Am. J. Hum. Genet. 54:447–453, 1994

Severe Neonatal Marfan Syndrome Resulting from a De Novo 3-bp Insertion into the Fibrillin Gene on Chromosome 15

Dianna M. Milewicz and Madeleine Duvic

Departments of Internal Medicine, Pediatrics, and Dermatology, University of Texas Medical School at Houston, Houston

Summary

Severe neonatal Marfan syndrome has features of the Marfan syndrome and congenital contractural arachnodactyly present at birth, along with unique features such as loose, redundant skin and pulmonary emphysema. Since the Marfan syndrome and congenital contractural arachnodactyly are due to mutations in different genes, it has been uncertain whether neonatal Marfan syndrome is due to mutations in the fibrillin gene on chromosome 15 or in another gene. We studied an infant with severe neonatal Marfan syndrome. Dermal fibroblasts were metabolically labeled and found to secrete fibrillin inefficiently when compared with control cells. Reverse transcription and amplification of the proband's fibroblast RNA was used to identify a 3-bp insertion between nucleotides 480-481 or 481-482 of the fibrillin cDNA. The insertion maintains the reading frame of the protein and inserts a cysteine between amino acids 160 and 161 in an epidermal growth-factor-like motif of fibrillin. This 3-bp insertion was not found in the fibrillin gene in 70 unrelated, unaffected individuals and 11 unrelated individuals with the Marfan syndrome. We conclude that neonatal Marfan syndrome is the result of mutations in the fibrillin gene on chromosome 15 and is part of the Marfan syndrome spectrum.

Introduction

The Marfan syndrome is an inherited disorder of connective tissue with cardiovascular, ocular, and skeletal features, with an estimated prevalence of 1/10,000 (Pyeritz and McKusick 1979; Pyeritz 1993). It is inherited as an autosomal dominant disorder, but approximately one-quarter of patients are born to parents who are not clinically affected and are felt to result from new mutations in parental germ cells. The cardiovascular features include aortic root dilatation and dissection, mitral valve prolapse, and mitral and aortic valve regurgitation. Eye involvement includes lens dislocation and myopia. Skeletal features include dolichostenomelia, arachnodactyly, anterior chest deformity, scoliosis, and joint laxity (Beighton et al. 1988). Fibrillin, a glycoprotein found in microfibrils, has recently been shown to be the defective gene product causing the Marfan syndrome (Dietz et al. 1991; Lee et al. 1991; Maslen et al. 1991; Tsipouras et al. 1992). The gene maps to the long arm of chromosome 15 (FBN1) (Lee et al. 1991; Magenis et al. 1991), and several mutations have been identified in this gene in patients with the Marfan syndrome (Dietz et al. 1991, 1992, 1993; Kainulainen et al. 1992).

Congenital contractural arachnodactyly (CCA), an autosomal dominant condition, shares some of the phenotypic features of the Marfan syndrome (Beals and Hecht 1971; Hecht and Beals 1972). Clinical manifestations of CCA include dolichostenomelia and arachnodactyly, contractures of large joints, and abnormal pinnae formation. A second fibrillin-like gene has been localized to chromosome 5 and has been linked to CCA, indicating that CCA is a separate syndrome from the Marfan syndrome and may result from mutations in this fibrillin-like gene (Lee et al. 1991; Tsipouras et al. 1992).

A severe neonatal form of the Marfan syndrome has cardiovascular, skeletal, and ocular complications of

Received May 25, 1993; accepted for publication November 15, 1993.

Address for correspondence and reprints: Dianna M. Milewicz, M.D. Ph.D., Department of Internal Medicine, University of Texas Medical School, 6431 Fannin, MSB 1.614, Houston, TX 77030.

