



A novel *FBNI* heterozygous mutation identified in a Chinese family with autosomal dominant Marfan syndrome

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ABSTRACT. The purpose of this study was to identify the clinical features and mutations in the fibrillin-1 gene (*FBNI*) in a large Chinese family with autosomal dominant Marfan syndrome (MFS). Seventeen members from a Chinese family of 4 generations were included in the study. All members underwent complete ophthalmic examination. Molecular genetic analysis was performed on all subjects. All exons of *FBNI* were amplified by polymerase chain reaction, sequenced, and the sequences were compared with a reference database. Variations were evaluated in family members as well as 100 normal controls. Changes in structure and function of the protein induced by amino acid variation were predicted by bioinformatic analysis. Ectopia lentis,

dolichostenomelia, arachnodactyly, and tall stature were present in all patients diagnosed with MFS. The novel heterozygous missense mutation c.2243 T>G (p.C781W) in exon 19 of *FBNI* was identified in all 5 patients, but not in other family members or 100 normal controls. This mutation caused an amino acid substitution of cysteine to tryptophan at position 781 (p.C781W) of the FBNI protein. This mutation occurred in a highly conserved region and may cause structural and functional changes in the protein according to our bioinformatic analysis. Our results suggest that the novel mutation C781W of *FBNI* is responsible for the pathogenesis of MFS in this pedigree.

Key words: Ectopia lentis; Exon; *FBNI*; Marfan syndrome; Mutation

INTRODUCTION

Marfan syndrome (MFS) is a multisystemic, autosomal dominant disorder with pleiotropic manifestation mainly involving cardiovascular, ocular, and skeletal tissues (Milewicz et al., 1992). The estimated prevalence of MFS is 1 in 10,000-20,000 individuals (Dong et al., 2012), without gender or ethnic predisposition (Mátyás et al., 2002). The cardiovascular complications of the disorder include aortic root dilatation and dissection, mitral valve regurgitation, and mitral valve prolapse. These complications can lead to premature death if they are untreated (Murdoch et al., 1972). Skeletal abnormalities include scoliosis, joint laxity, arachnodactyly, anterior chest deformities, and tall stature (Pyeritz and McKusick, 1979). Ocular features include myopia, retinal detachment, and lens dislocation. The latter, also known as ectopia lentis, was observed in around 80% of MFS patients (Micheal et al., 2012). Ectopia lentis is characterized by the displacement or malposition of a lens from its normal location, causing the zonular filaments to be stretched or discontinued (Liang et al., 2011).

Genetic factors are known to play important roles in the pathogenesis of MFS. Two genes, including fibrillin-1 (*FBNI*) and transforming growth factor beta receptor II were found to be MFS-associated genes (Zhao et al., 2013). *FBNI* consists of 65 exons, spanning 235 kb of genomic DNA on chromosome 15q21 and encodes a secreted 350-kDa extracellular matrix glycoprotein that serves as a structural component of 10-12-nm calcium-binding microfibrils (Ramirez and Sakai, 2010). These microfibrils provide force-bearing structural support in elastic and non-elastic connective tissue throughout the body (Hubmacher et al., 2006). *FBNI* is mainly composed of 3 types of repeated modules, including a cysteine-rich epidermal growth factor (EGF)-like domain, transforming growth factor beta-1 binding protein-like module, and a hybrid module (Meng et al., 2011).

There are 47 such EGF-like domain modules in *FBNI*, and 43 contain a calcium-binding (cb) consensus sequence and are thus referred to as cbEGF-like modules (Handford et al., 1991). To date, more than 1200 mutations have been detected in *FBNI*, and most affect one of the cbEGF domains, often involving 1 of the 6 highly conserved cysteine residues within the cbEGF domains (Micheal et al., 2012).

The age-related nature of some clinical manifestations as well as variable phenotypic expression of MFS may prevent accurate diagnosis, particularly in children (Loeys et al., 2001). As such, in situations of clinical uncertainty, molecular analysis of *FBNI* appears to be a logical method for the clinical diagnosis of MFS (Robinson et al., 2006). To understand the

genetic background of a 4-generation Chinese family with MFS, the primary candidate gene *FBNI* was analyzed in this study.

