A new missense mutation of fibrillin in a patient with Marfan syndrome

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Abstract
A patient with Marfan syndrome was shown to be heterozygous for a G to A transition at nucleotide 3952 of the FBN1 gene. This would result in a cysteine to tyrosine substitution at amino acid 1223 in the fibrillin protein.

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Marfan syndrome (MFS) is an autosomal dominant inherited disorder of connective tissue with a frequency of 1 in 10,000. It classically affects three systems, cardiovascular, ocular, and skeletal. The fibrillin gene FBN1 is the disease causing locus for MFS. Fibrillin is a 350 kDa protein that is an abundant component of 10-12 microfibrils. A full length cDNA copy of the FBN1 mRNA was screened for mutations using 16 sets of overlapping primers and the polymerase chain reaction (PCR) to search for single stranded conformation polymorphisms (SSCP). SSCP analysis on the SaqI digestion fragments of the amplification product of primers G2S (5' AGGGAAGTATCAGTGCTGGC 3') and G2AS (5' TCCTGCAGTCTGACATGCTG 3') gave a band of aberrant mobility (data not shown). Sequencing of this region showed patient 2521 to be heterozygous for a G to A transition at nucleotide 3952. This would be predicted to change cysteine 1223 of the fibrillin polypeptide to a tyrosine. Screening of the unaffected members of the pedigree was performed using hybridisation of allele specific oligonucleotides to either the G or the A allele (fig 1).

None of the unaffected members of the pedigree was found to carry the A allele (fig 1). ASO screening of 100 controls and 58 other MFS patients showed it was neither a common cause of MFS nor a common polymorphic variant.

Patient 2521 is a 66 year old female whose parents were unaffected by MFS. She has bilateral dislocated lenses, myopia, and severe kyphoscoliosis. She has arachnodactyly in her left hand only, and one foot is longer than the other. She was diagnosed as having MFS when she gave birth to a daughter with a pronounced marfanoid habitus. The child had bilateral dislocated lenses, long fingers, hands, and feet, pectus excavatum, kyphoscoliosis, and poorly developed musculature. She died at 15 months from congenital heart disease complicated by bronchopneumonia. Necropsy showed that the pulmonary valve had only two cusps. There was no evidence of either mitral or aortic valve involvement in either patient 2521 or her affected daughter.

There was further evidence to suggest the mutation was causal. No sample was available from either of her unaffected parents, so it was not possible to show directly that the first appearance of the MFS phenotype in the pedigree coincided with a de novo mutation at cysteine 1223. The pedigree was genotyped for the (CA)n polymorphism MTS2 within intron 8 of the FBN1 gene (fig 1). Each of the MTS2 alleles of patient 2521 was shared by at least one of her unaffected sibs. This showed that the mutation in patient 2521 was a de novo

Figure 1 Screening of the proband (arrowed) and her pedigree by ASO hybridisation. G2S and G2S (5' ATGATCTCTGTCAGGATT 3') primed PCR amplification products of genomic DNA from pedigree members were filter immobilised. Amplifications were carried out according to standard conditions, with a temperature profile of: 94°C for 0.5 minutes; 58°C for 1.0 minute; 72°C for 1.0 minute (30 cycles). No samples were available from any of the dead persons. Directly below each extant family member is shown the autoradiograph from hybridisation of filter with oligonucleotides complementary to either the wild type (GYS 1223) allele or the mutant (TYR 1223) allele. The primers used were: CYS1223 ASO: (5' CCCGCGCTGACGCTAGTATT 3') and TYR1223 ASO: (5' AATGAGCTATCGAGCGG 3'). The mismatched base of each oligonucleotide is underlined. Washing was carried out at 58°C (Tyr1223 ASO) or 60°C (CYS1223 ASO) according to standard procedures. The MTS2 polymorphism genotypes are also shown on the pedigree. The MTS2 alleles were amplified and recognised under the conditions detailed in reference 9.
Cysteine 1. CYS 1242 TYR
CYS 1117 TYR

Cysteine 2. CYS 1249 SER

Cysteine 3. CYS 1663 ARG
CYS 2221 SER
CYS 2307 SER

Figure 2 A schematic diagram of EGF-like domain number 15 of fibrillin. The six highly conserved cysteines are shaded. The cysteine residue involved in the mutation described in this patient (CYS 1223 TYR at cysteine 5) and others reported in different EGF-like domains are indicated by arrowed boxes.

mutation that must have occurred in the germ-line of one of her parents.

The C1223Y mutation is located within the 15th EGF-like repeat of fibrillin. It is not the first cysteine substitution to be described in Marfan syndrome. At a recent meeting eight other MFS patients were shown to be heterozygous for missense mutations at key cysteine residues in fibrillin.\(^\text{10-17}\) All these cysteine substitutions were unique, and six of them occurred at highly conserved residues within EGF-like repeats.\(^\text{10-17}\) There are 47 EGF-like repeats in the fibrillin molecule.\(^\text{3}\) Fibrillin EGF-like repeat 15 is shown schematically in fig 2, with the cysteines numbered 1 to 6; cysteine 1223 occurs at cysteine number 5 in this repeat. The substitution of a tyrosine residue here would clearly disrupt the disulphide bond between cysteine number 5 and cysteine number 6. The relative positions of the other six cysteine mutations that occur in other EGF-like repeats are also shown in fig 2.

The precedent for disease related substitutions at cysteine residues in EGF-like repeats is not limited to fibrillin and MFS. Similar mutations have been detected in human factor IX and low density lipoprotein receptor in cases of haemophilia B\(^\text{20}\) and familial hypercholesterolaemia.\(^\text{21-23}\)

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