

Original Article

A novel mutation in fibrillin-1 gene identified in a Chinese family with marfan syndrome

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Abstract: Marfan syndrome (MFS) is an autosomal dominant inheritary disorder of the connective tissue. We report clinical features of a Chinese family with MFS and identify mutations in fibrillin-1 gene (FBN1). In this study, all three members of this family underwent complete ophthalmologic examinations. Two of the three members were diagnosed with MFS. Molecular genetic analysis was performed on the three members. All coding exons of FBN1 were amplified by polymerase chain reaction (PCR). The amplified products were sequenced and compared with a reference sequence in the database. Possible structural and functional changes of the protein induced by amino acids variance were predicted by bioinformatic analysis. A novel heterozygous mutation c.4504 T>A (p.C1502S) in exon 36 was identified in the two affected members, but not in the unaffected member. To our knowledge, this FBN1 mutation has not been reported before in MFS or other patients.

Keywords: Marfan syndrome, fibrillin-1, genetic analysis, exons, PCR, mutation

Introduction

Marfan Syndrome is known as an autosomal dominant hereditary disorder of the connective tissue with many clinical manifestations in the cardiovascular, skeletal, and ocular systems [1]. Most MFS patients have a family history. The incidence of MFS ranges from 1:10,000 to 1:20,000 [2]. There is no ethnic, geographical, or gender predisposition in MFS patients [3, 4].

A number of genes, including FBN1, transforming growth factor-beta receptor-1 (TGFB1) [5], and transforming growth factor-beta receptor-2 (TGFB2) [6], were found to be related to the pathogenesis of MFS. FBN1 was first found to be associated with MFS in 1991 [7]. Up to date, more than 3000 mutations have been identified in FBN1, including missense mutations, nonsense mutations, deletions, insertions and splice site mutations (<http://www.umd.be/FBN1/>).

FBN1, 235 kb in size, lies on the long arm of chromosome 15 at 15q15-q21.1 consisting of

65 exons and encodes a 350-kDa extracellular matrix glycoprotein fibrillin-1 [8]. Fibrillins polymerize into supramolecular fibrillar structures, microfibrils. FBN1 gene mutations disrupt microfibril formation, leading to fibrillin protein abnormalities and forms structurally inferior connective tissues [4].

In this study, we used gene-sequencing technology to analyze a small Chinese family with MFS. A novel mutation in the exon 36 of FBN1 (c.4504 T>A) was identified in the two affected members, but not in the unaffected member.

Material and methods

Family recruitment and clinical examination

One ethnic Han Chinese family with MFS was recruited at Shenzhen Eye Hospital. This family had three members (the parents and their daughter) with father as the proband. The parents were not related by consanguinity. The research was approved by the Medical Ethics Committee of Shenzhen Eye Hospital of Jinan

Table 1. Clinical details of the patients in the family

Patients	Proband	Proband's daughter
Age (years)	29	4
Gender	M	F
Ectopia lentis	+	+
Myopia	+	+
Strabismus	-	-
Glaucoma	-	-
Retinal detachment	-	-
Height (cm)	183	120
Arm span (cm)	192	124
AS/H	1.05	1.03
Thin body	+	+
Arachnodactyly	+	+
Aortic root aneurysm	+	-

University in Shenzhen, Guangdong Province, and performed according to the principles of the Declaration of Helsinki. The written informed consent was obtained from the three participants before blood samples were taken for analysis. The complete physical, ophthalmic, and cardiovascular examinations were performed on the three members.

Molecular genetic analysis

Two ml of peripheral venous blood samples were obtained from the three individuals. Genomic DNA was extracted from 200 µl peripheral blood cells using a Qiamp Blood Kit (Qiagen, Hilden, Germany). DNA integrity was detected by 1% agarose gel electrophoresis.

All 65 exon-intron boundaries of the FBN1 were amplified from genomic DNA of each participant by PCR using a MyCycler thermocycler (Bio-Rad, Hercules, CA). The forward and reverse primers were designed based on previously described primers [9]. PCR was conducted using 30 µl PCR amplification reaction mixture that contained 30 ng genomic DNA, 1.0 µM of each of the forward and reverse primers, and 15 µL of 2 × TaqMaster Mix (SinoBioBiltech Co. Ltd, Shanghai, China).

