A novel *FBN1* mutation causes autosomal dominant Marfan syndrome

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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.

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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

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

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
Ectopialentis	+	+
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.

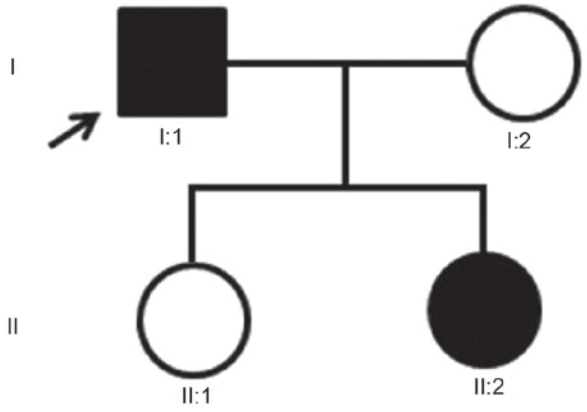

Figure 1. Pedigree of the family with Marfan syndrome. Solid symbols indicate affected patients, open symbols indicate unaffected subjects and arrow indicates the proband in this family. Squares represent males and circles represent females.

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

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

Table II. Continued.

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

Table II. Continued.

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

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

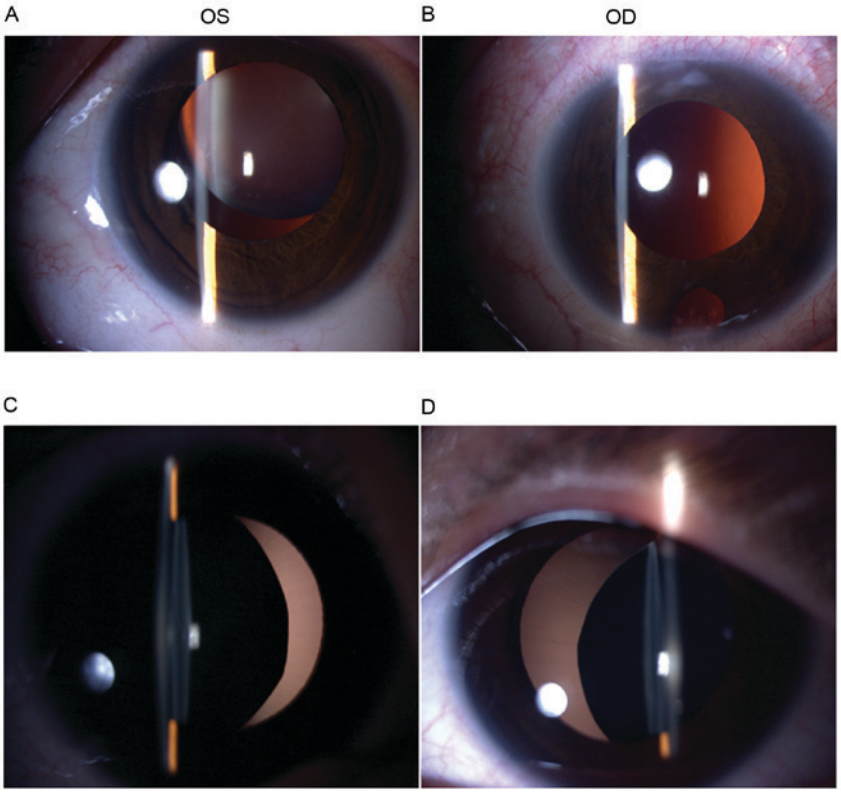

Figure 2. Slitlamp photograph of the proband (I:1) and his daughter (II:2) in the family with MFS. (A) Left eye of the proband had ectopialentis. (B) Lensectomy and vitrectomy combined with silicone oil tamponade was performed for the right eye of the proband following retinal reattachment and silicone oil removed 3 months later. (C and D) Ectopialentis 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 ectopialentis, 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

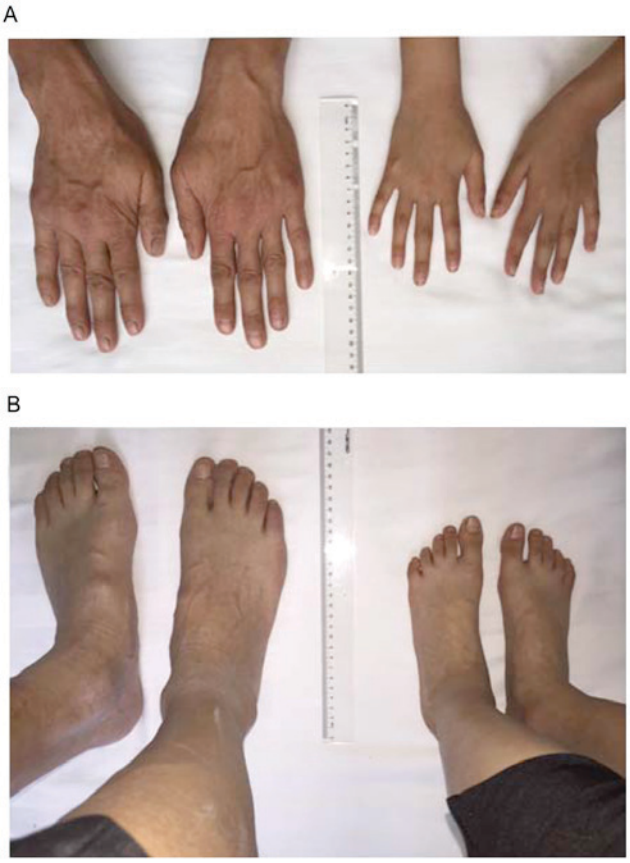

Figure 3. Arachnodactyly of the proband (I:1) and the affected daughter (II:2). (A) Proband (left) and his daughter (right) had (B) long fingers and (B) flat feet.

