

A novel *FBN1* mutation causes autosomal dominant Marfan syndrome

YING XIAO^{1*}, XIAOQI LIU^{2*}, XIAOXIN GUO^{2*}, LIPING LIU¹, LINXIN JIANG², QI WANG¹ and BO GONG²

¹Department of Ophthalmology, Shandong Provincial Hospital Affiliated to Shandong University, Jinan, Shandong 250021;

²Sichuan Provincial Key Laboratory for Disease Gene Study, Hospital of University of Electronic Science and Technology of China and Sichuan Provincial People's Hospital, School of Medicine, University of Electronic Science and Technology of China, Chengdu, Sichuan 610072, P.R. China

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Abstract. Marfan syndrome (MFS) is an inherited and systemic disorder. It has been reported that mutations in the fibrillin-1 gene (*FBN1*) account for ~90% of autosomal dominant cases of MFS. This study was conducted to screen mutations of *FBN1* in a Chinese family with autosomal dominant MFS; four individuals including two patients with MFS were recruited. The family members underwent complete physical, cardiovascular and ophthalmologic examinations. Genomic DNA samples were collected from the family along with 383 unrelated healthy subjects. *FBN1* coding regions were amplified by polymerase chain reaction and analyzed by direct sequencing. SIFT and PolyPhen-2 were used to predict the possible structural and functional alterations of the protein. A novel heterozygous mutation c.1708 T>G (p.C570G) in exon 14 was identified, which led to a substitution of cysteine by glycine at codon 570 (p.C570G). The mutation was identified as being associated with the MFS phenotype in the affected members of this family. However, the unaffected family members and the 383 normal controls lacked the mutation. Multiple sequence alignment of the human *FBN1* protein revealed that this novel mutation occurred within a

highly conserved region of the *FBN1* protein across different species and may induce structural alterations in this functional domain. The spectrum of MFS-associated mutations in the *FBN1* gene has been enriched from this study; this may improve understanding of the molecular pathogenesis and clinical diagnosis of MFS.

Introduction

Marfan syndrome (MFS) is an autosomal dominant hereditary disease comprising a disorder of fibrous connective tissue involving the ocular, skeletal and cardiovascular systems (1). According to the Ghent criteria, patients with malfunctions of at least two organ systems could be diagnosed with MFS (2). Aortic root dilatation/dissection and lens dislocation were two cardinal manifestations to establish an unequivocal diagnosis of MFS in patients with positive family history. Due to the large clinical variability of MFS, and several other connective tissue disorders with comparable clinical features, distinguishing MFS from those similar syndromes is still challenging.

Increasing evidence indicates that heredity holds a key role in the development of MFS. It has been reported that MFS generally results from mutations in the human fibrillin-1 (*FBN1*) gene (3,4). At present, >3,000 mutations have been identified in relation to MFS. Most mutations are specific to a family with MFS, whereas ~10% of *FBN1* mutations are shared by different families (5). Located at chromosome 15q-21.1 with 65 exons, the *FBN1* gene encodes a secreted 350 kDa glycoprotein (6). Human *FBN1* protein shares conserved sequences with other species. *FBN1* protein constitutes extracellular microfibrils and controls the stability, as well as the microfibril assembly. Mutations within the *FBN1* gene may disrupt microfibril formation, leading to abnormalities of fibrillin and eventually weakening the connective tissue (7).

In the present study, the entire coding region of *FBN1* was analyzed, and a novel mutation in exon 14 of *FBN1* was identified in all affected members. The newly identified *FBN1* mutation in a Chinese family with MFS further emphasizes the important role of *FBN1* in the mechanism of MFS development. The present study not only expanded the

Correspondence to: Dr Bo Gong, Sichuan Provincial Key Laboratory for Disease Gene Study, Hospital of University of Electronic Science and Technology of China and Sichuan Provincial People's Hospital, School of Medicine, University of Electronic Science and Technology of China, 32 Road West 2, the First Ring, Chengdu, Sichuan 610072, P.R. China
E-mail: gongbo2007@hotmail.com

Dr Qi Wang, Department of Ophthalmology, Shandong Provincial Hospital Affiliated to Shandong University, 324 Jingwu Road, Jinan, Shandong 250021, P.R. China
E-mail: drwangqi@hotmail.com

*Contributed equally

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mutation spectrum of *FBN1* resulting in MFS development in a Chinese family, but is also likely to aid understanding of the molecular pathogenesis and clinical diagnosis of *FBN1*-associated MFS.

