Weil et al. 1988; Gonzalez-Redondo et al. 1989; Bonadio et al. 1990; Lee et al. 1991*b*; de Boer et al. 1992; Pope et al. 1992).

It is interesting that another change was observed in the allele harboring the +1 position G→C transversion. All clones containing the mutation at the +1 position also had, at the +3 position, a T rather than a G, which was observed in the normal allele. This pattern was seen in all affected members who were examined. Unaffected relatives had neither change. Changes at the +3 position are also known to cause mutations (de Boer et al. 1992); however, there is some degree of polymorphism associated with that position. Although the vast majority of +3 donor splice site position bases are G or A, some intronic sequences with a T at that position have been described (Padgett et al. 1986; Shapiro and Senapathy 1987; Gonzalez-Redondo et al. 1989; Jacob and Gallinaro 1989; Sutton et al. 1989). Therefore, to assess whether the +3 change is unique to this family or a more common polymorphism, we performed restriction mapping of 50 unrelated chromosomes but failed to identify any with possible +3 position alterations. The change noted at the +3 position in the kindred described here may still be a rare polymorphism and not directly related to the exon-skipping mutation. Alternatively, the +3 change may be unique to this family's abnormal allele and may contribute to the misplicing. Nevertheless, it is evident that the G→C change at the invariant +1 position would cause misplicing.

Most mutations currently known are single-base substitutions. One mutation in a large kindred has been shown to be a genomic deletion which includes three EGF-like exons. It is noteworthy that only the proband had ectopia lentis, and it was unilateral, which is rare in Marfan syndrome. Other affected relatives have no reported lens dislocation. Deletion or skipping of EGF-like exons may be envisaged, in certain regions of the fibrillin gene, to produce a shortened protein that retains sufficient structural integrity to provide enough support in the ciliary zonule but not in the other systems where the pleiotropic manifestations are observed. This does not necessarily mean that all Marfan families without ectopia lentis will have exon deletions or, alternatively, that families in which a deletion or skipping of an exon is observed will not have ectopia lentis. In fact, families with point mutations in an EGF-like exon and without ectopia lentis have already been observed (Dietz et al. 1992*a*). A caveat to this possibility may explain the presence of unilateral lens dislocation in one member of this kindred. For example, trauma or other circumstances may precipitate lens subluxation because of the a priori molecular defect in fibrillin. It is the hope that the elucidation of molecular defects will enable the construction of genotype-phenotype correlations. These, coupled with immunochemical and biochemical findings, may become of prognostic value in the clinical management of the Marfan syndrome.

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References

Arpaia F, Dumbrille-Ross A, Maler T, Neote K, Tropak M, Troxel C, Stirling JL, et al (1988) Identification of an altered splice site in Ashkenazi Tay-Sachs disease. Nature 333:85–86

Atweh GF, Wong C, Reed R, Antonarakis SE, Zhu D, Ghosh PK, Maniatis T, et al (1987) A new mutation in IVS-1 of the human β globin gene causing β thalassemia due to abnormal splicing. Blood 70:147–151

Bonadio J, Ramirez F, Barr M (1990) An intron mutation in the human alpha 1(I) collagen gene alters the efficiency of pre-mRNA splicing and is associated with osteogenesis imperfecta type II. J Biol Chem 265:2262–2268

Botkin JR, Alemagno S (1992) Carrier screening for cystic fibrosis: a pilot study of the attitudes of pregnant women. Am J Public Health 82:723–725

Bridges AB, Faed M, Boxer M, Gray JR, Bundy C, Murray A (1992) Marfan syndrome in a large family: response of family members to a screening programme. J Med Genet 29:81–85

Child A, Poloniecki J, Camm AJ (1992) Reproductive decisions and the potential demand for prenatal diagnosis. Paper presented at the Second International Symposium on the Marfan Syndrome, San Francisco, November 7–9

Chimienti G, Capurso A, Resta F, Pepe G (1992) A G→C change at the donor splice site of intron 1 causes lipoprotein lipase deficiency in a Southern-Italian family. Biochem Biophys Res Commun 187:620–627

Chomczynski P, Sacchi S (1987) Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal Biochem 162:156–159

Cole WG, Chiodo AA, Lamande SR, Janeczko R, Ramirez F, Dahl H-HM, Chan D, et al (1990) A base substitution at a splice site in the COL3A1 gene causes exon skipping and generates abnormal type III procollagen in a patient with Ehlers-Danlos syndrome type IV. J Biol Chem 265:17070–17077

de Boer M, Bolscher BGJM, Dinauer MC, Orkin SH, Smith CIE, Åhlin A, Weening RS, et al (1992) Splice site mutations are a common cause of X-linked chronic granulomatous disease. Blood 80:1553–1558

