A Recurring \textit{FBN1} Gene Mutation in Neonatal Marfan Syndrome

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\textbf{Background:} Marfan syndrome is an autosomal dominant disorder of connective tissue caused by mutations in the fibrillin 1 gene (\textit{FBN1}). \textit{FBN1} mutations have been associated with a broad spectrum of phenotypes. Neonatal Marfan syndrome has unique clinical manifestations and mutations.

\textbf{Objective:} To determine if there is a discernible genotypic-phenotypic correlation associated with the unique mutation in neonatal Marfan syndrome.

\textbf{Study Design:} A newborn exhibited many typical characteristics of neonatal Marfan syndrome, including arachnodactyly; contractures of both elbows, knees, and ankles; small-joint laxity; dilated cardiomyopathy; valvular dysplasia and insufficiency; congestive heart failure; and pulmonary emphysema. Three atypical features were also discovered: a right diaphragmatic hernia, a myocardial mass, and left main-stem bronchomalacia. She died at 3 1/2 months of age. Total RNA was extracted from skin fibroblasts and amplified by means of reverse transcriptase polymerase chain reaction amplification with \textit{FBN1}-specific primers. The complementary DNA fragments were sequenced.

\textbf{Results:} A single T-to-C transition at nucleotide 3276 (T3276C) was identified and confirmed at the DNA level by sequencing of genomic DNA. This results in a substitution of threonine for isoleucine.

\textbf{Conclusions:} Neonatal Marfan syndrome is a unique clinical entity with recurring mutation hot spots in exons 24 to 27 and 31 to 32 of the \textit{FBN1} gene. Some clinical features in this case report are unusual for neonatal Marfan syndrome. This is the third report of this T3276C mutation in the \textit{FBN1} gene with unusual clinical manifestations. We conclude that there is a genotypic-phenotypic correlation associated with this mutation.

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\textbf{MARFAN syndrome (MFS) is an autosomal dominant disorder of connective tissue with pleiotropic manifestations involving the cardiovascular, ocular, and skeletal systems. Neonatal MFS (nMFS) is a clinical diagnosis defined by Morse et al.} It lies at the most severe end of the MFS clinical spectrum, sharing some characteristics of MFS, but with additional unique manifestations. Patients exhibit congestive heart failure, mitral or tricuspid insufficiency, crumpled ears, flexion contractures, pulmonary emphysema, and loose skin, giving a “senile” appearance. These patients frequently die of congestive heart failure secondary to mitral or tricuspid insufficiency in the first year of life.

The molecular basis of MFS is a mutation in the fibrillin 1 gene (\textit{FBN1}), which encodes a key structural component of the elastin-associated microfibrils of the extracellular matrix of connective tissue. Patients with nMFS have mutations in 1 of 2 “hot spots” in the \textit{FBN1} gene: exons 24 to 27 and exons 31 to 32. However, genotypic-phenotypic correlations have been slow to emerge because of the wide range of interfamily and intrafamily phenotypic variability. We identified an infant with a mutation in exon 24 whose unusual clinical findings suggest a distinct subgroup of patients with nMFS.

\textbf{PATIENT REPORT}

A 2615-g (approximately 10th percentile) female neonate was delivered by normal spontaneous vaginal delivery to a 20-year-old Bengali woman. The father was 36 years old, and the family history was negative for birth defects or connective-tissue disorders. The postterm pregnancy had been complicated by intrauterine growth retardation evidenced by a small weight-length ratio. Serial ultrasound scans performed during pregnancy had shown advanced total fetal length.
The newborn examination showed a birth length of 52 cm (90th percentile) and a head circumference of 32.5 cm (5th-10th percentile). She had craniofacial dysmorphism consisting of deep-set eyes, high arched palate, and large floppy pinnae and musculoskeletal abnormalities, including arachnodactyly (Figure 1); flexion contractures at both elbows, knees, and ankles; and small-joint laxity. The length of the middle finger was 4.5 cm (>97th percentile); hand, 7.5 cm (80th percentile), and ear, 4.5 cm (97th percentile).

During her initial neonatal hospitalization, the patient became cyanotic with respiratory distress, at which time a chest radiograph showed a right hemidiaphragm bulging up against the right lung, consistent with a diaphragmatic hernia. Echocardiography showed a dilated 4-chamber heart with a patent foramen ovale; aortic, tricuspid, and pulmonic insufficiency; dysplastic and prolapsing tricuspid and mitral valves; and aortic root dilation. At 7 weeks, repeat echocardiography showed progression of mitral, aortic, and tricuspid valve regurgitation. Also noted was an echodense mass in the coronal septum of the heart, without outflow tract obstruction. Magnetic resonance imaging showed a 7 × 4-mm area of high density in the epicardium near the right coronary artery, without enhancement, suggesting a myxomatous collection of proteinaceous material or lipoma. Plain skull films were consistent with craniosynostosis. Ophthalmologic examination showed no ectopia lentis.