Materials and methods

Subjects. A family with MFS was recruited from the Shandong Provincial Hospital Affiliated to Shandong University (Jinan, China) (Fig. 1). This study was conducted in accordance to the tenets of The Declaration of Helsinki and was approved by the Institutional Review Boards of the Hospital of University of Electronic Science and Technology of China and Sichuan Provincial People's Hospital (Chengdu, China), and the Shandong Provincial Hospital Affiliated to Shandong University. A total of 383 ethnically matched, unrelated and normal healthy individuals were recruited from the Hospital of University of Electronic Science and Technology of China & Sichuan Provincial People's Hospital (255 males and 128 females; mean age at recruitment 55.26 ± 8.78 years). These control individuals had no medical history associated with any related diseases. Written informed consent was obtained from all participants prior to the study.

Clinical diagnosis. Two of the family members were diagnosed with MFS according to the revised Ghent criteria (2). Non-consanguineous marriages were found in the family; clinical information of the affected family members is summarized in Table I. All members of this family underwent complete physical, cardiovascular and ophthalmologic examinations. Unrelated healthy individuals also underwent the same examinations.

Mutation screening. Genomic DNA samples were extracted from peripheral blood using a Blood DNA extraction kit (Thermo Fisher Scientific, Inc., Waltham, MA, USA). The whole coding region of *FBN1* (NM_000138.4) was amplified by polymerase chain reaction (PCR) with 35 cycles (30 sec at 95°C for initial denaturation, 30 sec for annealing at different temperatures as shown in Table II, and 30 sec at 72°C for extension), using a GeneAmp® PCR system 9700 (Applied Biosystems; Thermo Scientific Inc.). Sequencing primers of all the exons were designed using Primer 5.0 (Premier Biosoft International, Palo Alto, CA, USA; Table II). Amplified PCR products were purified and sequenced directly (BigDye Terminators Sequencing kit) with an Automated Genetic Analysis system 3130 (both from Applied Biosystems; Thermo Fisher Scientific, Inc.). Comparative amino acid sequence analysis of the human *FBN1* protein was performed across different species using HomoloGene (<https://www.ncbi.nlm.nih.gov/homologene/?term=FBN1>). The potentially damaging effects of the mutation on the structure and function of *FBN1* was predicted using SIFT (<http://sift.jcvi.org>) and PolyPhen-2 (<http://genetics.bwh.harvard.edu/pph2/>).

Results

Clinical findings. The parents and two daughters of a family from Shandong, China, were included in the present study (Fig. 1). Other relatives of this family were not willing

Table I. Clinical details of the patients with Marfan syndrome in the family.

Characteristic	Proband (I:1)	Proband's daughter (II:2)
Age (years)	44	8
Gender	M	F
Ectopia lentis	+	+
Myopia	+	+
Strabismus	+, exotropia	+, exotropia
Glaucoma	-	-
Retinal detachment	+	-
Height (cm)	184	134
Arm span (cm)	186	137
AS/H	1.01	1.02
Overgrowth of the long bones	+	+
Arachnodactyly	+	+
Scoliosis	-	-
Pectus excavatum	-	-
Pectus carinatum	+	-
Flatfeet	+	+
Mitral valve prolapse	-	-
Aortic aneurysm	+ (ruptured 5 years ago then formed aortic dissection; Bentall surgery was performed at that time)	
Aortic root dimension (mm)	25.0 (artificial vessel diameter)	29.1

M, male; F, female; AS, arm span; H, Height.