0002-9297/94/5403-0007$02.00

the Marfan syndrome present at birth, as well as features of CCA, such as congenital contractures and abnormal ears (Shankar et al. 1967; Day and Burke 1986; Gross et al. 1989; Huggon et al. 1990; Raghunath et al. 1993). In addition, these infants often have loose, redundant skin, a typical facies, and pulmonary emphysema. Death of these affected children often occurs within the first year of life, from congestive heart failure. Because children with neonatal Marfan syndrome have a more severe and complex phenotype, it is unclear whether the syndrome is due to more detrimental mutations in either the fibrillin gene on chromosome 15 or the gene causing CCA, possibly the fibrillin gene on chromosome 5, or another gene. Previous studies of dermal fibroblasts have demonstrated defects in fibrillin protein processing by the cells from patients with neonatal Marfan syndrome (Milewicz et al. 1992; Raghunath et al. 1993), suggesting a fibrillin gene mutation. Here we report a child who has the neonatal Marfan phenotype with a unique mutation in the chromosome 15 fibrillin gene and demonstrate that this neonatal syndrome is part of the Marfan syndrome spectrum.

Material and Methods

Clinical Summary

The patient's history has been previously reported in detail (Gross et al. 1989). This was a male infant who was born with dolichostenomelia, arachnodactyly, joint contractures, large ears, hypotonia, pectus carinatum, and loose redundant skin over the face and neck (fig. 1). He also had bilateral ectopia lentis present at birth, and echocardiogram showed aortic root dilatation and a dysplastic mitral valve. The patient had progressive aortic and mitral insufficiency documented by echocardiogram and was ventilator dependent by 7 mo of age because of progressive respiratory failure. He died at age 21 mo. At autopsy, findings included an enlarged aortic root, a dilated left atrium and ventricle, and redundant mitral valve. The aortic valve and pulmonary valve were also abnormal. There was pulmonary emphysema with numerous bullae throughout the lungs.

Metabolic Labeling and Electrophoretic Analysis of Fibrillin

Dermal fibroblasts were obtained from the proband after appropriate parental consent. Fibroblasts were explanted and maintained in culture as described elsewhere (Milewicz et al. 1992). To radiolabel synthesized proteins, 250,000 dermal fibroblasts were plated in 35-mm dishes (Corning Glass, Corning, NY) and were allowed to attach and spread for 72 h in Dulbecco-Vogt modified Eagle medium (DMEM) supplemented with 10% FCS (Irving Scientific, Santa Ana, CA). The medium was replaced with DMEM lacking FCS and cysteine but was supplemented with sodium ascorbate (50 μg/ml) and incubated for 2 h. The cells were labeled with [^{35}S]cysteine (1,300 Ci/M; Amersham, Arlington Heights, IL) for 30 min (50 μCi/150 μl DMEM). Medium was collected, the cell layer was washed in DMEM, and the cells were incubated with DMEM for varying periods of time up to 20 h. The medium was collected and cell lysate was harvested in 50 mM Tris-HCl pH 8.0, 1% NP40, and 1 mM phenylmethylsulfonyl fluoride. After the cell lysate was aspirated from the dish, the material remaining on the dish was scrapped into the same buffer with a rubber policeman, and the insoluble proteins were collected by cen-

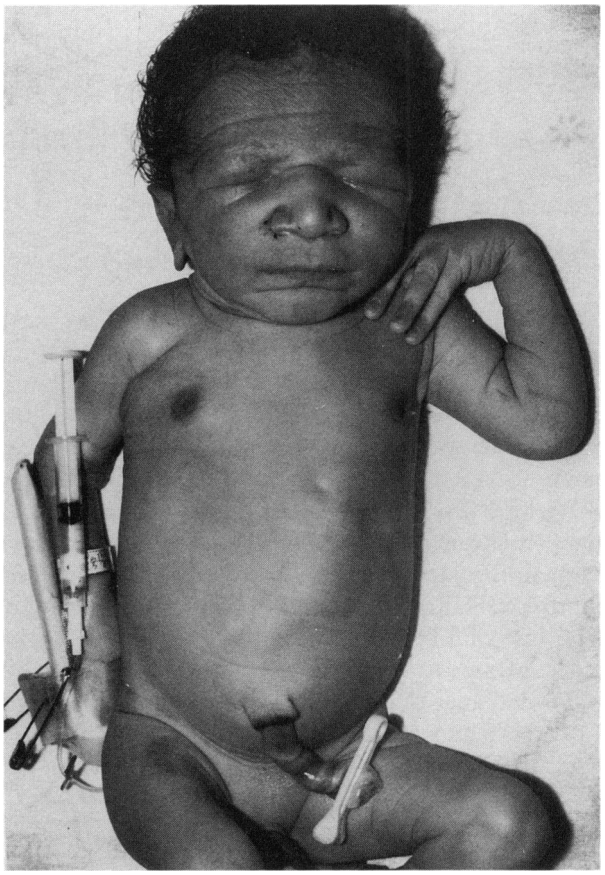

Figure 1 Patient as a neonate. Note the redundant skin, micrognathia, arachnodactyly, and joint contractures.