MATERIAL AND METHODS

Family recruitment and clinical examination

A 4-generation pedigree (Figure 1) with ectopia lentis was recruited from Shenzhen Eye Hospital (Shenzhen, Guangdong, China). Consanguineous marriage was noted in the family (Figure 1). The study was approved by the Medical Ethics Committee of the Shenzhen Eye Hospital. Informed consent was obtained from all participants according to the principles of the Declaration of Helsinki. All family members included in the study underwent examinations, including slit-lamp biomicroscopy, gonioscopy, and fundus examination. All individuals in the control group were healthy and had no history of other familial inherited diseases.

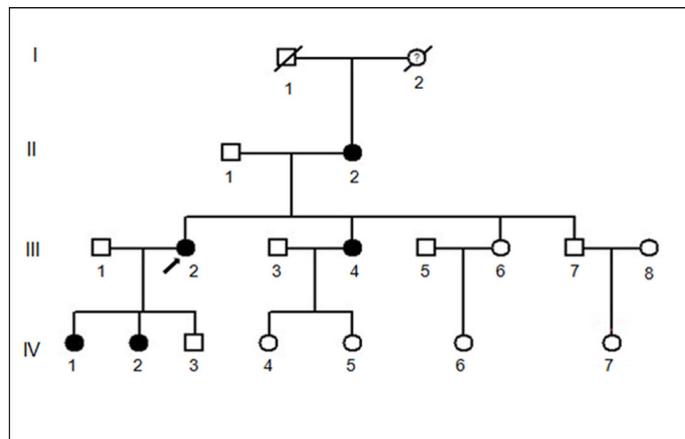


Figure 1. Pedigree of the Chinese family with MFS. Squares and circles indicate males and females, respectively, and the filled symbols represent the affected members. Slashed symbols denote deceased subjects. Symbol with a question mark in the center indicates that the diagnosis was unclear. Arrow indicates the proband. The parents of proband (II: 1 and II: 2) were consanguineous.

Molecular genetic analysis

Genomic DNA was extracted from 200 μ L venous blood using a Qiam Blood Kit (Qiagen, Hilden, Germany). All procedures were performed according to manufacturer instructions. DNA integrity was evaluated by 1% agarose gel electrophoresis. All 65 exons of the *FBNI* gene were amplified by polymerase chain reaction (PCR) using previously described primers (Liang et al., 2011). PCR amplification was conducted in a MyCycler thermocycler (Bio-Rad, Hercules, CA, USA). The 30- μ L PCR mixtures included 30–40 ng genomic DNA, 1.0 μ M of each forward and reverse primers, and 15 μ L 2X Taq Master Mix (including 1X PCR buffer, 2.5 mM $MgCl_2$, 0.3 mM of each dNTP, and 1.5 U *Pfu* DNA polymerase). All reagents used in this procedure were purchased from SinoBio Biltech Co., Ltd. (Shanghai, China). Sequence data were compared pairwise with the published *FBNI* sequences. Muta-

tions were named according to the nomenclature recommended by the Human Genomic Variation Society.

RESULTS

Clinical findings of the pedigree

Five of the 17 members in this 4-generation family were diagnosed as having MFS. All patients showed similar clinical manifestations, including bilateral lens dislocation (Table 1 and Figure 2). Skeletal system abnormalities such as tall stature, thin body habitus, long limbs, and arachnodactyly were observed in all patients (Figure 3). However, no patients displayed cardiovascular system abnormalities. Individuals I: 1 and I: 2 died many years ago; according to the memories of their family members, I: 2 had poor visual acuity, but no related medical records were available.

Table 1. Clinical details of the patients in the family.

Patients	II: 2	III: 2	III: 4	IV: 1	IV: 2
Age (years)	65	36	34	16	11
Gender	Female	Female	Female	Female	Female
Ectopia lentis	+	+	+	+	+
Myopia	-	-	-	-	-
Strabismus	+	+	+	+	+
Glaucoma	-	-	-	-	-
Retina detachment	-	-	-	-	-
Height (cm)	168	174	171	165	162
Arm span (cm)	169	175	172	167	165
Thin body	+	+	+	+	+
Arachnodactyly	+	+	+	+	+
Cardiovascular system	-	-	-	-	-