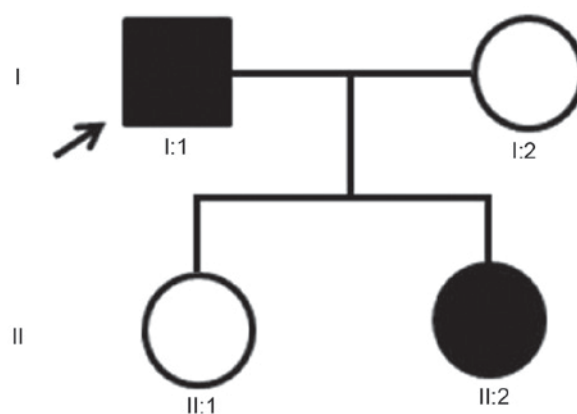


Figure 1. Pedigree of the family with Marfan syndrome. Solid symbols indicate affected patients, open symbols indicate unaffected subjects and arrow indicates the proband in this family. Squares represent males and circles represent females.

to be tested and so additional clinical details were unattainable. Two affected patients (I:1 and II:2) exhibited similar

Table II. Primers used for mutation screening of the *FBN1* gene.

Primer name	Primer sequence (5'-3')	Product size (bp)	Annealing temperature (°C)
<i>FBN11&2F</i>	TCGGGGATTGTCTCTGTGT	434	59
<i>FBN11&2R</i>	GCCCGTTGTTCTGGATCTTG		
<i>FBN13F</i>	ACCAACCCAGCATTGAGTCT	308	60
<i>FBN13R</i>	TTCTAAGGCTCCCCATGCAA		
<i>FBN14F</i>	TTGTGAGGGACCTGAGAACC	296	59
<i>FBN14R</i>	TTGCAGGAAAGAGGAAAGCC		
<i>FBN15F</i>	CAACTCCTGTGAGCTGTTGC	278	60
<i>FBN15R</i>	AAACATGCTGTGTCCCAGGT		
<i>FBN16F</i>	GTCCTTCCAGAGGACCACAA	228	60
<i>FBN16R</i>	CAGCTTTAGGTACCAGCATGTC		
<i>FBN17F</i>	GCATGATGGTTCCTGCTTTT	380	60
<i>FBN17R</i>	GCAGTCAGCGAAATTGTGAA		
<i>FBN18F</i>	TTCCAAATATTGTGATGGACAAA	448	60
<i>FBN18R</i>	ACAGGGTTTTTCTGGTCCAA		
<i>FBN19F</i>	GCTGTTTCCAGGGACATGAT	441	60
<i>FBN19R</i>	TTTATGGGAGGCAAAACGTC		
<i>FBN110F</i>	AGCCCCAGTGTGAAGTATGG	396	60
<i>FBN110R</i>	TTCCCTGGACGTCATCTCTT		
<i>FBN111F</i>	TGACTTCTGTGGGCCTATGA	300	59
<i>FBN111R</i>	TAACTTGAACAATGCAAGAAAAA		
<i>FBN112F</i>	TTGTCACCAGACGACCTTTG	383	60
<i>FBN112R</i>	CCACCAAGTTTGGGGTAAGTT		
<i>FBN113F</i>	AAAAGGAACCCAGAAAGTCTTAGAA	295	60
<i>FBN113R</i>	CTTCCGGCATGGGTATTTA		
<i>FBN114F</i>	GGAGGGAGGGGAAATAAA	244	60
<i>FBN114R</i>	ACTGCAATGGAAGGAGAGGA		
<i>FBN115F</i>	GATCTTATTTGGATGAAAGTTAGCC	400	59
<i>FBN115R</i>	AGTCAGGTTTCCCAAACCAA		
<i>FBN116F</i>	TTCCCCATTTTCAAGGGTTA	294	61
<i>FBN116R</i>	CGTTTGTTACCATTTGGCCTT		
<i>FBN117F</i>	GGGGGTTCATCTGTTTGA	242	60
<i>FBN117R</i>	CAGTACGAGGGCATCTCCAT		
<i>FBN118F</i>	ACCAAGGGCAGGATCTACCT	188	60
<i>FBN118R</i>	ACCCACAAGAAAGCCTGATG		
<i>FBN119F</i>	CCTGTAGCTCCTAAGGTCATTACA	300	60
<i>FBN119R</i>	CTCCCAGCAATGAAAGAAGG		
<i>FBN120F</i>	CAAAGTTTGGGCCCTTTTGA	226	59
<i>FBN120R</i>	TGGCATTCCAAAAGATAGCA		
<i>FBN121F</i>	GGCCCAAGACTAGATTTTAGCA	243	60
<i>FBN121R</i>	TTTTGCAGGAAAAGCTGACA		
<i>FBN122F</i>	AATGTCAGCTTTTCTGCAA	368	59
<i>FBN122R</i>	TGAAATACTAGGCTTCCCCTTT		
<i>FBN123F</i>	TGTCAGAACTGCAAAGTCTGG	204	60
<i>FBN123R</i>	GACAGCTTTATCCAGTCCGAGT		
<i>FBN124F</i>	TGCTATTCAGGCACCCTAGA	400	59
<i>FBN124R</i>	TGGAGTGTGTGTCTGTACCTGA		
<i>FBN125F</i>	AACAGAGTGTGGCAGTTTGG	373	60
<i>FBN125R</i>	CTGAGATCATGAAAATGCATCC		
<i>FBN126&27F</i>	GACCTCCTGACTGCTTGCTC	494	60
<i>FBN126&27R</i>	CAAAGCTTCATGGAATCCTTCT		
<i>FBN128&29F</i>	GAGTGCTTGGTCTGGTGAG	564	61
<i>FBN128&29R</i>	AGCGATGAAAACAAAACCTCAGA		