trifugation at 10,000 *g* for 5 min at 4°C. Proteins were dissolved in sample buffer containing SDS and β-mercaptoethanol, separated by electrophoresis in 4% acrylamide slab gels containing SDS, and localized by autoradiography.

Amplification, Cloning, and Sequencing Fibrillin cDNA Fragments

Total cellular RNA was harvested from fibroblast cell strains from the proband, an unaffected individual, and other individuals with the Marfan syndrome by using RNAzol B (Cinna/Biotecx Laboratories, Houston). Fifteen primer pairs, spaced at approximately 500-bp intervals, with 100 bp overlap at the 5′ and 3′ ends, were necessary to span part of the cDNA sequence of fibrillin (Maslen et al. 1991). The downstream primer of each set was used to prime reverse transcription of the fibrillin cDNA using total cellular RNA, and both primers of a set were used to amplify the fibrillin cDNA fragment by using Cetus reagents and thermal cycler. The mutation was identified using primers BA (5′-TTGTCGACCACCATTCATTATGCTGCA-3′, with an *Sal*I restriction site at the 5′ end) and BS (5′-CATTGGCAGCTTTAAGTGCAGG-3′) that amplified nucleotides 446–927 of the fibrillin cDNA. PCR conditions were denaturing at 94°C for 30 s, annealing at 52°C for 30 s, extension at 72°C for 30 s, for a total of 35 cycles, then a final extension at 72°C for 10 min. The amplified cDNA fragments were analyzed on a 6% acrylamide slab gel and stained with ethidium bromide.

Amplified cDNA fragments corresponding to nucleotides 446–927 were directionally cloned into the plasmid Bluescript by using *Sal*I and *Sma*I restriction sites (Stratagene, La Jolla, CA), the plasmid DNA harvested using Magic Mini-Prep (Promega, Madison, WI), and purified plasmid DNA from four clones sequenced using Applied Biosystems automated sequencer (University of Texas Medical School at Houston Molecular Genetics Core Facility).

Screening Genomic DNA for the Mutation

A set of primers (BS and BA-2) were used to amplify a 67-bp fragment of the exon of the fibrillin gene containing the identified mutation (corresponding to nucleotides 447–514 of the fibrillin cDNA sequence) from genomic DNA. The amplified DNA fragments were separated on an 8% polyacrylamide gel containing 7 M urea and were detected by silver staining using Gelcode system (Pierce, Rockford, IL). The sequence for the BA-2 primer was 5′-TGTGCAGTTCCTTTCTTCAGAATC-3′.