At 9 weeks of age, the patient contracted a respiratory syncytial virus infection resulting in shifting lobar atelectasis, causing respiratory failure and requiring intubation and mechanical ventilation. On recovery from atelectasis, causing respiratory failure and requiring intubation and mechanical ventilation. On recovery from atelectasis, causing respiratory failure and requiring intubation and mechanical ventilation. On recovery from atelectasis, causing respiratory failure and requiring intubation and mechanical ventilation. On recovery from atelectasis, causing respiratory failure and requiring intubation and mechanical ventilation. On recovery from atelectasis, causing respiratory failure and requiring intubation and mechanical ventilation. On recovery from atelectasis, causing respiratory failure and requiring intubation and mechanical ventilation. On recovery from atelectasis, causing respiratory failure and requiring intubation and mechanical ventilation. 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isolated from the patient by means of primers Ex 25F (GAGTGCAAGATGATA CCCAG) and Ex 25R (CCT TAA GGC TCA TTA ACT AGA).

Polymerase chain reaction was carried out at annealing temperatures specific for each primer set, initial denaturation of 5 minutes at 95°C, secondary denaturation of 20 seconds at 96°C, extension of 1 minute at 72°C for 35 cycles in total volume of 50 µL with the use of 0.5 U of Taq polymerase (Promega US, Madison, Wis), and XL3 Buffer (Boehringer Mannheim, Indianapolis, Ind). Polymerase chain reaction fragments were separated by agarose gel electrophoresis. The fragments were then excised from the gel and purified with QIAGEN columns according to the manufacturer’s instructions (QIAGEN, Inc, Valencia, Calif). Purified fragments were sequenced by means of internal primers with a sequencing kit (Beckman Coulter, Inc, Fullerton, Calif) for automated sequencing under the conditions described by the manufacturer. Products from the sequencing reaction were separated and analyzed on a sequencer (Beckman Coulter, Inc, model CEQ 2000).

**RESULTS**

Analysis of the coding sequence of the FBN1 gene identified a single thymine-to-cytosine transition at nucleotide position 3276 in exon 24. This mutation was confirmed at the DNA level by sequencing of exon 24 amplified from a genomic DNA sample from the patient. The patient was therefore heterozygous at nucleotide 3276, as opposed to the normal control individual, who was homozygous for the wild-type sequence (Figure 3). This mutation predicts the substitution of a threonine for an isoleucine at amino acid position 1048 of the fibrillin 1 molecule.

**COMMENT**

Mutations in FBN1 have been found in only 78% of patients with MFS, perhaps because of the size of the gene or overdiagnosis. FBN1 is found on chromosome 15, spans approximately 200 kilobases of genomic DNA, and contains 65 exons. More than 200 FBN1 mutations have been discovered to date, and only 11 of these represent recurrent mutations. Of cases of MFS, 25% to 30% represent new mutations. Most mutations are unique to one family or patient, suggesting a high mutation rate. Mutations have been found in every exon in this gene and are spread evenly throughout the gene, with the exception of hot spots in exons 24 to 27 and 31 to 32 associated with nMFS. This nMFS region comprises perhaps the only accepted genotypic-phenotypic correlation for fibrillin 1–MFS described to date. Most mutations have been missense mutations; however, exon-skipping mutations are also common.

FBN1 encodes fibrillin 1, a 350-kd glycoprotein. This protein is intracellularly processed, including adding mannose residues to its 14 N-glycosylation sites. It is then secreted into the extracellular matrix and cleaved from its signal peptide by a member of the paired basic amino acid cleaving enzyme convertase enzyme family to its active form, fibrillin 1. The resultant fibrillin 1 monomers polymerize in a parallel head-to-tail fashion. Parallel bundles of fibrillin polymers form the major component of the 10- to 12-nm microfibrils. It seems that the protein’s N terminal is particularly important in correct microfibril assembly. Most mutations in fibrillin 1 are thought to exert a dominant negative effect, whereby the mutant fibrillin inhibits global microfibrillar function, perhaps through defective polymerization or aggregation. Microfibrils form the scaffolding in the deposition of tropoelastin and resulting elastic fiber formation. Elastin is an amorphous molecular aggregate, laid down along microfibrils, which is essential for giving elastic fibers the elastic recoil necessary to function. Tropoelastin precursors are laid down alongside microfibrils and then cleaved to active elastin proteins, which then polymerize. Microfibrils give structure and direction to elastic fibers and are thought to function as the anchoring component, connecting elastic fibers to cells and basement membranes. They are present in tissues that resist load and stress, such
as the aortic adventitia, and in the skin. However, microfibrils are also found in tissues not linked to elastin, such as ciliary zonules.

Patients with MFS have been found to have defects in fibrillin synthesis, secretion, and extracellular matrix assembly. Some patients exhibit a lack of extracellular matrix assembly, and some a defective microfibril assembly, leading to frayed and shortened microfibrils.