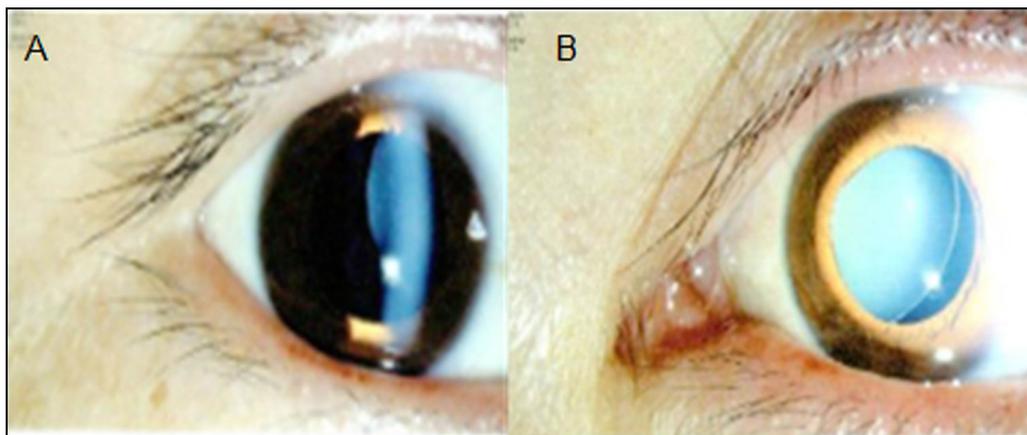


Figure 2. Anterior segment of proband (III: 2). **A** and **B** show ectopia lentis in both eyes. The pupils are dilated. The lens is dislocated nasally in the right eye (**A**); in the left eye (**B**), the lens is dislocated superiorly.



Figure 3. Arachnodactyly of the patient IV: 2.

Mutation identification and bioinformatic analysis

Sequence analysis of *FBNI* revealed a heterozygous missense mutation, c.2243 T>G (p.C781W), in exon 19 of all 5 patients (Figure 4). No mutation was identified in other asymptomatic members of the family or 100 normal control subjects included in the study.

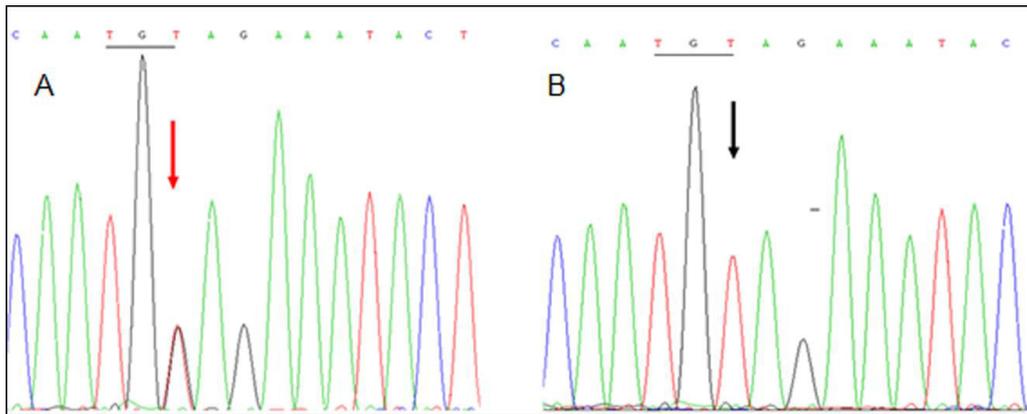


Figure 4. Sequencing results of the *FBNI* gene. **A.** A heterozygous T to G cysteine substitution at codon 781 in one patient from the family (red arrow). **B.** Wild-type sequence from an unaffected member.

The mutation c.2243 T>G in exon 19 of *FBNI* resulted in replacement of cysteine by tryptophan. Cysteine at position 781 was highly conserved in *FBNI* based on the analysis of orthologs from 8 different species according to the online ClustalW tool (Figure 5). The p.C781W mutation of *FBNI* was predicted to be “likely damaging” by polymorphism phenotyping with a score of 0.99 and “damaging” by sorting intolerant from tolerant analysis with a score of 0.00, indicating that protein function was affected by amino acid changes (a score <0.05 indicates that the amino acid change affects the protein function). Secondary structure prediction using the GORIV method suggested that the p.C781W mutation resides in the 12th cbEGF domain, affecting 1 of the most highly conserved cysteine residues.