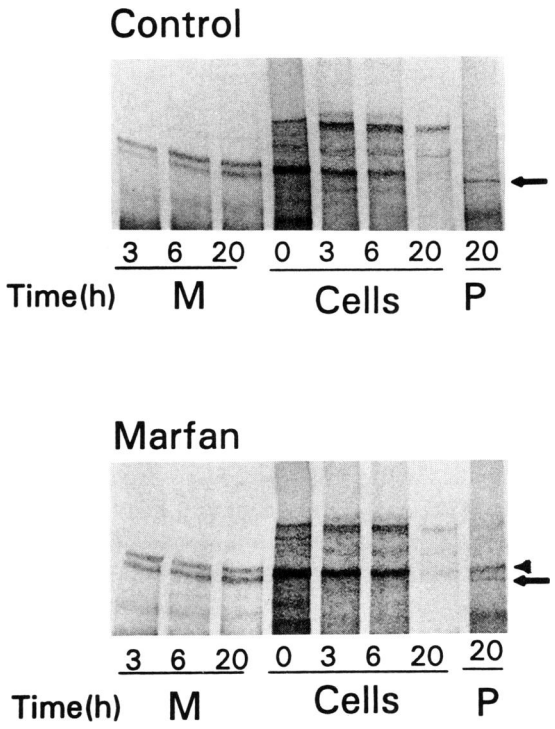

Figure 2 Altered secretion of fibrillin by cells from the patient. Cells from the patient and age-matched control were incubated with [^{35}S]cysteine for 30 min and then chased for up to 20 h. The media (M), intracellular proteins (Cells), and pericellular matrix (P) were harvested separately. The majority of the fibrillin is chased from the control cells at the end of 6 h, while a substantial amount remains in the cells from the affected child. The fibrillin is secreted from the cell and processed to a faster-migrating form. The processed form of fibrillin is deposited in the pericellular matrix by the control cells and, to a lesser extent, by the patient's cells (arrows). The patient's cells also have unprocessed fibrillin in the matrix proteins, whereas the control cells do not (arrowhead).

Results

Delayed Secretion of Fibrillin by the Patient's Dermal Fibroblasts

Although skin fibroblasts from the proband synthesized normal amounts of fibrillin, the secretion of fibrillin was delayed. Figure 2 demonstrates a pulse/chase experiment with cells from the proband and age-matched control cells. After a 30-min pulse of [^{35}S]cysteine (0 time) the two cell strains synthesized similar amounts of fibrillin, judging by label incorporation. By 6 h of chase, the control cells had secreted the majority of fibrillin from the cell, whereas the cells from the proband still contained a significant pool of intracellular fibrillin. Once in the media, the fibrillin

appears as a double band because of extracellular processing to a lower-molecular-weight form, approximately 20 kD smaller. The secreted and processed fibrillin was deposited in the extracellular matrix by both the control cells and the proband's cells (fig. 2, arrows). When compared with the control cells, the proband's cells showed less processed fibrillin in the matrix. In addition, the proband's cells had unprocessed fibrillin present in the matrix material (fig. 2, arrowhead).

Identification of a 3-bp Insertion into the Fibrillin cDNA

Fibrillin cDNA synthesized from mRNA from the patient's cells, a control cell strain, and cells from five unrelated individuals with the Marfan syndrome was PCR amplified with 15 sets of primers spaced at approximately 600-bp intervals spanning part of the cDNA sequence of fibrillin (Maslen et al. 1991). When primers BS and BA that amplified nucleotides 446–927 (Corson et al. 1993) (or nucleotides 3140–3621 [Pereira et al. 1993]) of the fibrillin cDNA were used and the DNA fragments analyzed on a 6% polyacrylamide gel, an abnormally migrating band was observed in the patient's amplified cDNA fragments (fig. 3*A*). The patient's DNA contained a slower-migrating fragment (fig. 3*A*, arrowhead) along with a fragment that comigrated with the other DNA fragments amplified (fig. 3*A*, arrow). The DNA fragments from the proband were cloned into a plasmid vector, and four plasmid clones were sequenced. Two clones contained the normal fibrillin sequence between nucleotides 446 and 927, and two clones had a 3-bp insertion between nucleotides 480 and 481 or 481 and 482 (or nucleotides 3174 and 3175 or 3175 and 3176) (fig. 3*B*). The exact location of the insertion could not be determined from the sequence. In either location, the insertion maintained the reading frame for protein and inserted a cysteine between amino acids 160 and 161 in the fibrillin protein (or amino acids 1058 and 1059) (fig. 3*C*). The cysteine is inserted into one of the motifs of fibrillin that has homology to the tandem repeats in human epidermal growth-factor (EGF) precursor (Lee et al. 1991; Maslen et al. 1991).

Population Screening

Population screening was done to confirm that the insertion was not a polymorphism in the fibrillin gene. Genomic DNA from 70 random individuals was screened for the insertion. In addition, genomic DNA from 11 unrelated Marfan patients was screened for the mutation. The 3-bp insertion was not observed in any of these individuals. Genomic DNA from the proband's family was not available to study.

