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C596G mutation in *FBN1* causes Marfan syndrome with exotropia in a Chinese family

Fengyun Wang, Bo Li, Lan Lan, Lin Li

Department of Ophthalmology, The First Affiliated Hospital, Henan University of Science and Technology, Luoyang, Henan, China

Purpose: To screen mutations in the fibrillin-1 (*FBN1*) gene in a Chinese family with autosomal dominant Marfan syndrome (MFS).
Methods: Patients and unaffected family members were given ophthalmic, cardiovascular, and physical examinations with a 5-year follow-up. Genomic DNA was extracted from the leukocytes of venous blood from all patients and their relatives. The entire coding region of the *FBN1*gene was screened with an ABI 9700 GeneAmp PCR System. The mutation identified was screened in 100 healthy and ethnically unrelated Chinese individuals.
Results: Mutation screening in *FBN1* identified a T>G transition at position c.1786 in exon 14, leading to substitution of cysteine for glycine at codon 596 (C596G) in this four-generation Chinese family. The C596G mutation was associated with the disease phenotypes in all six patients but not found in 14 unaffected family members or the 100 ethnically unrelated and healthy controls.
Conclusions: A C596G mutation in *FBN1* was identified in a Chinese family with MFS. Our results expand the spectrum of *FBN1* mutations and contribute to the understanding of the role of *FBN1* in the pathogenesis of Marfan syndrome.

Marfan syndrome (MFS) is an inherited, autosomal dominant, systemic disorder of connective tissue. Estimated incidence of this disease is 1/5,000–1/10,000 [1] with over 25% sporadic cases [2]. It has been well documented that the ocular, skeletal, and cardiovascular systems are the three major systems affected by the disease. The clinical criteria for MFS require involvement of at least two organ systems to establish the diagnosis if the patient has no family history. Ocular manifestations of MFS mainly involve ectopia lentis, characteristic of the dislocation of the lens, and high-myopic eyes [3,4]. Strabismus is a condition that the eyes cannot be properly aligned with each other due to a lack of coordination between the extraocular muscles [5]. Strabismus is a minor feature in patients with reported MFS [3,4]. Genetic screening can be applied to help the diagnosis. A major clinical manifestation in one organ system is enough to make the diagnosis of MFS with the presence of a mutation in the fibrillin-1 (*FBN1;* OMIM 134797) gene [6]. *FBN1* has been identified as a major disease-causing gene of MFS [2], indicating that genetic factors play a critical role in the pathogenesis of MFS.

FBN1, located at chromosome 15q-21.1 with 65 exons [7,8], encodes 2871-aa structural protein fibrillin-1, a 350-kDa glycoprotein with a modular structure comprising 47 epidermal growth factor-like (EGF) domains and seven transforming growth factor-β1 binding protein-like (TB) domains. Fibrillin-1 is the major component of extracellular microfibrils and regulates microfibril stability and assembly [9]. Fibrillin-1 mutations disrupt microfibril formation, result in fibrillin protein abnormalities, and eventually weaken the connective tissue. Nearly 3,000 mutations including 1,745 missense mutations in the *FBN1* gene have been documented in the Universal Mutation Database [10]. Most mutations are unique for specific families with MFS, and only approximately 15% of the mutations recur in different families [11]. In this study, we report that a missense mutation in exon 14 of *FBN1* (c.1786T>G), resulting in the substitution of cysteine by glycine at codon 596 (p.C596G), is associated with patients with MFS from a four-generation, non-consanguineous Chinese family. Our data further confirm the important role of *FBN1* in the pathogenesis of MFS.

