·Basic Research·

A novel FBN1 missense mutation (p.C102Y) associated with ectopia lentis syndrome in a Chinese family

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Abstract

• AIM: To characterize the disease-causing mutations in a Chinese family with ectopia lentis syndrome (ELS).

• METHODS: Patients and their family members were given complete physical, ophthalmic, and cardiovascular examinations. Genomic DNA samples were extracted from the peripheral blood of the pedigree members and 100 healthy controls. Mutation screening was performed in the fibrillin -1 (FBN1) gene by bi -directional sequencing of the amplified products. The mutation was analyzed using two bioinformatics methods.

• RESULTS: A novel heterozygous c.305G>A mutation in exon 3 of FBN1 was detected. As a result of this change, a highly conserved cysteine residue was replaced by a tyrosine residue (p.C102Y). Another mutation was found in the same exon (c.303T>C), which did not change the amino acid sequence. Both mutations were discovered in each affected individual, but not in the unaffected family members, or in 100 ethnically matched controls. A bioinformatics analysis predicted that mutation p.C102Y would affect protein function.

• CONCLUSION: In the first epidermal growth factor-like module, we identified a novel FBN1 mutation (p.C102Y), which caused ELS in the family. Our study presented a unique phenotype, including some distinct ophthalmic findings, such as hypoplasia of the iris and anisometropia. Our results expanded the mutation spectrum of FBN1 and enriched the overall knowledge of genotype-phenotype correlations due to FBN1 mutations.

• KEYWORDS: Marfan syndrome; fibrillin-1; ectopia lentis syndrome; cysteine residue

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INTRODUCTION

Marfan syndrome (MFS, OMIM #154700) is a dominantly inherited disorder of the connective tissue, with ocular, skeletal, and cardiovascular manifestations. It is clinically diagnosed according to the Ghent criteria [1,2]. Mutations in the fibrillin-1 (FBN1) gene have been characterized in patients with Marfan and Marfan-related disorders. The revised Ghent nosology has designed a new scoring system with more weight attached to the cardiovascular manifestations and includes mutational status[2]. A series of fibrillinopathies, including ectopia lentis syndrome (ELS), MASS phenotype (mitral valve prolapse, aortic root enlargement, and skeletal and skin alterations), and mitral valve prolapse syndrome (MVPS), are also associated with FBN1 gene mutations[2, 3].

FBN1 encodes a ubiquitous connective tissue microfibrillar protein named FBN1. FBN1 has three classes of modules: the first class has 47 cysteine-rich repeat modules called epidermal growth factor (EGF)-like module, while 43 of them contain a calcium binding consensus sequence [4,5]. The other two classes include latent transforming growth factor β1 binding protein (LTBP) modules and "hybrid modules"[6]. To date, more than 1200 mutations are known in the FBN1 gene, while missense mutations account for a majority (60%) of these mutations [4]. Most of the mutations that lead to neonatal MFS are found clustered in exons 24-32, which encode a central stretch of twelve cbEGF repeats [7]. Nevertheless, it should be noted that distinct mutations in this region may also lead to atypically severe MFS and classic MFS. Mutations located in exons 59-65 (the last seven exons of FBN1) are associated with mild fibrillinopathies without aortic dilatation[4].

In the present study, we investigated a three-generation family with ELS, and detected a novel heterozygous mutation c.305G>A in FBN1,which cosegregated with the disease in the family.

SUBJECTS AND METHODS

The research protocols adhered to the guidelines of the Declaration of Helsinki and were approved by the Medical Ethics Committees of the Second Affiliated Hospital of

Medical College, Zhejiang University (Hangzhou, Zhejiang Province, China). Appropriate informed consent from each participant was obtained.

Clinical Evaluation A three-generation Chinese ELS pedigree composed of twenty-one individuals, including four affected individuals, formed the basis of this study. Nineteen family members participated in the study (three affected and sixteen unaffected, Figure 1), and 100 unrelated control subjects were recruited. The proband (III:8) was a 14-year-old girl who was diagnosed with ELS according to the Ghent criteria at her first hospital visit. The family history revealed four affected members over three generations, one of whom was deceased. Available individuals (II:2, II:4, II:6, II:8, II:9, II:10, III:8, and III:9) were given complete physical, ophthalmic, and cardiovascular examinations.

