Double mutant fibrillin-1 (FBN1) allele in a patient with neonatal Marfan syndrome

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Abstract
It is now well established that defects in fibrillin-1 (FBN1) cause the variable and pleiotropic features of Marfan syndrome (MFS) and, at the most severe end of its clinical spectrum, neonatal Marfan syndrome (nMFS). Patients with nMFS have mitral and tricuspid valve involvement and aortic root dilatation, and die of congestive heart failure, often in the first year of life. Although mutations in classical MFS have been observed along the entire length of the FBN1 mRNA, mutations in nMFS appear to cluster in a relatively small region of FBN1, approximately between exons 24 and 34. Here we describe the appearance of two FBN1 mutations in a single allele of an infant with nMFS. The changes were within six bases of each other in exon 26. One was a T3212G transversion resulting in an I1071S amino acid substitution and the second was an A3219T transversion resulting in an E1073D amino acid substitution. This is the first reported double mutant allele in FBN1. (J Med Genet 1996;33:760–763)

Key words: Marfan syndrome; fibrillin; mutation.

Recently, in describing the identification of two cystic fibrosis transmembrane conductance regulator (CFTR) mutations in a single allele, Savov et al. asked whether double mutant alleles were rare. Analysis of the CFTR gene in several populations has shown the appearance of double mutant alleles. In fact, different clinical phenotypes have been associated with the presence of double mutant alleles. Similar double mutant alleles have been observed in the acid β-glucosidase gene in Gaucher disease1 and in the p53 gene in some human tumours.6

Marfan syndrome (MFS) is an autosomal dominant heritable connective tissue disorder manifested by abnormalities in the skeletal, ocular, and cardiovascular systems.7 Neonatal Marfan syndrome (nMFS) is at the most severe end of the phenotypic expression of MFS.8 Patients with nMFS share many of the skeletal features seen in classical MFS, but their cardiovascular phenotype includes acute multiavalvular disease that is rarely seen in classical MFS. These patients often die of congestive heart failure within the first year of life.8

It is now well known that defects in the gene encoding fibrillin-1 (FBN1) cause a wide spec- trum of microfibrilopathies. These include MFS,1,10-23-26 nMFS,23-25 30 31 a family with relatively mild skeletal findings,22 ectopia lentis,24 31 and late onset aortic aneurysms.32 Interestingly, all documented mutations in nMFS patients have clustered between exons 24 and 34. Here we describe a double mutant allele in a nMFS patient. The mutations, in exon 26, are a T3212G transversion that results in an I1071S amino acid substitution and an A3219T transversion resulting in an E1073D amino acid substitution. Although probably caused by a single mutational event, this is the first reported double mutant fibrillin-1 allele.

Materials and methods

CASE REPORT
A black female infant with clinical features of severe nMFS was born to a 28 year old primigravida at 34 weeks’ gestation. Cranial malformation, diaphragmatic abnormality, limb anomaly, and tricuspid and pulmonary insufficiency were noted prenatally. Birth weight, length, and head circumference were normal. She had scaphocephaly, plagiocephaly, and widely open anterior and posterior fontanelles. The ears were large with flat pinnae. The chest showed pectus carinatum and profound left thoracolumbar scoliosis. Cardiac findings were significant for dextrocardia. There were bilateral flexion contractures of the elbows, wrists, hips, knees, and ankles. She had arachnodactyly, camptodactyly, loose redundant skin, and decreased muscle mass. Ophthalmological evaluation showed bilateral optic nerve coloboma with no lens subluxation. Echocardiogram, at birth, showed situs solitus, dextrocardia, and prolapsing tricuspid and mitral valves, with tricuspid and mitral insufficiency and right ventricular hypertrophy. She had evantration of the right hemidiaphragm which was surgically corrected at 1½ months. Chromosome analysis from amniocytes, blood lymphoblasts, and skin fibroblasts was 46,XX. The patient’s hospital course was complicated by several episodes of spontaneous pneumothorax requiring persistent intubation. She died at the age of 3 months following a tracheostomy procedure. Permission for necropsy was not granted.

DNA EXTRACTION

Extraction of DNA from dermal fibroblasts was carried out as previously described.25

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DOUBLE MUTANT FIBRILLIN-1 ALLELE IN A PATIENT WITH NEONATAL MARFAN SYNDROME

POLYMERASE CHAIN REACTION
Amplification of genomic DNA from the patient and controls was performed using intron primers flanking exon 26: forward 5' ATT TAA GGC TGT CCT GAG AC 3' and reverse 5' CAT GGA ATC CTT CTC TTT CTG 3' at the following conditions: denaturing 94°C/1.5 minutes, annealing 58°C/2.5 minutes, and extension 72°C/2.5 minutes for 30 cycles.

HETERODUPLEX ANALYSES
Amplified products were screened for the presence of possible mutations using Mutation Detection Enhancement (MDE) (FMC Corp) gels. Primer sequences and amplification conditions were as described by Niibrook et al. In order to maximise the formation of heteroduplexes, 10 μl of PCR product was mixed with 2 μl of gel loading buffer (50% sucrose, 0.6% xylene cyanol, 0.6% bromophenol blue) and incubated at 95°C/three minutes, 75°C/five minutes, and 37°C for at least five minutes until all samples were loaded onto the gel. Samples were resolved on 0.5 x MDE gels at 800 volts for approximately five hours according to the manufacturer’s protocol. After electrophoresis, gels were stained in a solution of 0.6 x TBE containing 1 μg/ml ethidium bromide and DNA was visualised under UV light.