Table II. Continued.

Primer name	Primer sequence (5'-3')	Product size (bp)	Annealing temperature (°C)
<i>FBN130F</i>	GGGACAGACATCCAAACCAT	249	62
<i>FBN130R</i>	CAAAGCCTGGGCCCTAAAC		
<i>FBN131F</i>	CTCACTGAACAGTGGAACCAA	280	59
<i>FBN131R</i>	GCTCTCTTTGGAATGCTGGT	280	59
<i>FBN132F</i>	GAATCTTTCTATCACTGACCCAAAC		
<i>FBN132R</i>	TCGAGGGGAAAGTACTCAATG	325	59
<i>FBN133&34F</i>	CATTTGTGCTGAGCCTTTTTC	495	60
<i>FBN133&34R</i>	GAATGCCTGGCTTCTCTGAC		
<i>FBN135F</i>	TGCTGCACTGGAAAGTTGAT	231	60
<i>FBN135R</i>	AGTGGCTTCCCCATCAGTTA		
<i>FBN136F</i>	TGCCCAGATTGGTGTTAGAT	400	59
<i>FBN136R</i>	CAGGTCTGAGAAAAGGTATCTGTG		
<i>FBN137&38F</i>	AGATTGGGCCCTGTTCTTTT	819	60
<i>FBN137&38R</i>	TTGGGAATAAGGTCCCCTCT		
<i>FBN139&40F</i>	TCAGACGGGCAGAGTAACAA	496	59
<i>FBN139&40R</i>	CCATATTCTGGTTTTGCAGGT		
<i>FBN141F</i>	AGGCCATTCCAAAATGTGAA	249	60
<i>FBN141R</i>	TTGTGAGCTCTTCTCTTTGT		
<i>FBN142F</i>	ATTTCCCATGATGGCATCAC	300	60
<i>FBN142R</i>	TGCTTCCTTCGCTAAGACTGA		
<i>FBN143F</i>	CTATCCTCCCATCCCACCTT	273	60
<i>FBN143R</i>	CAGGGTGTTTGCACAGTTTG		
<i>FBN144F</i>	CACAGGGATCATGTGCTGTC	315	60
<i>FBN144R</i>	TCCACACCATGCCCTTTACT		
<i>FBN145F</i>	GGCTTTGTGACTGGACACC	218	62
<i>FBN145R</i>	GTAGGCATGTCCAGCCTGTG		
<i>FBN146F</i>	GAGCTAGGATTACTCCTGAGAATGA	398	59
<i>FBN146R</i>	TCATGTTCAGATTGCCAAAGA		
<i>FBN147F</i>	GGCCTGGTGAACCCTAAAT	247	60
<i>FBN147R</i>	TTCTTTTGCTGATGCACAAT		
<i>FBN148F</i>	TGCTGGGATTATGACATCTTG	292	60
<i>FBN148R</i>	TTTTCTCCAGGTTTCCAGA		
<i>FBN149F</i>	CCAGTGGAACCTCTTCCTT	205	60
<i>FBN149R</i>	GACACCCGACACTCCTCATT		
<i>FBN150F</i>	TGATGTCTCCATCGTGTTTTG	208	61
<i>FBN150R</i>	ATTGAAAGCCCAAAGCCTTC		
<i>FBN151F</i>	GGAAAGCAACTGAAGGGTGT	263	590
<i>FBN151R</i>	GCCTACAGTCTTACTTACATCATGG		
<i>FBN152&53F</i>	GGAGAAGCTTGTAATGAATTGCT	594	60
<i>FBN152&53R</i>	AACTTATTTCACTGCCATCTTGG		
<i>FBN154F</i>	TTTGACACATTCTTGTTTTT	207	60
<i>FBN154R</i>	CAACCAATTGTTCCAGGAT		
<i>FBN155F</i>	CCTTTTGTTGCTGTCCATGAT	249	60
<i>FBN155R</i>	AGGGAAGCTTTGAGGGACAT		
<i>FBN156F</i>	TCATACTCAACAGAGCAGAAGGA	363	59
<i>FBN156R</i>	CAAGAACTCAGAGCCCAGGT		
<i>FBN157F</i>	AAGGAACAAAGGGAGGGAAG	392	60
<i>FBN157R</i>	CAGTCATTACGGCATCTCCA		
<i>FBN158F</i>	CTGACATCCCCTTTGCCATA	277	61
<i>FBN158R</i>	TCCCTGCAAGTATTTTTGGAC		
<i>FBN159&60F</i>	CACTGAAGTGACCCCTACA	600	60
<i>FBN159&60R</i>	TGAGGGGCAATGGTCAAT		