METHODS

Patients and clinical data: This study was approved by the First Affiliated Hospital, Henan University of Science and Technology Joint Committee on Clinical Investigation and performed according to the tenets of the Declaration of Helsinki for Human Subjects. After we had obtained informed consent from each participant, all participants underwent complete physical, cardiovascular, and ophthalmologic examinations, and patients with MFS were diagnosed according to revised Ghent criteria [12]. Clinical data were

Correspondence to: Fengyun Wang, Department of Ophthalmology, The First Affiliated Hospital, Henan University of science and Technology, 24 Jinghua Lane, Jianxi Qu, Luoyang, Henan, 471003, China. Phone: 86-379-69823178; FAX: 86-379-69823178; email: wangfengyun2015@126.com

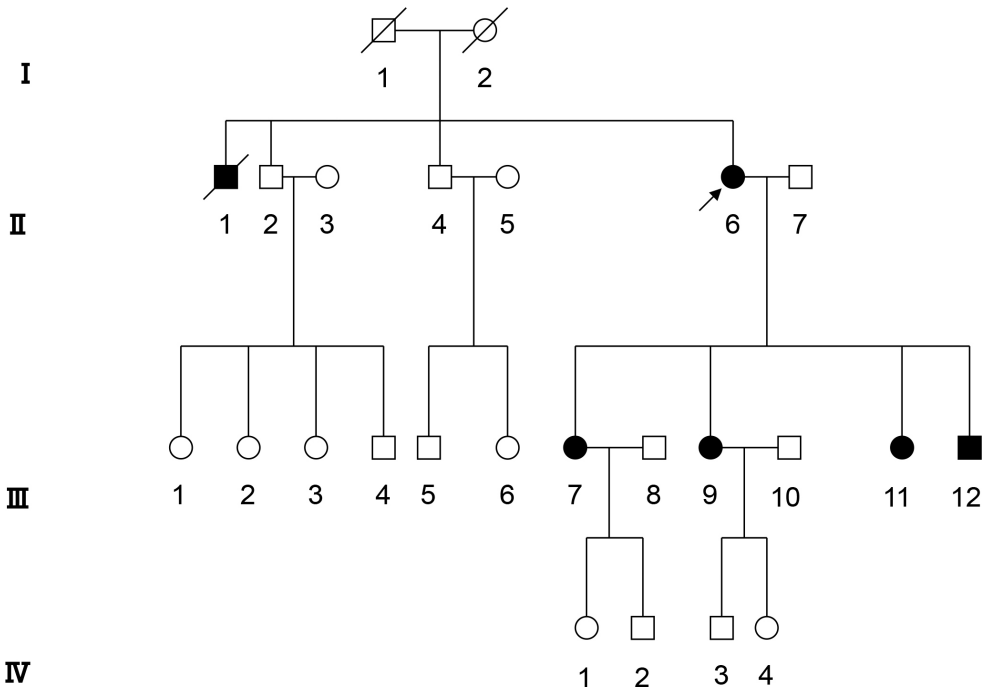

Figure 1. The pedigree of the family. Squares and circles represent men and women, respectively. The darkened symbols indicate the affected members. The patient above the arrow is the proband. Slashes denote deceased members. I to IV represent generations; 1 to 12 represent members in each generation.

collected from 11 family members (six patients: II:1, II:6, III:7, III:9, III:11, and III:12; five unaffected: II:2, II:7, III:4, III:5, and IV:2). One hundred healthy and ethnically unrelated Chinese controls were also recruited.

DNA sample collection and mutation screening of FBN1: To identify constitutional mutations, 5 ml of peripheral blood was obtained by venipuncture from a Bai Chinese family with MFS, and genomic DNA was extracted from peripheral blood cells according to standard protocols (Roche Diagnostics Corporation, Indianapolis, IN). The entire coding region of *FBN1* was amplified with PCR from genomic DNA. Primers for 65 exons and exon-intron boundaries of *FBN1* were designed with the Primer 3 program. PCR reactions were each performed in a 50 µl reaction solution containing 5 µl 10 × PCR buffer, 1 µl dNTP (10 µM), 1 µl DNA template, 1 µL primer-F (10 µM), 1 µl primer-R (10 µM)), 0.5 µl rTaq (2 U/µl), and 40.5 µl ddH_2O. Amplification was performed with initial denaturation for 5 min at 95 °C, followed by 40 cycles of denaturation at 95 °C for 30 s, annealing at 55 °C for 30 s, extension at 72 °C for 1 min, and a final extension at 72 °C for 5 min (ABI Gene AmpPCR System 9700, Life Technologies, Grand Island, NY). For direct sequencing, the PCR products were purified (DNA TIANgel Midi Purification Kit, Beijing, China), and the purified PCR products were sequenced using a DNA capillary tube sequencer (ABI 3730×l). The sequencing results were assembled and analyzed using a Genetic Analyzer (Applied Biosystem, Foster City, CA) with the published DNA sequence for *FBN1* (GenBank accession number NC-000015.9). The novelty of the variant was searched in the following databases: 1000 Genomes; NHLBI Exome Variant Server, Human Gene Mutation Database, the Genome Database, dbSNP, Human Genome Variation Database, KMDB/Mutation View, and the Universal Mutation Database.