RESULTS
Genomic DNA, amplified with intron primers to exon 26, showed heteroduplex formation when run on an MDE gel (fig 1A). Direct sequencing of the amplified fragment showed two nucleotide substitutions (data not shown). In order to determine whether both changes were on a single allele, the amplicon was cloned and subsequently sequenced. Sequence analysis of the clones showed that both the T3212G and A3219T transversions were on the same FBN1 allele (fig 1B). The former mutation resulted in an I1071S amino acid substitution and the latter transversion resulted in an E1073D amino acid substitution. Study of the parents confirmed that neither harboured any of the mutations (data not shown). Studies using several highly informative markers were also consistent with paternity.

DISCUSSION
It is becoming increasingly evident that a clustering of some FBN1 mutations in what Kainulainen et al have called “the neonatal region” results in disease at the most severe end of the Marfan syndrome phenotype spectrum, namely nMFS. Recent studies have expanded the region to include exons 24 to 34 of FBN1. Importantly, not all patients with mutations in the “neonatal region” have nMFS. Some have classical MFS, but at the severe end of the spectrum, while others have missense mutations that cause typical or mild MFS. A compound heterozygous MFS patient, whose parents had classical MFS, has already been described. His two mutations caused very severe disease and early death.

The patient reported here had the typical cardiovascular features seen in nMFS, but also had dextrocardia. There are three major causes of dextrocardia: (1) situs inversus, a mirror image inversion of all the normally asymmetrical structures; (2) dextroversion, failure of the cardiac apex to rotate from right to left; and (3) dextroposition, a mechanical shift of the heart to the right owing to either a mass effect in the left chest or lack of normal lung volume in the right chest. Echocardiograms and plain chest and abdominal radiographs showed the position of the liver, spleen, stomach, and heart in our patient. The cardiac situs was determined to be solitus (atria were in the correct right-left orientation). Also, there was no abdominal heterotaxia. This finding rules out laterality disorders, such as Kartagener syndrome and the asplenia and polysplenia disorders. We believe this patient had dextroposition secondary to her severely misshapen thoracic cage, despite the raised right hemidiaphragm. Unfortunately, no necropsy was performed that would have confirmed this suspicion.

Although many hundreds of CFTR mutations have been reported, from the thousands of CF patients screened, few double mutant alleles have been identified. Savov et al.

Figure 1  (A) Mutation detection enhancement (MDE) gel analysis of an amplicon of exon 26 from genomic DNA. The heteroduplex band can be seen in the lane from the patient (arrow). The remaining lanes represent analysis of the same amplified region in controls, but do not exhibit heteroduplex formation. (B) Sequence analysis of cloned fragments showed two transversions within seven nucleotides, T3212G and A3219T, in the "mutant" clone when compared to the "normal" clone.
correctly point out that once the common mutations have been identified in patients screening stops. It is indeed possible that if the entire CFTR was screened in all CF patients, additional double mutant alleles would be uncovered. Since almost all MFS patients and families have unique FBN1 mutations, most protocols call for the screening of the entire coding region and flanking splice junctions.

The recent publication of intron primers for all 65 FBN1 exons has greatly aided the effort to identify mutations in MFS patients and in those with overlapping phenotypes. In our routine screening through the neonatal region first and subsequently the entire complement of the remaining FBN1 exons, we identified a nMFS patient with two changes in FBN1. Since the initial sequencing was performed directly from PCR amplicons, we were unsure whether both changes were in the same allele. Cloning of the PCR products showed that both changes were in a single allele. It was still unclear whether one change was a polymorphism and the issue of paternity needed to be addressed. Paternity was confirmed by examining four highly polymorphic markers. Amplification and sequencing of parental DNA showed that neither parent harboured either the T3212G or A3219T transitions. Thus, both changes had occurred sporadically and, given their proximity, were most probably a single mutational event. Conformational studies of the folding region of FBN1 some 50 bases upstream and downstream from the mutations do not show any particularly stable DNA secondary structures that might interfere with replication. It may be significant that both mutations are transversions, however. Both of the substituted positions are conserved in the precursor epidermal growth factor (EGF)-like calcium binding domains of FBN1. The I1071S amino acid substitution appears to be the most significant, since the substitution of a hydrophobic residue for a polar amino acid may disrupt the hydrophobic core of fibrillin. The E1073D amino acid substitution appears, on the surface, to be fairly conservative, since both aspartate and glutamate are negatively charged with relatively small side chains. However, the glutamic acid is invariant in the FBN1 EGF-like domains and is adjacent to one of the cysteine residues that are also invariant. The substitution of aspartic acid for glutamic acid may influence the formation of disulphide bridges. A substitution at the 1073 residue causing nMFS has in fact been described. In that case, however, the substitution for glutamic acid was for lysine, a basic amino acid with a much larger side chain. It is likely that both the I1071S and E1073D amino acid substitutions played a role in altering the structure of fibrillin-1 and the microfibrils in this case of nMFS. Double mutant alleles are probably rare in most disorders, but, when present, may influence the course of the clinical outcome.

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