Discussion

The patient presented here represents a case of severe neonatal Marfan syndrome, a unique phenotype with features of both the Marfan syndrome and CCA. The most salient features of this phenotype include dolichostenomelia, arachnodactyly, loose and redundant skin, characteristic facies with micrognathia, abnormal pinnae, hyperextensible joints, flexion contractures, chest wall deformities, skeletal muscle hypoplasia, pulmonary emphysema, and ectopia lentis (Shankar et al. 1967; Lababidi and Monzon 1981; Day and Burke 1986; Gross et al. 1989; Huggon et al. 1990; Raghunath et al. 1993; Royce and Steinmann 1993). Cardiac lesions in these patients include mitral valve prolapse and regurgitation, tricuspid valve prolapse and regurgitation, and aortic root dilatation. Most of these children die of congestive heart failure during the first year of life.

The Marfan syndrome and CCA are due to mutations in different genes (Lee et al. 1991; Tsipouras et al. 1992). Because of the overlap in the phenotype of patients with severe neonatal Marfan syndrome, it was uncertain whether this syndrome was the result of more detrimental mutations in the fibrillin gene on chromosome 15 or the gene that causes CCA, possibly the fibrillin-like gene on chromosome 5, or another related or unrelated gene. Our patient was found to have a de novo mutation in FBN1, establishing that neonatal Marfan syndrome can result from mutations in this fibrillin gene.

The proband had no family history suggestive of the Marfan syndrome or CCA, and the proband's parents, both between 20 and 30 years of age, were evaluated clinically and did not have either the Marfan syndrome or CCA. The second fibrillin allele was not analyzed at all sites for mutations, so we cannot exclude the possibility that the patient was a compound heterozygote for mutations in both fibrillin genes. In addition, we cannot exclude the possibility that the patient was a compound heterozygote for mutations in both the fibrillin gene and fibrillin-like gene on chromosome 5. However, since heterozygous mutations in these gene are associated with the Marfan syndrome or possibly CCA, the fact that the parents were phenotypically normal helps to exclude both these possibilities.

The 3-bp insertion is a unique mutation in the fibrillin gene. The first mutation in the fibrillin gene on