Table II. Continued.

Primer name	Primer sequence (5'-3')	Product size (bp)	Annealing temperature (°C)
<i>FBN161&62F</i>	TGTTGGCTTGACTCAAATGC	600	61
<i>FBN161&62R</i>	CCTCCACAAGGATTCACCAG		
<i>FBN163F</i>	TGGTGGCTCTGCTTCTTTT	178	60
<i>FBN163R</i>	GCCATGCATCTTGAGAGTGA		
<i>FBN164F</i>	AAGTGGCCAGATCCAATGTC	334	60
<i>FBN164R</i>	ACCATGACCAGGAAGAGCAC		
<i>FBN165F</i>	CATCTATGCTCCCCTTCTGC	243	60
<i>FBN165R</i>	TTCCACCACAGGAGACATCA		
<i>FBN166F</i>	GCAGCATAAGGCAGAAAATTG	583	60
<i>FBN166R</i>	TGATTCTGATTGGGGGAAAA		

FBN1, fibrillin-1; F, forward; R, reverse; bp, base pair.

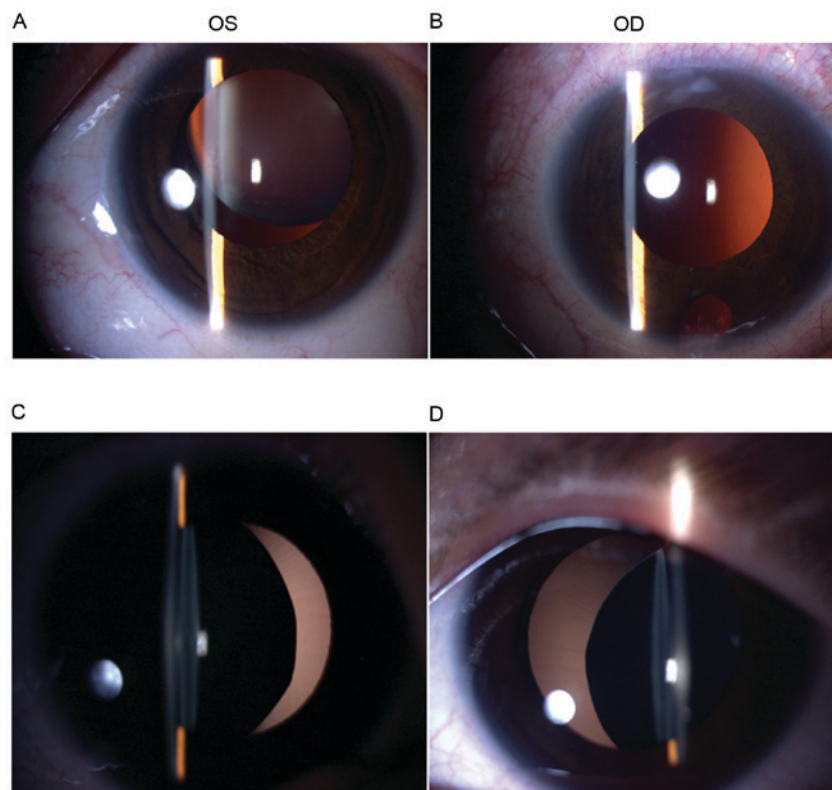


Figure 2. Slitlamp photograph of the proband (I:1) and his daughter (II:2) in the family with MFS. (A) Left eye of the proband had ectopia lentis. (B) Lensectomy and vitrectomy combined with silicone oil tamponade was performed for the right eye of the proband following retinal reattachment and silicone oil removed 3 months later. (C and D) Ectopia lentis of the proband's daughter (II:2). (C) Lens nasal deviation occurred in her left eye and (D) nasal-inferior dislocation in her right eye. OS, oculus sinister (left eye); OD, oculus dexter (right eye).

clinical symptoms, including ectopia lentis, myopia and strabismus (Fig. 2 and Table I). The left eye of the proband (I:1) underwent refractive lensectomy and vitrectomy combined with silicone oil tamponade after retinal detachment 2 years prior to the current study; following retinal re-attachment, silicone oil was removed 3 months later. The two patients both had the same facial and skeletal features, including arachnodactyly, flat feet and dilation of the aortic root (Fig. 3 and Table I). The proband had pectus carinatum and aortic aneurysm. The patient received Bentall surgery and underwent aortic arch

replacement 5 years prior to the current study, as their aortic aneurysm ruptured and formed aortic dissection (Fig. 4). The other two members of the family had no features of MFS.