The PCR products were purified and directly sequenced by an ABI 377XL automated DNA sequencer (Applied Biosystems, Foster City, CA). Sequence data were compared with the FBN1 sequences from the Human Genome database (GenBank NC_000015.10).

Bioinformatics analysis

To analyze the evolutionary conservation of the mutant region, the online Clustalw2 (<http://www.ebi.ac.uk/Tools/msa/clustalw2/>) tool was used to align the protein sequences from eight different species. The possible functional impact of an amino acid change induced by mutation was predicted by sorting intolerant from tolerant analysis (SIFT) (<http://sift.jcvi.org/>) and Polyphen-2 (<http://genetics.bwh.harvard.edu/pph2/>).

Results

Clinical findings

This family was composed of the parents and their 4-year-old daughter. The father and daughter were MFS patients, the mother (27-year-old) had no clinical manifestations of MFS. Clinical data of the patients were showed in **Table 1**. The proband was the father (29-year-old) presented with aortic root aneurysm. Both father and daughter manifested lens dislocation (**Figure 1**), high myopia, and abnormalities of the skeletal system including tall stature and arachnodactyly (**Figure 2**). Aortic root aneurysm was not presented in the daughter. The mother, an unaffected family member, appeared to be normal.

Mutation identification

In order to detect the genetic defects of this family, we screened the exons of FBN1 by the Sanger sequencing. The mother, as a control, was also analyzed. A novel heterozygous missense mutation, c.4504 T>A (**Figure 3**) in exon 36, was identified in the father and daughter, but not in the mother. This heterozygous mutation resulted in the replacement of wild-type cysteine by serine (p.C1502S).

Bioinformatics analysis

The mutational site (p.C1502S) is located at a highly conserved region of the cbEGF-like22 calcium binding domain of fibrillin-1. It affects the first conserved cysteine (C1) of the cbEGF-like domain (<http://www.umd.olyphen-2be/FBN1/>). This highly conserved region was found to be completely conserved in 8 different species by using the online Clustalw2 tool (**Figure 4**). To analyze the protein function, the p.C1502S

A novel mutation of MFS

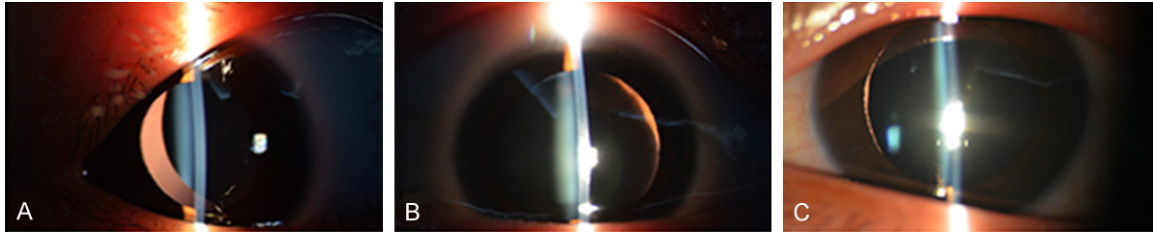


Figure 1. Slit lamp photographs of the eye of the affected family members showing ectopia lentis after the pupils were dilated. The lens is dislocated temporal in the right eye (A) of proband's daughter; the proband's eyes, right (B) and the left (C), are both nasally lens dislocation.