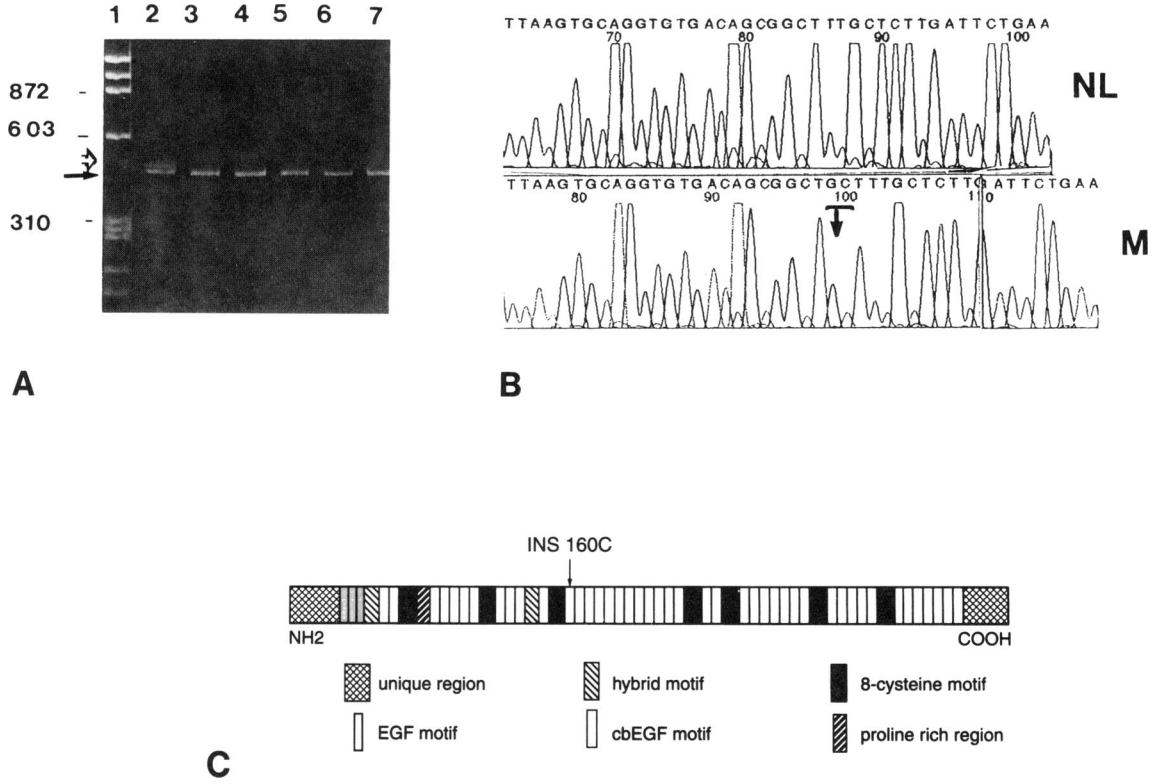

Figure 3 Identification of the fibrillin gene mutation in the patient. *A*, Polyacrylamide gel analysis of a 481-bp cDNA fragment from the proband (lane 2), four unrelated patients with the Marfan syndrome (lanes 3, 4, 6, and 7), and one control individual (lane 5). An abnormally migrating band (arrowhead) was observed in the sample from the proband (lane 2). Molecular weight markers were ΦX174 DNA digested with *Hae*III (lane 1). *B*, Sequence of the normal (NL) and mutant (M) alleles. A 3-bp insertion is revealed in the cDNA between nucleotides 480 and 481 or 481 and 482 (Corson et al. 1993) (or nucleotides 3174 and 3175 or 3175 and 3176 [Pereira et al. 1993]) (arrow). *C*, Location of the inserted cysteine (arrow) into one of the EGF-like domains of fibrillin, which is shown on a schematic map of the fibrillin protein (Corson et al. 1993). The shaded boxes depict motifs found in the fibrillin protein as indicated.

chromosome 15 was a de novo missense mutation resulting in an arginine for proline at codon 239 (Dietz et al. 1991). Other published mutations in the fibrillin gene include a large in-frame deletion of 366 bases of the fibrillin mRNA, a G-to-A transition resulting in a premature stop codon (Kainulainen et al. 1992), a point mutation at nucleotide 4226 leading to a cysteine-to-serine substitution at codon 1409 (Dietz et al. 1992), and a nonsense mutation at base 3645 leading to altered splicing of the exon containing the mutation (Dietz et al. 1993). All these mutations have occurred in patients with classic features of the Marfan syndrome.

The 3-bp insertion maintains the reading frame for fibrillin and inserts a cysteine into one of the 47 identified cysteine-rich domains of fibrillin that have homology to the EGF (Corson et al. 1993). EGF has six cysteines that form intrachain disulfide bonds (Cooke et al. 1987). In the EGF-like repeats of fibrillin, these residues are completely conserved and probably form the same secondary structure as EGF (Maslen et al. 1991). For EGF, the specific secondary structure is maintained by disulfide bridges between the first and third, the second and fourth, and the fifth and sixth cysteine residues (Cooke et al. 1987). The insertion of a new cysteine into an EGF-like domain could be predicted to disrupt proper disulfide bond formation in a number of ways. First, the extra cysteine could prevent proper pairing of cysteine within the immediate EGF-like domain. Alternatively, the additional reactive cysteine might be available for irregular interaction with other cysteines in adjacent domains, such as an EGF-like domain and a TGF-β binding protein–like domain, also cysteine-rich, on the other side. Last, evidence suggests that intermolecular disulfide bonds are important for fibrillin aggregation into microfibrils, and the extra cysteine may also disrupt these cross-links (Maddox et al.

1989). All these factors may contribute to producing a severe phenotype.

The pulse-chase studies of fibrillin processing showed delayed secretion of fibrillin synthesized by the proband's cells. This indicates that some of the fibrillin molecules are slowly attaining a folded structure requisite for secretion and therefore are retained within the cell for a period longer than normal. This abnormal folding may be the result of intramolecular mispairing of cysteine, which supports the first and second hypotheses mentioned.