Mutation screening of *FBN1*. Direct sequencing of the whole coding region of *FBN1* detected a novel missense mutation c.1708 T>G (p.C570G), situated at nucleotide 570 in exon 14 of the coding region (Fig. 5A). This heterozygous mutation was detected in the two affected patients (I:1 and II:2) but was not



Figure 3. Arachnodactyly of the proband (I:1) and the affected daughter (II:2). (A) Proband (left) and his daughter (right) had (B) long fingers and (B) flat feet.

found in the unaffected mother and daughter (I:2 and II:1) of the family and in the 383 ethnically matched healthy subjects. Therefore, c.1708 T>G (p.C570G) cosegregated to the patients with MFS in this family. Multiple sequencing alignment of human *FBN1* protein with various species revealed that the novel mutation occurred within a highly conserved region of the calcium binding epidermal growth factor-like (cbEGF) domain (Fig. 5C). This mutation is a T>G transition, converting cysteine to glycine at amino acid 570 (p.C570G). This amino acid substitution in the *FBN1* protein was predicted to be damaging by SIFT and PolyPhen-2.

Discussion

It has been reported that MFS is mainly caused by mutations in the *FBN1* gene, which was the first gene identified to cause MFS (8). Of all the identified mutations in the *FBN1* gene, 38.6% result in a truncated *FBN1* protein and 60.3% represent missense mutations across different ethnic groups (9). *FBN1* mutations may cause abnormalities in the formation of microfibrils and fibrillin. As a result, connective tissues weaken (10). A novel *FBN1* heterozygous missense mutation, c.1708 T>G (p.C570G) was identified within a Chinese family associated with MFS in the present study.

FBN1 is an important component of microfibrils and is expressed in many human tissues, including in zonules, the cardiovascular system, cartilage, tendon and cornea. The

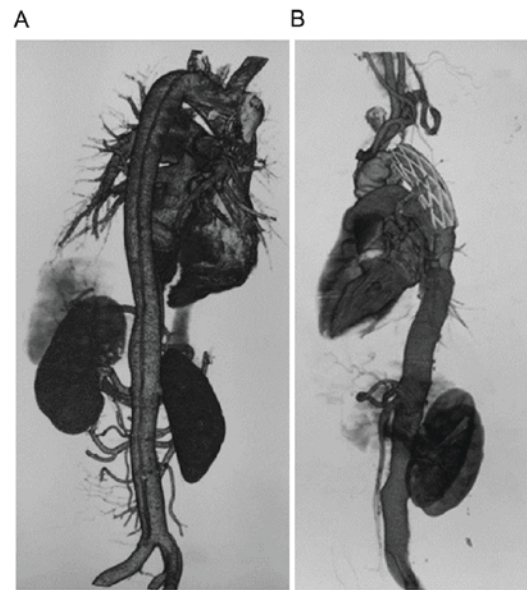


Figure 4. CTA of the aortic vessels of the proband. (A) CTA confirmed the formation of aortic dissection. (B) CTA image of the artificial vessel of the proband after Bentall surgery. CTA, computed tomography angiogram.