Decruyenaere M, Evers-Kiebooms G, Denayer L, Van Den Berghe H (1992) Cystic fibrosis: community knowledge and attitudes towards carrier screening and prenatal diagnosis. Clin Genet 41:189–196

Denayer L, Evers-Kiebooms G, DeBoeck K, Van Den Berghe H (1992) Reproductive decision making of aunts and uncles of a child with cystic fibrosis: genetic risk perception and attitudes toward carrier identification and prenatal diagnosis. Am J Med Genet 44:104–111

Dietz HC, Cutting GR, Pyeritz RE, Maslen CL, Sakai LY, Corson GM, Puffenberger EG, et al (1991) Marfan syndrome caused by a recurrent de novo missense mutation in the fibrillin gene. Nature 352:337–339

Dietz HC, Pyeritz RE, Puffenberger EG, Kendzior RJ, Corson GM, Maslen CL, Sakai LY, et al (1992*a*) Marfan phenotype variability in a family segregating a missense mutation in the epidermal growth factor-like motif of the fibrillin gene. J Clin Invest 89:1674–1680

Dietz HC, Saraiva JM, Pyeritz RE, Cutting GR, Francomano CA (1992*b*) Clustering of fibrillin (FBN1) missense mutations in Marfan syndrome patients at cysteine residues in EGF-like domains. Hum Mutat 1:366–374

Dietz HC, Valle D, Francomano CA, Kendzior RJ, Pyeritz RE, Cutting GR (1993) The skipping of constitutive exons in vivo induced by nonsense mutations. Science 259:680–683

Evans MI, Drugan A, Koppitch FC III, Zador IE, Sacks AJ, Sokol RJ (1989) Genetic diagnosis in the first trimester: the norm for the 1990s. Am J Obstet Gynecol 160:1332–1339

Fojo SS, Beisiegel U, Beil U, Higuchi K, Bojanovski M, Gregg RE, Greten H, et al (1988) Donor splice site mutation in the apolipoprotein (Apo) C-II gene (Apo C-II Hamburg) of a patient with Apo C-II deficiency. J Clin Invest 82:1489–1494

Godfrey M (1993) The Marfan syndrome. In: Beighton P (ed) McKusick's heritable disorders of connective tissue, 5th ed. CV Mosby, St Louis, pp 51–135

Godfrey M, Menashe V, Weleber RG, Koler RD, Bigley RH, Lovrien E, Zonana J, et al (1990) Cosegregation of elastin-associated microfibrillar abnormalities with the Marfan phenotype in families. Am J Hum Genet 46:652–660

Gonzalez-Redondo JM, Stoming TA, Kutlar F, Kutlar A, McKie VC, McKie KM, Huisman TH (1989) Severe Hb S-β-thalassaemia with a TTC substitution in the donor splice site of the first intron of the β-globin gene. Br J Haematol 71:113–117

Gotoda T, Yamada N, Kawamura M, Kozaki K, Mori N, Ishibashi S, Shimano H, et al (1991*a*) Heterogeneous mutations in the human lipoprotein lipase gene in patients with familial lipoprotein lipase deficiency. J Clin Invest 88:1856–1864

Gotoda T, Yamada N, Murase T, Inaba T, Ishibashi S, Shimano H, Koga S, et al (1991*b*) Occurrence of multiple aberrantly spliced mRNAs upon a donor splice site mutation that causes familial lipoprotein lipase deficiency. J Biol Chem 266:24757–24762

Hollister DW, Godfrey M, Sakai LY, Pyeritz RE (1990) Immunohistologic abnormalities of the microfibrillar-fiber system in the Marfan syndrome. N Engl J Med 323:152–159

Jacob M, Gallinaro H (1989) The 5′ splice site: phylogenetic

evolution and variable geometry of association with U1RNA. Nucleic Acids Res 17:2159–2180

Kainulainen K, Sakai LY, Child A, Pope FM, Puhakka L, Ryhänen L, Palotie A, et al (1992) Two mutations in Marfan syndrome resulting in truncated fibrillin polypeptides. Proc Natl Acad Sci USA 89:5917–5921

Koenigsberg M, Factor S, Cho S, Herskowitz A, Nitowsky H, Morecki R (1981) Fetal Marfan syndrome: prenatal ultrasound diagnosis with pathological confirmation of skeletal and aortic lesions. Prenat Diagn 1:241–247