RESULTS

Clinical findings: A four-generation family, including nine men and 11 women, in Henan province, China, was identified and diagnosed with MFS (Figure 1). The inheritance pattern in this family appeared to be autosomal dominant. In 2008, six individuals of this pedigree were found to have MFS based on clinical examinations and hospital records. The median onset age of these patients was 29 years, ranging from 22 to 62 years old. All six patients in the family manifested similar clinical symptoms, mainly in the ocular and skeletal systems (Table 1), and the unaffected family members appeared normal. Ocular symptoms included bilateral lens dislocation, high myopia, and exotropia, a form of strabismus in which the eyes deviate outward. Abnormalities of the skeletal system in MFS such as joint laxity, dolichostenomelia, pectus excavatum or pectus carinatum, and arachnodactyly were observed. Cardiovascular abnormalities were noted only in patient II:1, who had an aortic aneurysm and mitral valve prolapse, in this family. The proband had exotropia of both

Table 1. Clinical features of affected family members.

Patient ID	II:1	II:6	III:7	III:9	III:11	III:12
Age (year)	62	54	30	28	24	22
Sex	M	F	F	F	F	M
Ocular system						
Ectopia lentis	+	OCL*	+	+	+	+
Myopia	+	OCL*	+	+	+	+
Exotropia	+	+	+	+	+	+
Glacouma	-	+	-	-	-	-
Retinal detachment	-	+	-	-	-	-
Cardiovascular system						
Aortic root dimension (mm)	30.2	29.3	28.5	27.8	27.6	28.4
Mitral valve prolapse	+	-	-	-	-	-
Aortic aneurysm	+	-	-	-	-	-
Skeletal system						
Height (H: cm)	172	165	164	165	166	171
Arm span (AS: cm)	175	170	170	171	170	176
AS/H	1.02	1.03	1.04	1.04	1.02	1.03
Scoliosis	-	+	-	-	-	-
Arachnodactyly	+	+	+	+	+	+
Joint hypermobility	-	-	-	-	-	-
Pectus excavatum	+	+	-	-	+	+
Pectus carinatum	-	-	+	+	-	-
						Other manifestations
Hyperextensible skin	+	+	+	+	+	+
Striae	+	+	+	+	+	+
Hernia	-	-	-	-	-	-

*OCL: Operated for ectopia lentis. II:1 The First of Second generation

eyes (Figure 2A), bilateral lens dislocation (Figure 2B), and arachnodactyly (Figure 2C). The proband underwent removal of ectopia lentis and reattachment of the retina in both eyes. Patient II:1 did not receive treatment and died of a dissecting aneurysm before the age of 62. Patient III:11 underwent squint correction of exotropia.

Mutation analysis: Direct sequencing of the 65 exons of *FBN1* revealed a heterozygous missense mutation in exon 14, c.1786T>G (Figure 3A), which resulted in the substitution of cysteine by glycine at codon 596 (p.C596G). The mutation was detected in all six patients. No mutation was observed in the unaffected family members or in any of the 100 ethnically unrelated and healthy controls (Figure 3B and data not shown). Therefore, the c.1786T>G mutation was linked to the disease phenotype in all patients.