protein serves a role in the formation of zonules and is secreted from ciliary bodies of non-pigmented cells (11). *FBN1* protein is composed of repeated modules, including cbEGF and transforming growth factor-1 binding protein-like domains, and is responsible for maintaining microfibrils in an ordered arrangement (12,13). The majority of identified missense mutations in *FBN1* are localized in cbEGF (14). The mutated monomer of *FBN1* could interfere with the polymerization of fibrillin and microfibril aggregation (15). *FBN1* mutations within cbEGF modules may disrupt the stability of elastic fibers and render *FBN1* susceptible to proteolysis. As a result, the transforming growth factor- β signaling activity that affects extracellular matrix formation may malfunction (4,16).

In the present study, a novel c.1708 T>G (p.C570G) heterozygous missense mutation of the *FBN1* gene was reported in a Chinese family with MFS. Three similar missense mutations: c.1709G>A (p.C570Y) (17), c.1709G>C (p.C570S) (18) and c.1709G>C (p.C570R) (19) have been reported in sporadic cases; however, clinical data in these studies were not obtained. In this pedigree, c.1708 T>G (p.C570G) in *FBN1* was detected in the two patients with MFS (I:1 and II:2). The proband (I:1) initially came to Shandong Provincial Hospital to see an ophthalmologist and was found to suffer from ectopia lentis, myopia and strabismus in both eyes. The proband and the affected daughter (II:2) had similar facial and skeletal features of MFS, including arachnodactyly, flat feet and dilation of aortic root. In addition, pectus carinatum, aortic dissection and retinal detachment were also detected in the proband. These findings suggested that the clinical manifestations of the patient with MFS became more evident with age. This mutation was not included in the Exome Aggregation Consortium dataset; c.1708 T>G (p.C570G) of *FBN1* was not detected in the mother (I:2) and another daughter (II:1) of this family, or in the 383 unrelated normal controls during the mutation screening in the present study. This indicated that c.1708 T>G (p.C570G) of *FBN1* cosegregated with affected MFS patients