Kuivaniemi H, Kontusaari S, Tromp G, Zhao M, Sabol C, Prockop DJ (1990) Identical G+1 to A mutations in three different introns of the type III procollagen gene (COL3A1) produce different patterns of RNA splicing in three variants of Ehlers-Danlos syndrome IV. J Biol Chem 265:12067–12074

Landels EC, Green PM, Ellis IH, Fensom AH, Bobrow M (1992) β-Hexosaminidase splice site mutation has a high frequency among non-Jewish Tay-Sachs disease carriers from the British Isles. J Med Genet 29:563–567

Lee B, Godfrey M, Vitale E, Hori H, Mattei M-G, Sarfarazi M, Tsipouras P, et al (1991*a*) Linkage of Marfan syndrome and a phenotypically related disorder to two different fibrillin genes. Nature 352:330–334

Lee B, Vitale E, Superti-Furga A, Steinmann B, Ramirez F (1991*b*) G to T transversion at position +5 of a splice donor site causes skipping of the preceding exon in the type III procollagen transcripts of a patient with Ehlers-Danlos syndrome type IV. J Biol Chem 266:5256–5259

Maddalena A, Bick DP, Schulman JD (1992) Molecular diagnosis of genetic disease. J Reprod Med 37:437–444

Miller SR, Schwartz RH (1992) Attitudes toward genetic testing of Amish, Mennonite, and Hutterite families with cystic fibrosis. Am J Public Health 82:236–242

Mononen I, Heisterkamp N, Kaartinen V, Mononen T, Williams JC, Groffen J (1992) Aspartylglycosaminuria in a non-Finnish patient caused by a donor splice mutation in the glycoasparaginase gene. J Biol Chem 267:3196–3199

Padgett RA, Grabowski PJ, Konarska MM, Seiler S, Sharp PA (1986) Splicing of messenger RNA precursors. Annu Rev Biochem 55:1119–1150

Pope FM, Nicholls AC, Palan A, Kwee ML, De Groot WP, Hausmann R (1992) Clinical features of an affected father and daughter with Ehlers-Danlos syndrome type VIIB. Br J Dermatol 126:77–82

Sambrook J, Fritsch EF, Maniatis T (1989) Molecular cloning: a laboratory manual, 2d ed. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY

Shapiro MB, Senapathy P (1987) RNA splice junctions of different classes of eukaryotes: sequence statistics and functional implications in gene expression. Nucleic Acids Res 15:7155–7174

Sutton VR, Kienzle BK, Knowles RW (1989) An altered splice site is found in the DRB4 gene that is not expressed in HLA-DR7,Dw11 individuals. Immunogenetics 29:317–322

Tsipouras P, Del Mastro R, Sarfarazi M, Lee B, Vitale E, Child AH, Godfrey M, et al (1992) Linkage analysis demonstrates that Marfan syndrome, dominant ectopia lentis, and congenital contractural arachnodactyly are linked to the fibrillin genes on chromosomes 15 and 5. N Engl J Med 326:905–909

Vasan NS, Kuivaniemi H, Vogel BE, Minor RR, Wootton JAM, Tromp G, Weksberg R, et al (1991) A mutation in the proα2(I) gene (COL1A2) for type I procollagen in Ehlers-Danlos syndrome type VII: evidence suggesting that skipping of exon 6 in RNA splicing may be a common cause of the phenotype. Am J Hum Genet 48:305–317

Watson EK, Marchant J, Bush A, Williamson B (1992) Attitudes toward prenatal diagnosis and carrier screening for cystic fibrosis among the parents of patients in a paediatric cystic fibrosis clinic. J Med Genet 29:490–491

Weil D, Bernard M, Combates N, Wirtz MK, Hollister DW, Steinmann B, Ramirez F (1988) Identification of a mutation that causes exon skipping during collagen pre-mRNA splicing in an Ehlers-Danlos syndrome variant. J Biol Chem 263:8561–8564

Weil D, D'Alessio M, Ramirez F, Eyre DR (1990) Structural and functional characterization of a splicing mutation in the pro-alpha 2(I) collagen gene of an Ehlers-Danlos type VII patient. J Biol Chem 265:16007–16011

Wertz DC, Janes SR, Rosenfield JM, Erbe RW (1992) Attitudes toward the prenatal diagnosis of cystic fibrosis: factors in decision making among affected families. Am J Hum Genet 50:1077–1085