Of note, there is a subgroup of mutations that have normal synthesis, secretion, and extracellular matrix deposition. Few genotypic-phenotypic correlations have been discovered to date. However, a specific subgroup of patients with severe impairment in extracellular matrix deposition has been found to have an increased incidence of aortic dissection.

Characteristics seen in MFS include arachnodactyly, dolichostenomelia, scoliosis, chest-wall deformities like pectus carinatum or excavatum, tall stature, ligamentous laxity, abnormal joint mobility, and protrusio acetabuli. Ectopia lentis is seen in about 80% of patients with MFS. Cardiovascular manifestations are associated with the most morbidity and mortality.

The most common cardiovascular complications of MFS involve prolapse or insufficiency of the aortic-ventricular valves and dilation of the aorta. Morbidity and mortality commonly result from progressive aortic root dilation with concomitant aortic valve insufficiency or aortic dissection. The diagnosis of MFS can be made according to the Gent nosology criteria. Conversely, there are patients with fibrillin 1 mutations who do not meet clinical criteria for MFS but who have related connective-tissue disorders. These related disorders are termed type I fibrillinopathies. The phenotypic spectrum in type 1 fibrillinopathies is very broad. Few patients fulfill the diagnostic criteria of MFS. Patients with findings limited to a single organ system are likely to have less severe abnormalities of fibrillin 1 that are influenced by environmental factors or the effects of modifying genes.

Neonatal MFS is unique in its clinical characteristics and severity. Our patient met the criteria for nMFS defined by Morse et al. She also exhibited some unusual features, including a myxomatous tissue mass surrounding the aortic-ventricular valves, bronchomalacia, respiratory failure, and diaphragmatic hernia. The case reported by Bresters et al. and similar features, in whom the same substitution was identified and predicted to result in a substitution of a threonine for an isoleucine, which produces a single T-to-C transition at nucleotide 3276 (Table). The 2 other cases were reported by Lonnqvist et al. The discrepancy in the numbering of the T3276C mutation in this report and that of Bresters et al. compared with Lonnqvist et al. reported it as T3143C, results from their numbering the sequence from the beginning of the translation start site, rather than the beginning of the messenger RNA. Lonnqvist et al showed that this mutation resulted in a novel N-glycosylation site at Asn1046 that is used to add a mannoside residue. N-glycolysation is important in the correct folding and stability of proteins, as well as in protein-protein interactions. In addition, excessive N glycosylation may inhibit the extracellular secretion of fibrillin 1 and severely impair microfibril formation. Delayed secretion with over-N glycosylation was also noted in a nonsense mutation in the terminal FBN1 exon in a patient with MFS. However, it is not known whether delayed secretion causes over-N glycosylation or if N glycosylation causes functional impairment directly. Lonnqvist and coworkers’ studies found that their patient with nMFS was completely devoid of fibrillin 1 and 10-nm microfibrillar structures.

### CONCLUSIONS

We have described an infant with nMFS and unusual features. A single T-to-C transition at nucleotide 3276 of FBN1 was identified and predicted to result in a substitution of a threonine for an isoleucine, which produces a novel N-glycosylation site at Asn1046. This same mutation has been reported previously in 2 infants with nMFS and similar features, in whom the same substitution was identified. We conclude that the severe phenotype and unusual clinical features seen in these 3 patients are secondary to this rare mutation and represent a novel genotypic-phenotypic correlation within the group of pa-

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**Clinical Findings in 3 Cases of Neonatal Marfan Syndrome With T-to-C Transition at nt3276 of FBN1**

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| Arachnodactyly, dilatation of 4 chambers and aorta, bronchomalacia, emphysematous changes, respiratory failure, diaphragmatic hernia, myxomatous tissue mass surrounding atriocentrival valves | Eventration of right hemidiaphragm, myxomatous tissue surrounding atriocentrival node and atriocentrival, pulmonary, and aortic valves | Dilated aortic root, mitral and tricuspid regurgitation, sustained fractures of the clavicle and humerus, respiratory failure |**What This Study Adds**

Neonatal Marfan syndrome is a rare disorder of connective tissue. It is caused by a mutation in FBN1. FBN1 mutations have been associated with a broad spectrum of clinical phenotypes. There is currently limited correlation between genotype and phenotype in this disorder. We attempted to describe unique somatic features observed in our patient with nMFS and to correlate them with an FBN1 mutation, T3276C, previously identified in 2 other patients. We conclude that there is a genotypic-phenotypic correlation associated with this disorder.

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Patients with nMFS. Additional investigation is needed to demonstrate the molecular pathologic consequences of this mutation in the pathway from N glycosylation to the defective microfibril assembly. Similar studies will result in additional genotypic-phenotypic correlations and a better understanding of the pathogenesis of this complex disorder.

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