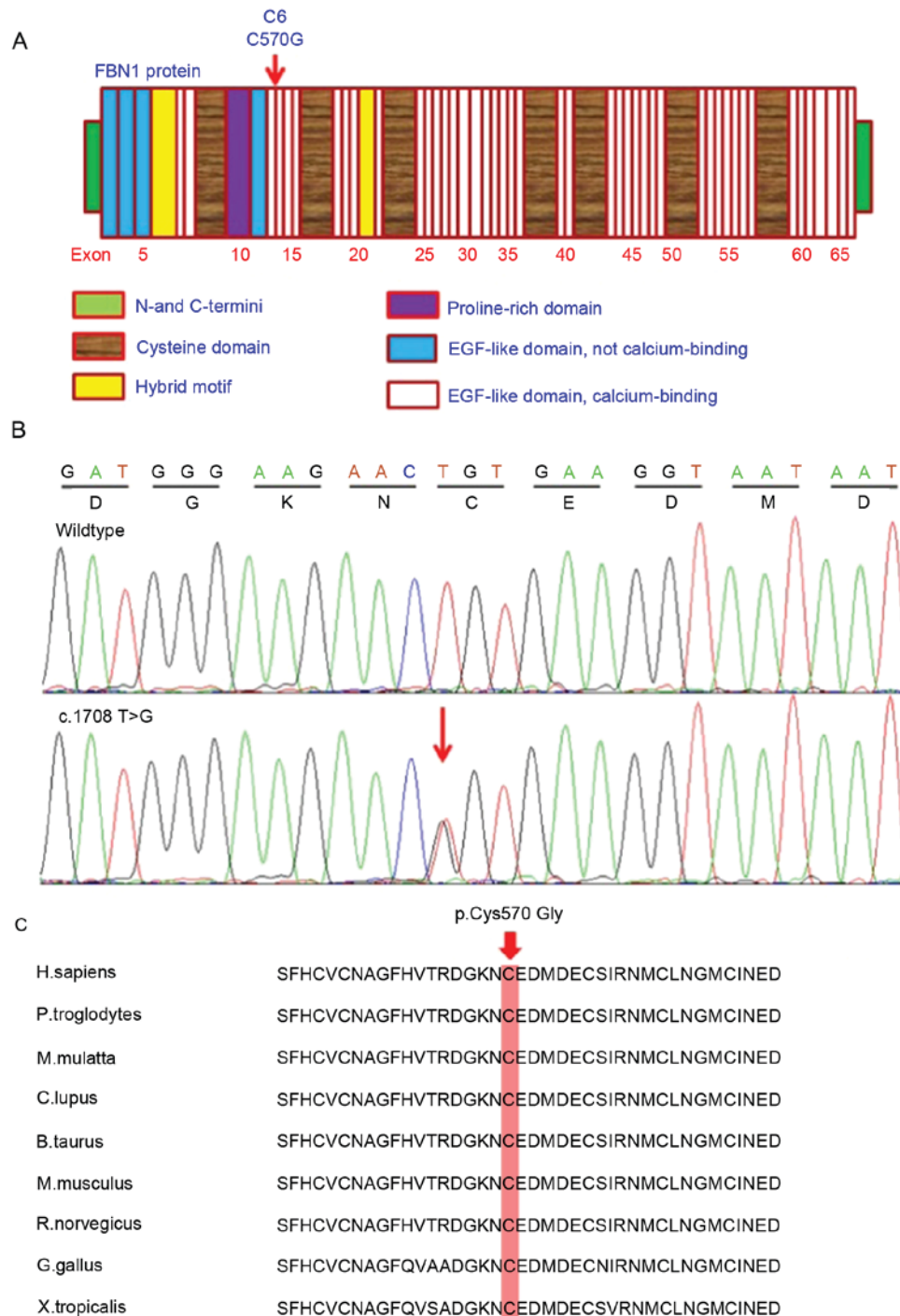


Figure 5. Representative chromatogram of *FBN1* sequence. (A) *FBN1* has different functional regions and the p.C570G mutation occurs in the calcium binding EGF-like domain. (B) Normal sequence from an unaffected individual (I:2) (upper sequence), and a heterozygous T to G substitution at codon 570 from affected subjects (I:1 and II:2) (lower sequence). (C) Orthologous protein sequence alignment of *FBN1* from different species, the mutated residue showing conservation of cysteine at codon 570 was shaded in red. EGF, epidermal growth factor; *FBN1*, fibrillin-1.

and may serve an important role in the pathogenesis of MFS development in this pedigree.

The p.C570G mutation of *FBN1* identified in this family with MFS resulted in a substitution of a highly conserved cysteine residue for glycine in a cbEGF domain of *FBN1*. This mutation is predicted to abolish one disulfide bond and thus affect the sixth conserved cysteine (C6) of the cbEGF domain; disulfide bonds are essential for the correct EGF-like domain structure. SIFT and PolyPhen-2 predictions indicated

that this mutation is critical to protein function, supporting a possible pathogenic effect of this mutation. Evidence has revealed that most *FBN1* mutations are clustered in exons 24-32, a hot spot region associated with classic and severe forms of MFS (17,20); mutations in exons 12-15 encoding cbEGF-like domains (C3-C6) cause a mild phenotype of MFS with possible late cardiovascular involvement (21). Evidence from the present study consistently indicated that the identified heterozygous mutation, c.1708T>G, is located at exon 14 and

that this cysteine substitution detected in the proband resulted in pectus carinatum and aortic dissection. These two factors correlated with increasing age. However, evident symptoms were not detected in the young affected daughter (II:2), even though significant dilation of the aortic root was identified. Nevertheless, further functional analyses are required to confirm the role of *FBN1* and its underlying mechanisms in MFS.

In conclusion, a novel heterozygous mutation, c.1708 T>G (p.C570G), in the *FBN1* gene was identified in a Chinese family associated with MFS. The results from the present study enrich the spectrum of MFS-associated mutations of *FBN1* and may aid presymptomatic molecular diagnosis of undetermined cases of MFS.

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