DISCUSSION

Mutations of the *FBN1* gene cause MFS [2] or Marfan-related diseases [8,13]. *FBN1* was the first disease-causing gene identified for MFS [14], and mutation of this gene is associated with the majority of the patients with MFS [15,16]. Thus far, almost 3,000 mutations have been reported [10]. FBN1 is widely expressed in zonules, the cardiovascular system, cartilage, tendon, cornea, and other tissues, and is an important element of microfibrils. FBN1 is secreted by non-pigmented cells from ciliary bodies and is involved in the formation of zonules [8,17]. FBN1 is comprised mainly of repeated modules such as EGF domains and TB domains [18] and plays an important role in maintaining an ordered arrangement of microfibers [8,19]. Most mutations of *FBN1* occur in the EGF domains [18,20] that disrupt microfibril formation, which result in fibrillin protein abnormalities and subsequently weaken the connective tissue [7,15,18,21,22].

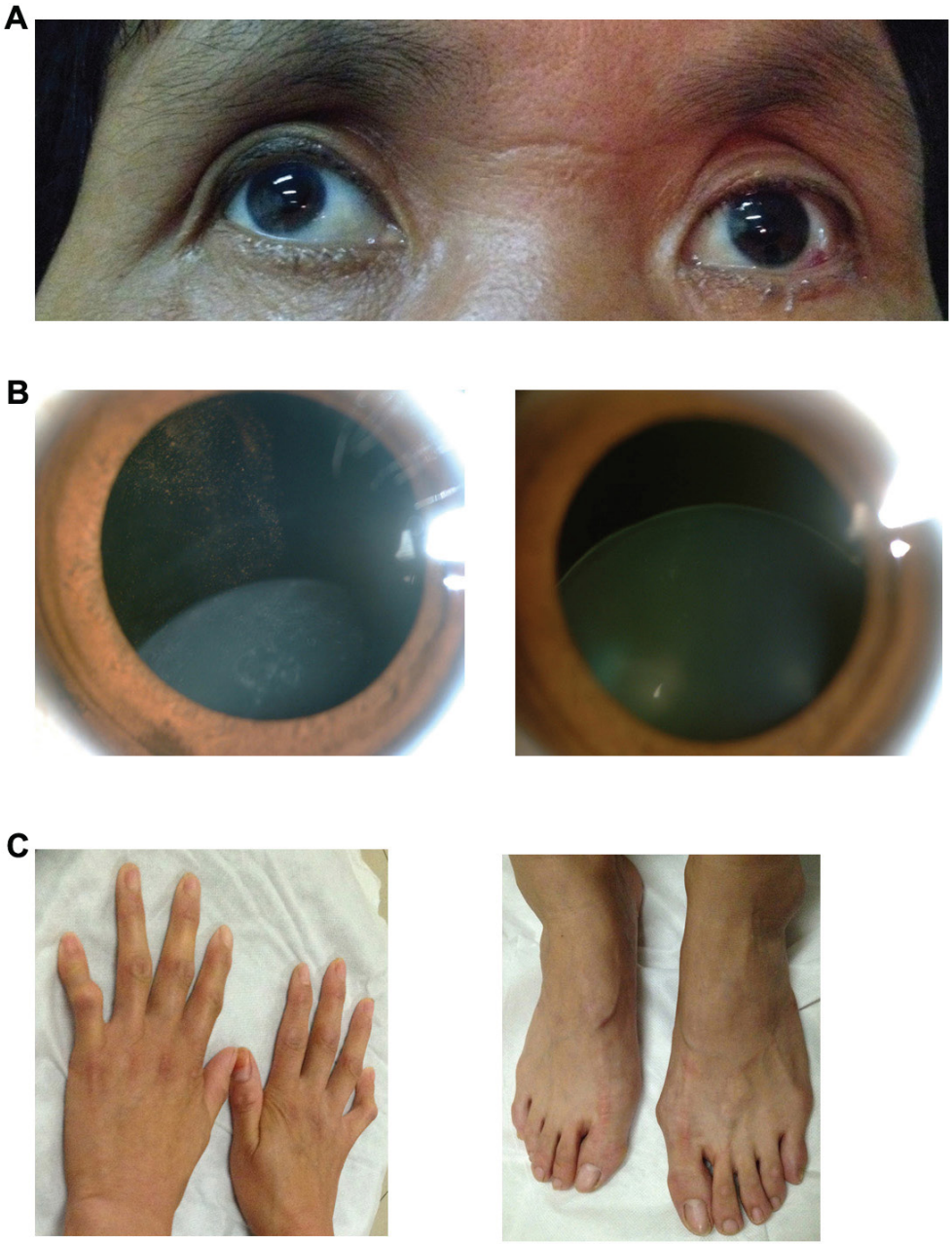

Figure 2. Photographs of the proband. **A**: Exotropia. Exotropia of the proband with cataract extraction and retinal reattachment surgery. Exotropia was unchanged after surgery. **B**: Ectopia lentis. After pupil dilation, images were taken with a slit lamp. The lens was dislocated downward with laxity of the upper suspensory ligaments in both eyes. **C**: Arachnodactyly.

Four hypotheses have been proposed to explain the mechanisms by which mutations of *FBN1* lead to MFS: 1) The mutated monomer of FBN1 interferes polymerization of fibrillin and microfiber aggregation [7,23]. 2) *FBN1* mutations destroy the stability of elastic fibers [7,24]. 3) Mutations in calcium-binding EGF modules render FBN1 susceptible to proteolysis [7,25]. 4) The mutations lead to loss of function of transforming growth factor beta signal activity on extracellular matrix formation, contributing to the pathogenesis of MFS [7,26,27].

Through the *FBN1* mutation screening in a Bai Chinese family diagnosed with MFS, we identified a heterozygous missense mutation c.1786T>G (p.C596G) in the pedigree. The mutation c.1786T>G cosegregated with all patients with MFS because it was not detected in unaffected family members or 100 ethnically unrelated and healthy controls. The main MFS symptoms in the family were ocular manifestations (ectopia lentis, high myopia, and exotropia) and skeletal manifestations (excessive development of extremities and arachnodactyly) (Table 1). Cardiovascular abnormalities were observed