Mutation Screening Peripheral blood was collected by venipuncture in EDTA-coated Becton-Dickinson (New Jersey, USA) Vacutainer tubes, and was stored at -20℃. Genomic DNA was extracted from peripheral lymphocytes through standard procedures using the QIAamp Blood kit (Qiagen, Duesseldorf, Germany). Sixty-five coding exons and flanking intronic sequences of FBN1 were amplified by polymerase chain reaction (PCR), using previously reported specific primers [8] with the exception of exons 12, 19, and 42 (primer sequences seen in Table 1). All coding exons of TGFBR2 were amplified by primer pairs previously reported [9]. The cycling conditions of PCR were as follows: 95℃ preactivation for 5min, 10 cycles of touchdown PCR with 1℃ down per cycle from 60℃ to 50℃, 25 cycles with denaturation at 95℃ for 25s, annealing at 55℃ for 25s and extension at 72℃ for 40s, and final extension at 72℃ for 10min. Each reaction mix (25 μL) contained 50 ng of genomic DNA, 10×PCR buffer, 1.5 mmol/L $MgCl_2$, 0.2 mmol/L dNTPs, 5 μmol each of sense and antisense primers, and 2.5U of Taq DNA polymerase (Takara Bio, Dalian, Liaoning Province, China). A bi-directional direct sequencing of the PCR products was then performed.

Bioinformatics Analysis The FBN1 amino acid sequences of different species were obtained from the NCBI Gene Database (http://www.ncbi.nlm.nih.gov/gene/). The multiple-sequence alignment of the amino acid sequences in the FBN1 from several different species was analyzed using the CLC Main Workbench 6.8.4 software (CLC bio, Aarhus, Denmark). Computational methods have been shown to be effective in predicting whether a specific amino acid substitution of a protein sequence is deleterious or neutral to the function of the protein. Two methods, including Sorting Intolerant from Tolerant amino acid substitutions (SIFT) and Polymorphism Phenotyping (PolyPhen), were used.

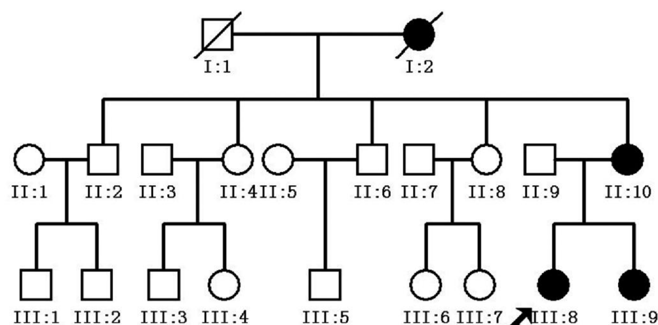

Figure 1 Pedigree of Chinese family with ELS.

Table 1 Three pairs of primers used in PCR of FBN1

Name	Prime sequence (5'-3')	Product length (bp)
Exon-12F	CAACCAGTCTTCAAATGGCTAATG	371
Exon-12R	CCATGGAACTCCTTTGAAGCC	
Exon-19F	GAGTTTTGCCTTTTTGCTTG	369
Exon-19R	TCAACTAAACTGGCATAACTGTC	
Exon-42F	TTATTGTTCTTTGCTGACCCCTATC	295
Exon-42R	CACAGGGTGTTTGCACAGTTTGT	

RESULTS

Clinical Evaluation Three affected family members (II:10, III:8, and III:9) were diagnosed with ELS based on the revised Ghent nosology for MFS [2], while the unaffected family members were not. However, further cardiovascular follow-up is needed through lifetime, since the patients would be reclassified as MFS if aortic root dissection is detected. The proband (individual III:8) had bilateral lens luxation and flattened corneas. Skeletal system abnormalities, such as tall stature, long limbs, long narrow head, flatfoot, arachnodactyly (Figure 2A), pectus carinatum, and scoliosis, were also present in the proband. Echocardiography detected mild regurgitation in the tricuspid and mitral valves, and a persistent left superior vena cava (PLSVC), but no aortic root aneurysm or dissection. The proband had a lentectomy and intraocular lens implantation 2y ago. The other affected family members showed similar symptoms to the proband, with minor differences (Table 2). Individual III:9, a 7-year-old girl, had bilateral posterior lens luxation into the vitreous. When we tried to dilate the pupils for a fundus examination, complete anterior lens dislocation occurred (Figure 2B-F). This individual (III:9) had mild regurgitation in the aortic and tricuspid valves, with no aortic root aneurysm or dissection. Individual II:10 had special ocular manifestations, including hypoplasia of the iris and anisometropia. Her pupils could only be dilated from 2.0 mm to 3.0 mm in diameter. The axial lengths of her right eye and left eye were 20.84 mm and 25.54 mm. Individual I:2 died of a stroke at the age of 70, and according to family members she also had tall stature, long limbs, and poor vision.