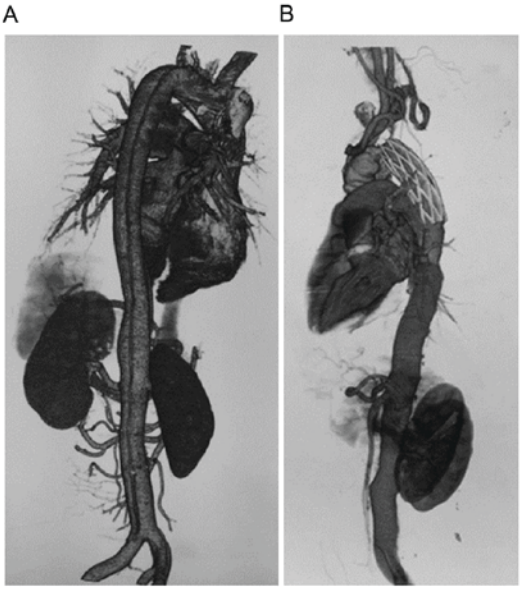

Figure 4. CTA of the aortic vessels of the proband. (A) CTA confirmed the formation of aortic dissection. (B) CTA image of the artificial vessel of the proband after Bentall surgery. CTA, computed tomography angiogram.

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 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 microfibers 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 microfiber 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 ectopialentis, 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

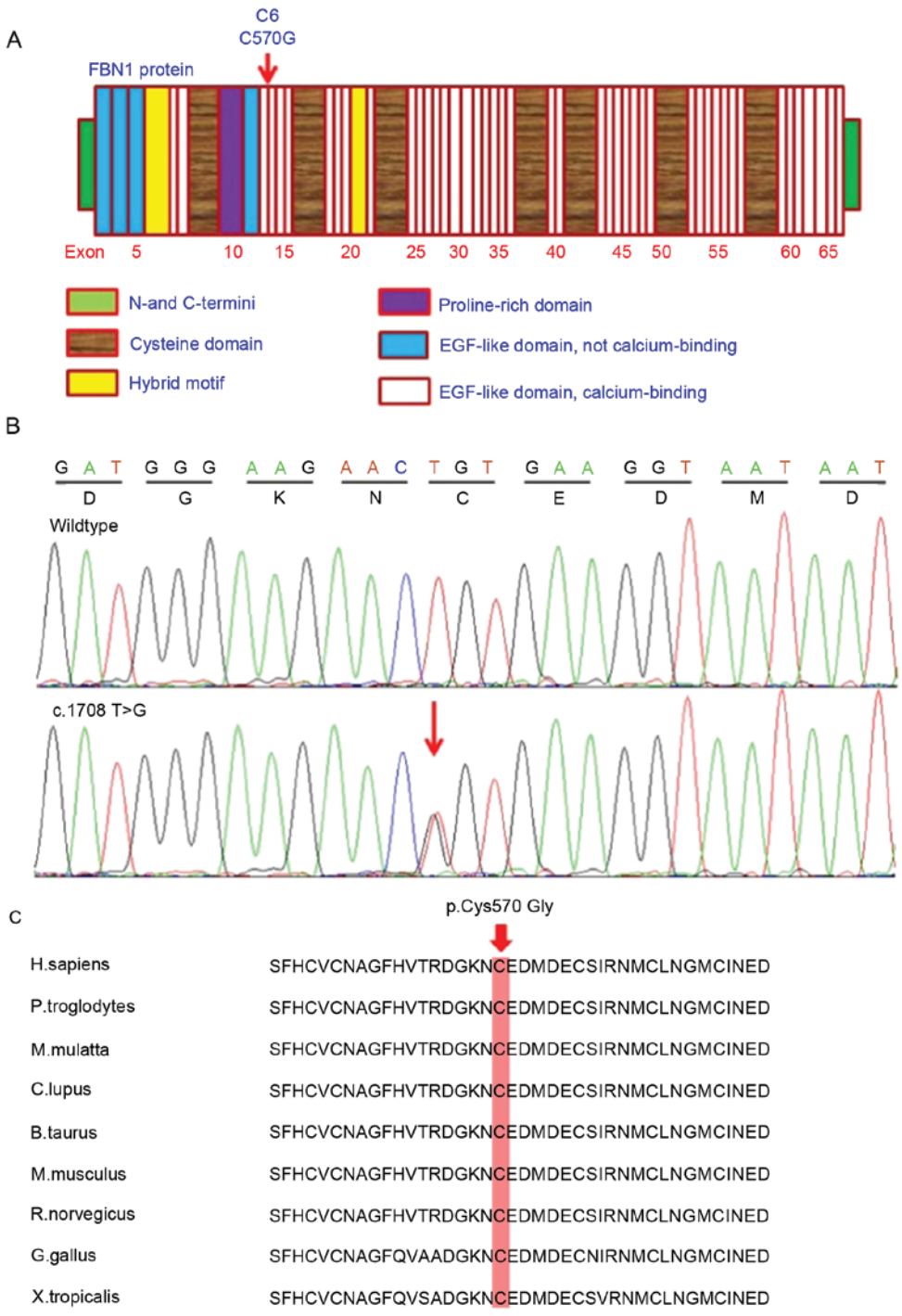

Figure 5. Representative chromatogram of *FBN1* sequence. (A) FBN1 has different functional regions and the p.C570G mutation occurs in the calcium binding EGF-like domain. (B) Normal sequence from an unaffected individual (I:2) (upper sequence), and a heterozygous T to G substitution at codon 570 from 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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