The patient's cells incorporated less processed fibrillin into the pericellular matrix, when compared with control cells (fig. 2). This result agrees with previous studies on cells from neonatal Marfan syndrome patients that have shown diminished incorporation of fibrillin into the extracellular matrix (Superti-Furga et al. 1992; Raghunath et al. 1993). The appearance of unprocessed fibrillin (or profibrillin) in the matrix of the patient's cells and not the control cells most likely is due to a pool of unprocessed, intracellular fibrillin remaining in the patient's cells for up to 20 h, whereas the labeled intracellular fibrillin is completely secreted by the control cells. When the intracellular proteins are harvested, cells are incompletely lysed. The matrix proteins are concentrated before they are analyzed, and any pool of fibrillin remaining within the cell will be seen in the matrix material. A less likely explanation is that the unprocessed form of fibrillin is incorporated into the pericellular matrix by the patient's cells and not by the control cells.

Fibrillin contains EGF-like repeats that show extensive homology to the EGF-like sequences found in numerous other proteins from many species, including mammalian tissue plasminogen activator (tPA) (Bassel-Duby et al. 1992), human low-density-lipoprotein (LDL) receptor (Davis et al. 1987), the *Drosophila Notch* locus (Kelley et al. 1987), and the *lin-2* gene of *Caenorhabditis elegans* (Greenwald et al. 1983). The functions of the EGF-like sequences have been studied in a number of these proteins. The EGF-like sequences of the LDL receptor are required for efficient binding of LDL and for acid-dependent release of the ligand by the receptor (Davis et al. 1987). The EGF-like domain of tPA is involved in binding tPA to a cellular receptor (Davis et al. 1987). Specific EGF repeats of *Notch* mediate cellular interactions during development (Rebay et al. 1991). These facts imply that the EGF-like domains of fibrillin are important in intermolecular interactions, possibly with other fibrillin molecules or other proteins in the extracellular matrix. Disruption of fibrillin intermolecular interactions in the matrix by the production of mutant fibrillin molecules may be the mechanism by which this dominant mutation exerts its effect, so called "dominant-negative" mutation (Herskowitz 1987).

In summary, we have shown that a mutation in the fibrillin 15 gene results in a severe neonatal form of the Marfan syndrome, indicating that this syndrome is part of the phenotypic spectrum of the Marfan syndrome. Future studies will help to elucidate how this novel mutation leads to the resulting severe phenotype.

Acknowledgments

We would like to thank Drs. Jacqueline Hecht and William Horton for helpful scientific discussion, Nancy Luzak and Jeanette Quimby for assistance in preparing the manuscript, Stone Cao for excellent technical assistance, and the members of the University of Texas Medical School at Houston Molecular Genetics Core Facility for prompt technical assistance. This work was supported by National Institutes of Health grant HD-24427, the National Marfan Foundation, and a Pfizer Scholars Award to D.M.M.

References

Bassel-Duby R, Jiang NY, Bittick T, Madison E, McGookey D, Orth K, Shohet R, et al (1992) Tyrosine 67 in the epidermal growth factor-like domain of tissue type plasminogen activator is important for clearance by a specific hepatic receptor. J Biol Chem 267:9668–9677

Beals RK, Hecht F (1971) Congenital contractural arachnodactyly: a heritable disorder of connective tissue. J Bone Joint Surg 53A:987–993

Beighton P, dePaepe A, Danks D, Finidori G, Gedde-Dahl T, Goodman R, Hall JG, et al (1988) International nosology of heritable disorders of connective tissue, Berlin, 1986. Am J Med Genet 29:581–594

Cooke RM, Wilkinson AJ, Baron M, Pastore A, Tappin MJ, Campbell ID, Gregory H, et al (1987) The solution structure of human epidermal growth factor. Nature 327:339–341

Corson GM, Chalberg SC, Dietz HC, Charbonneau NL, Sakai LY (1993) Fibrillin binds calcium and is coded by cDNAs that reveal a multidomain structure and alternatively spliced exons at the 5′ end. Genomics 17:476–484

Davis CG, Goldstein JL, Sudhof TC, Anderson RGW, Russell DW, Brown MS (1987) Acid-dependent ligand dissociation and recycling of LDL receptor mediated by growth factor homology region. Nature 326:760–765

Day DL, Burke BA (1986) Pulmonary emphysema in a neonate with Marfan syndrome. Pediatr Radiol 16:518–521