[<i>Homo sapiens</i>]	TYKICNSGYEVDSTGKNCVDINECVLNSLLCDNGGQCRNTPGSFVCT
[<i>Gallus gallus</i>]	TYKICNPGYEVDSTGKNCIDIDECVLNLLCDNGGQCRNTPGSFTCT
[<i>Mus musculus</i>]	TYKICNSGYEVDITGKNCVDINECVLNSLLCDNGGQCRNTPGSFVCT
[<i>Rattus norvegicus</i>]	TYKICNSGYEVDITGKNCVDINECVLNSLLCDNGGQCRNTPGSFVCT
[<i>Callithrix jacchus</i>]	TYKICNSGYEVDSTGKNCVDINECVLNSLLCDNGGQCRNTPGSFVCT
[<i>Bos taurus</i>]	TYKICNSGYEVDSTGKNCVDINECVLNSLLCDNGGQCRNTPGSFVCT
[<i>Pan troglodytes</i>]	TYKICNSGYEVDSTGKNCVDINECVLNSLLCDNGGQCRNTPGSFVCT
[<i>Macaca mulatta</i>]	TYKICNSGYEVDSTGKNCVDINECVLNSLLCDNGGQCRNTPGSFVCT

Figure 5. Mutation involved a highly conserved residue. The cysteine at position 781 is highly conserved for *FBNI*, which was demonstrated by analyzing orthologs from 8 different species.

DISCUSSION

FBNI was the first identified gene associated with the pathogenesis of MFS (Dietz et al., 1991). *FBNI* mutations are thought to exert a dominant negative effect (Erentug et al., 2006). To date, more than 1000 of the mutations in this gene have been described, and these mutations are spread throughout the entire gene without a clear predilection for any given region (Boileau et al., 2005). Most of the mutations are missense mutations, and most of these are cysteine substitutions.

The coding sequence of *FBNI* is spread over 65 coding exons. Previous studies revealed that mutations in *FBNI* frequently occur in exons 2, 15, 22, 27, 46, 55, and 62 (Faivre et al., 2008) rather than exon 19 (Micheal et al., 2012). To date, only 5 mutations have been identified in exon 19, including a frameshift mutation (2412delAT) observed in one Italian patient with clinical symptoms mainly involving the skeletal and cardiovascular systems (Pepe et al., 2001), 3 missense mutations in 3 sporadic patients (p.C781R, p.C781R, p.C776Y) in patients from Belgium with classical MFS and involvement of the cardiovascular system (Loeys et al., 2001), and a heterozygous missense mutation (c.2368T>A p.C790S) in all affected members presenting ectopia lentis, myopia, and glaucoma, but lacking the cardinal cardiovascular features of MFS in a large Pakistani family (Micheal et al., 2012). In our study, the novel heterozygous missense mutation c.2243 T>G (p.C781W) in exon 19 of *FBNI* was identified in all patients in the pedigree. All patients presented bilateral lens dislocation, strabismus, tall stature, thin body habitus, long limbs, and arachnodactyly, but without cardiovascular system abnormalities. In early reports, 4 mutations identified in exon 19 were found to be associated with cardiovascular system involvement; however, this was not the case in the family described in our study.

EGF-like domains of *FBNI* play a principal role in the pathogenesis of fibrillinopathies, as most of the mutations in these domains are associated with classic MFS (Schrijver et al., 1999). Among these mutations, most are located in cbEGF-like domains. Each cbEGF-like domain of fibrillin-1 contains 6 highly conserved cysteine residues that form 3 intra-domain disulfide bonds (C1-C3, C2-C4, C5-C6) and a consensus sequence for calcium binding in the NH2-terminal region (Reinhardt, 1997). Because 3 disulfide bonds are required to maintain cbEGF-like module fold, the loss or addition of cysteine residues can result in module mis-

folding (Downing et al., 1996). Higher frequency substitutions of cysteine were observed in MFS patients with ectopia lentis (Rommel et al., 2005). This result agrees with the clinical findings in the family described herein. In our study, we identified a mutation in cbEGF modules. A missense mutation in exon 19 of *FBNI* located in the cbEGF-like #12 domain results in replacement of cysteine by tryptophan (p.C781W). Cysteine deficiency may alter the intramolecular disulfide bond arrangement, changing the secondary structure of the protein (Dietz et al., 1993). The presence of these particular clinical features such as bilateral lens dislocation, strabismus, tall stature, thin body habitus, long limbs, and arachnodactyly in all affected individuals may be due to the novel mutation p.Cys781Trp or may be associated with additional genetic factors contributing to the disease phenotype in this family.

In summary, we identified the C781W mutation in MFS patients. This study added a novel mutation to the existing spectrum of *FBNI* mutations, suggesting that mutations in *FBNI* are correlated with MFS as observed in this family. This study adds support for the need for genetic diagnosis of inherited disorders.

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