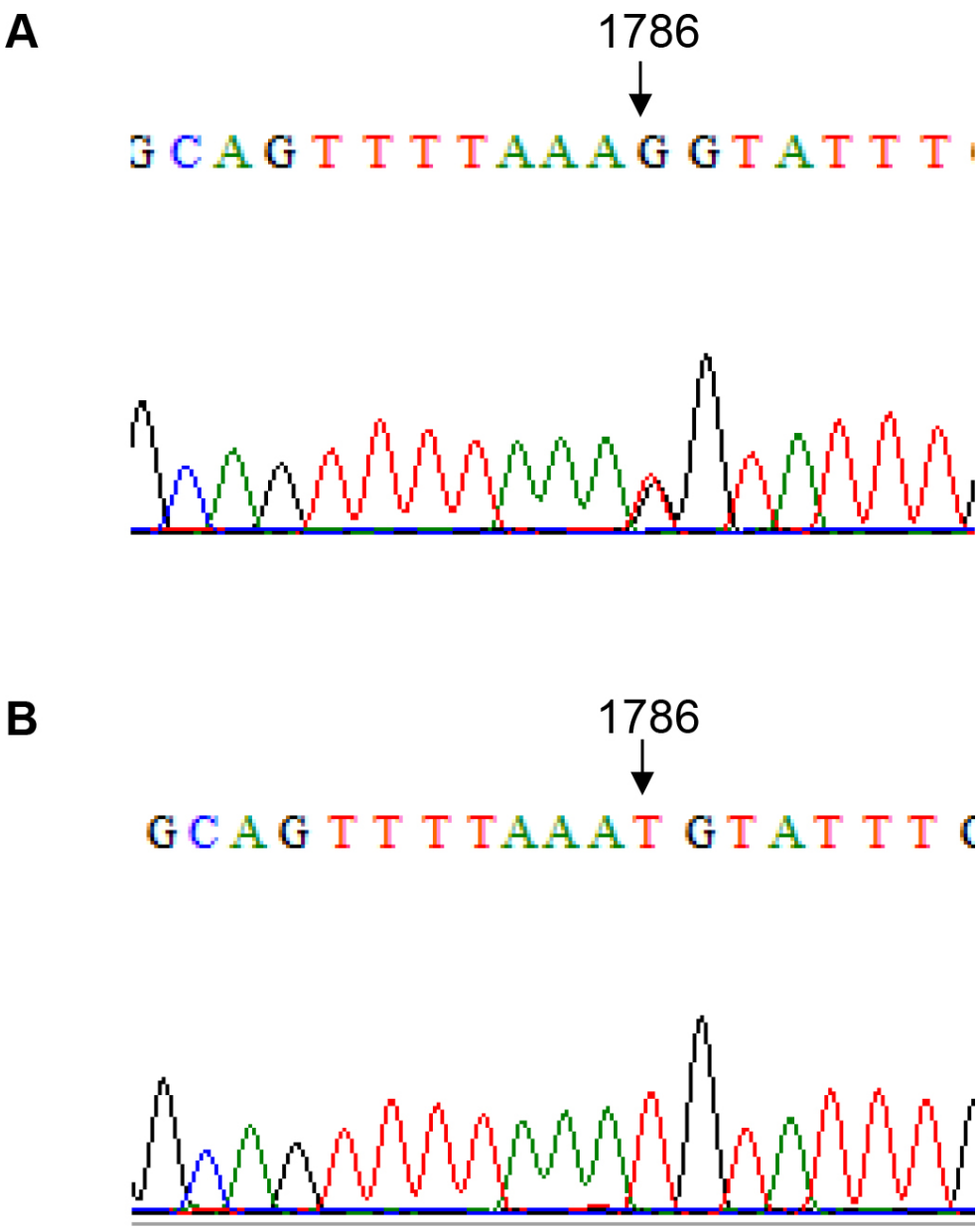

Figure 3. Identification of the C596G mutation in *FBN1* on exon 29. **A**: A heterozygous T>G change, causing the substitution of cysteine by glycine at codon 596 (p.C596G) in the proband. **B**: The corresponding normal sequence in an unaffected family member (II:2).

only in patient II:1. These symptoms are consistent with the diagnostic criteria for MFS [12]. Intriguingly, exotropia was observed in all six patients with MFS but not in 14 unaffected family members in the Bai Chinese family. This observation is in contrast to other MFS families in whom strabismus is a minor feature [3,4]. The occurrence rate of exotropia in patients with MS has been estimated at 11.7% (67 out of total 573 patients with MS) [28]. Thus, the mutation c.1786T>G might be uniquely associated with the higher prevalence of exotropia. Regardless, exotropia is a characteristic of patients with MFS, at least in this Bai Chinese family.

FBN1 mutations occur through all 65 exons. Mutations cluster in exons 24–32, a hot spot area associated with neonatal, classic, and other severe forms of MFS [16,29]. However, mutations in exons 12–15 encoding cbEGF-like domains 3–6 have caused a mild phenotype of MFS with possible late cardiovascular involvement [30-32]. The missense mutation c.1786T>G identified in our study is located on exon 14. The phenotypes of all the affected Bai Chinese family members manifested mainly in the ocular and skeletal organs except patient II:1, who had cardiovascular symptoms (Table 1), suggesting that the molecular mechanisms used by other mutations in exons 12–15 may

be involved in pathogenesis of MFS in the family with the mutation c.1786T>G in exon 14. Nevertheless, further investigations are required to confirm this hypothesis. The c.1786T>G mutation has been reported in one sporadic case from 53 Japanese probands suspected of having MFS, but no clinical information about the identified proband is available [33]. A similar missense mutation c.1786T>C (p.C596R) was found in a patient with ectopia lentis at the age of 3 [34]. This proband had no cardiovascular involvement, but the involvement of the skeletal system is unknown, showing that the type of amino acid mutated might affect the phenotype and severity.

In summary, a novel mutation of *FBN1* (c.1786T>G) in exon 14 was identified in a Bai Chinese family with MFS. The results expand the spectrum of *FBN1* mutations and help for early diagnosis in uncertain MFS cases.

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