Dietz HC, Cutting GR, Pyeritz RE, Maslen CL, Sakai LY,

Corson EG, Puffenberger A, 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 J, Corson GM, Maslen CL, Sakai LY, et al (1992) 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, Valle D, Francomano A, Kendzior RJ, Reed E, Pyeritz E, Cutting GR (1993) The skipping of constitutive exons in vivo induced by nonsense mutations. Science 259:680–683

Greenwald IS, Sternberg PW, Horvitz HR (1983) The *lin-12* locus specifies cell fates in Caenorhabditis elegans. Cell 34:435–444

Gross DM, Robinson LK, Smith LT, Glass N, Rosenberg H, Duvic M (1989) Severe perinatal Marfan syndrome. Pediatrics 84:83–89

Hecht F, Beals RK (1972) "New" syndrome of congenital contractural arachnodactyly originally described by Marfan in 1896. Pediatrics 49:574–579

Herskowitz I (1987) Functional inactivation of genes by dominant negative mutations. Nature 329:219–222

Huggon IC, Burke JP, Talbot JF (1990) Contractural arachnodactyly with mitral regurgitation and iridodonesis. Arch Dis Child 65:317–319

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

Kelley MR, Kidd S, Deutsch WA, Young MW (1987) Mutations altering the structure of epidermal growth factor-like coding sequences at the Drosophila *notch* locus. Cell 51:539–548

Lababidi Z, Monzon C (1981) Early cardiac manifestations of Marfan's syndrome in the newborn. Am Heart J 102:943–945

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

Maddox BK, Sakai LY, Keene DR, Glanville RW (1989) Connective tissue microfibrils: isolation and characterization of three large pepsin-resistant domains of fibrillin. J Biol Chem 264:21381–21385

Magenis RE, Maslen CL, Smith L, Allen L, Sakai LY (1991) Localization of the fibrillin gene to chromosome 15, band 15q21.1. Genomics 11:346–351

Maslen CL, Corson GM, Maddox BK, Glanville RW, Sakai LY (1991) Partial sequence of a candidate gene for the Marfan syndrome. Nature 352:334–337

Milewicz DM, Pyeritz RE, Crawford ES, Byers PH (1992) Marfan syndrome: defective synthesis, secretion and extracellular matrix formation of fibrillin by cultured dermal fibroblasts. J Clin Invest 89:79–86

Pereira L, D'Alessio M, Ramirez F, Lynch J, Sykes B, Pangilinan T, Bonadio J (1993) Genomic organization of the sequence coding for fibrillin, the defective gene product in Marfan syndrome. Hum Mol Genet 2:961–968

Pyeritz RE (1993) The Marfan syndrome. In: Royce PM, Steinmann B (eds) Connective tissue and its heritable disorders. Wiley-Liss, New York, pp 437–468

Pyeritz RE, McKusick VA (1979) The Marfan syndrome: diagnosis and management. N Engl J Med 300:772–777

Raghunath M, Superti-Furga A, Godfrey M, Steinmann B (1993) Decreased extracellular deposition of fibrillin and decorin in neonatal Marfan syndrome fibroblasts. Hum Genet 90:511–515

Rebay I, Flemming RJ, Fehon RG, Cherbas L, Cherbas P, Artavanis-Tsakonas S (1991) Specific EGF repeats of notch mediate interactions with delta and serrate: implications for notch as a multifunctional receptor. Cell 67:687–699

Royce PM, Steinmann B (1993) Connective tissue and its heritable disorders. Wiley-Liss, New York

Shankar KR, Hultgren MK, Lauer RM, Diehl AM (1967) Lethal tricuspid and mitral regurgitation in Marfan's syndrome. Am J Cardiol 20:122–128

Superti-Furga A, Raghunath M, Willems PJ (1992) Deficiencies of fibrillin and decorin in fibroblast cultures of a patient with neonatal Marfan syndrome. J Med Genet 29:875–878

Tsipouras P, DelMastro R, Sarfarazi M, Lee B, Child AH, Godfrey M, Devereux RB, et al (1992) Genetic linkage of the Marfan syndrome, ectopia lentis, and congenital contractural arachnodactyly to the fibrillin genes on chromosomes 15 and 5. N Engl J Med 326:905–909