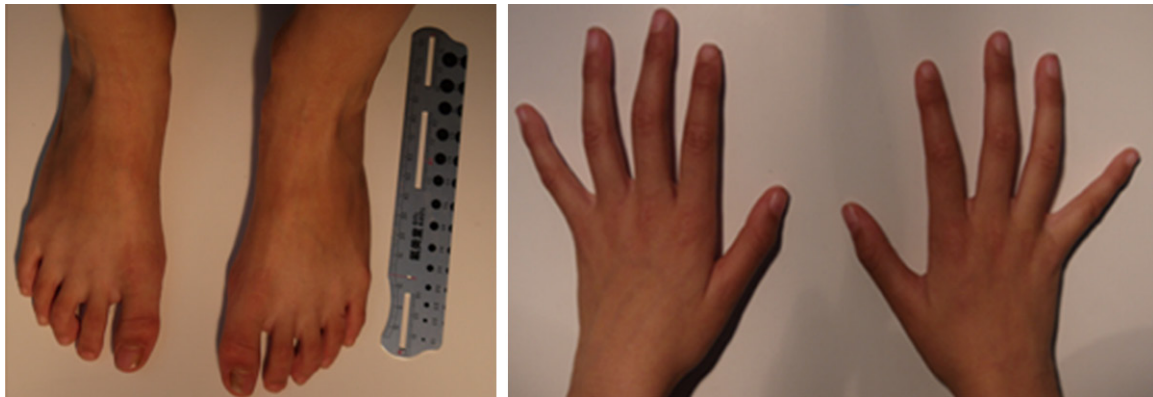


Figure 2. Arachnodactyly of the proband's daughter.

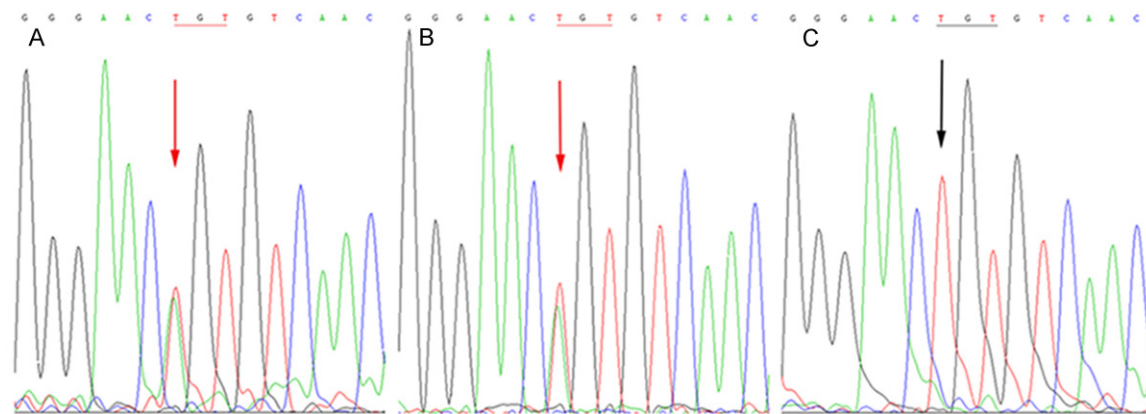


Figure 3. A FBN1 mutation in exon 36. A. A heterozygous T>A (red arrow) mutation of FBN1 was identified in the daughter and the proband (B). C. Wild type sequence from the unaffected member (black arrow).

mutation of FBN1 was predicted to be “likely damaging” by Polyphen-2 with a reliable score of 1.00 and “affect protein function” by SIFT with a reliable score of 0.00. The results of Polyphen-2 and SIFT were consistent, which indicate that p.C1502S mutation of FBN1 may result in the structural and functional changes of the protein.

Discussion

There are a wide variety of phenotypic characteristics in MFS patients, even within the same family. Cardiovascular complications are the most life-threatening manifestation of MFS, including aortic root aneurysm and dissection, mitral valve regurgitation, calcification of the

Homo sapiens	TDVNECLDPTTCISGNCVNTPGSYICDCPPDFELNPTRVGCVD
Macaca fascicularis	TDVNECLDPTTCISGNCVNTPGSYTCDCPPDFELNPTRVGCVD
Felis catus	TDVNECLDPTTCISGNCVNTPGSYTCDCPPDFELNPTRVGCVD
Canis lupus familiaris	TDVNECLDPTTCISGNCVNTPGSYTCDCPPDFELNPTRVGCVD
Bos Taurus	TDVNECLDPTTCISGNCVNTPGSYTCDCPPDFELNPTRVGCVD
Oryctolagus cuniculus	TDVNECLDPTTCISGNCVNTPGSYTCDCPPDFELNPTRVGCVD
Mus musculus	TDVNECLDPTTCISGNCVNTPGSYTCDCPPDFELNPTRVGCVD
Rattus norvegicus	TDVNECLDPTTCISGNCVNTPGSYTCDCPPDFELNPTRVGCVD

Figure 4. Mutation involved a highly conserved residue. The cysteine at position 1502 is highly conserved for FBN1, which was demonstrated by analyzing orthologs from 8 different species.

mitral valve annulus, dilatation or dissection of the descending aorta, and mitral valve prolapsed [2]. The ocular manifestations mainly include myopia, astigmatism, retinal detachment, and lens dislocation. Ectopia lentis is observed in around 80% of MFS patients [9]. The stretching or interrupt of zonular filaments causes displacement of the lens from its normal location [10]. Skeletal abnormalities include scoliosis, joint laxity, arachnodactyly, anterior chest deformities, and tall stature [11].

New diagnostic criteria, putting more weight on aortic root aneurysm and ectopia lentis, were released in 2010 [12]. These two clinical manifestations are sufficient for the diagnosis of MFS if there is no family history. In addition, FBN1 gene mutation is still considered to be an important diagnostic criterion for the MFS, and FBN1 testing has more weight in the diagnostic assessment [12]. The combination of an aortic aneurysm and a pathogenic FBN1 mutation is competent to confirm the diagnosis of MFS. But for the children and adolescents, diagnostic criteria exist differences because some symptoms, especially cardiovascular complications, may develop over time [11]. Therefore, the children with a positive family history and the manifestations of tall, thin body habitus, long limbs and arachnodactyly may be suggestive of a diagnosis of MFS [13]. In this study, the aortic root aneurysm, which was found in the father, is an important diagnostic criterion for MFS in this family. Moreover, the father and daughter presented with tall stature, slim body habitus, and dolichostenomelia, as well as lens dislocation and myopia. Furthermore, we identified a novel missense mutation in exon 36 (c.4504 T>A) of FBN1 in both father and daughter.

The pathogenesis of the MFS has not been fully elucidated. Previous studies showed that the disease-causing genes of MFS include FBN1 and TGFB2. Defects in FBN1 gene are much more common in MFS than that in TGFB2 genes [14, 15]. Fibrillin contains 47 EGF-like domains that are characterized by a highly conserved arrangement of six cysteine residues. These cysteine residues form 3 intra-domain disulfide bonds (C1-C3, C2-C4, C5-C6) that are required to maintain the right fold of cbEGF-like module [16]. Forty-three of the 47 EGF-like domains are the calcium-binding consensus sequences (cbEGF) that exist as tandem repeats. Most mutations of FBN1 occur in these cbEGF-like domains [17]. The mutation-sin cbEGF domains may result in eliminating or generating cysteine residues, or directly affecting the structure and function of the calcium binding domains [18]. CbEGF-like module would be misfolded if cysteine residues were altered [19]. It is likely that the loss, addition or replacement of cysteine residues disturb the formation of the three disulfide bonds, which in turn leads to malformation of stable and correct folded β -sheet. In our study, the missense mutation identified in cbEGF that results in the first cysteine (C1) being replaced by serine (p.C1502S). This replacement of cysteine may alter the intramolecular disulfide bonds arrangement, and thus change the secondary structure of the protein. In addition, FBN1 mutations have a dominant negative affection to microfibril [20] that disturbs the polymerization of fibrillin and assembly of microfibril. Therefore, FBN1 mutations could result in fibrillin protein abnormalities and subsequently weaken the connective tissue [21]. Mutated FBN1 may also destroy the stability of elastic fibers and are more susceptible to proteolysis. It was reported that FBN1 mutation was more

likely to be identified inpatients with ectopia lentis and cardiovascular complications. In addition, mutations involving a cysteine substitution in FBN1 were found to be much more common in the patients with ectopia lentis [22].

In conclusion, we identified a novel missense mutation in FBN1 (p.C1502S) in a Chinese Han family with MFS. Our results add a novel mutation to genotype-phenotype spectrum of FBN1 that causes MFS. The mutation involving cysteine substitutions in FBN1 could play a critical role in the pathogenesis of MFS in this family.

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Disclosure of conflict of interest

None.

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