Mutation Screening Mutation screening was performed for all exons, and a heterozygous change G>A at position 305

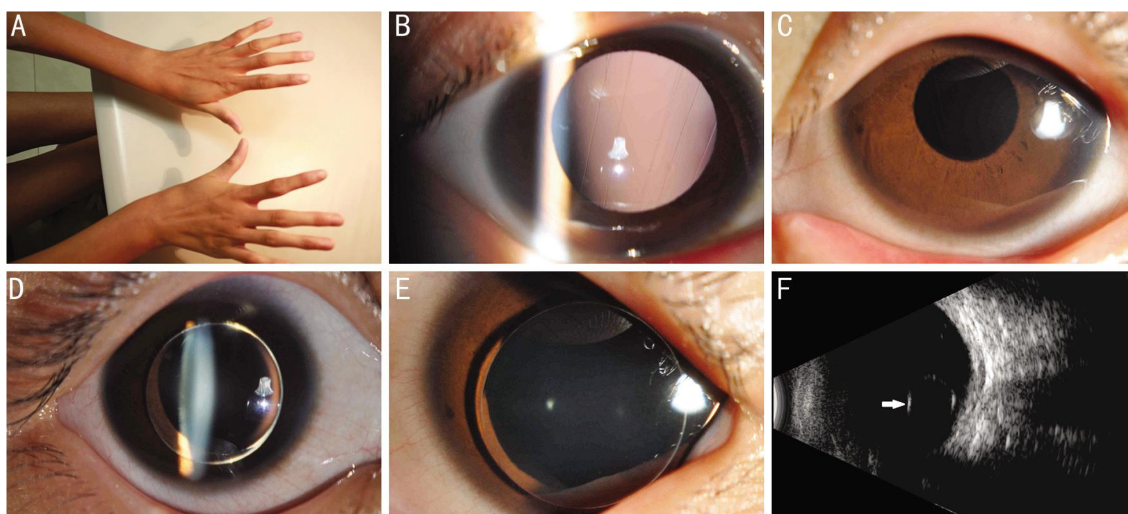

Figure 2 Clinical finding of Chinese family with ELS.

Table 2 Clinical evaluation of affected family members

Manifestation		Patient ID		
		II:10	III:8	III:9
Gender/age (a)		Female/41	Female/14	Female/7
Cardiovascular system				
I	Aortic root dimension (mm)[1]	28.1	24.9	22.4
II	Mitral valve prolapse	-	-	-
III	PLSVC	-	+	-
IV	Regurgitation in tricuspid and mitral valves	-	+	+
Ocular system				
I	Complete luxation of lens	+	+	+
II	Increased axial length[2]	-	-	-
III	Flattened corneas	+	+	+
IV	Anisometropia	+	-	-
V	Glaucoma	-	-	+
VI	Hypoplasia of the iris dilator muscle	+	-	-
Skeletal system				
I	Height (cm)	170	170	113
II	Arm span (cm)	172	172	114
III	Pectus carinatum	-	+	+
IV	Scoliosis	-	+	-
V	Flatfoot	+	+	+
VI	Arachnodactyly	+	+	+
VII	Facial features	+	+	+

PLSVC: Persistent left superior vena cava. [1]Normal range of aortic root dimension in adult is 20 to 37 mm; [2]Normal axial length in an adult is <23.5 mm[1].

(c.305G>A) was identified in exon 3 of the FBN1 gene. This mutation resulted in a substitution of a highly conserved cytosine residue by a tyrosine. (p.C102Y). In addition, another mutation (c.303T>C) was found in the same exon, which did not change the amino acid sequence (Figure 3). Both mutations were identified in the patients, and were not found in either the healthy family members or 100 controls with the same ethnic background. With the exception of several nonpathogenic single nucleotide polymorphisms, no other mutations were detected.

Bioinformatics Analysis We conducted a multiple-sequence alignment in the FBN1s from different species. The alignment data indicated that the cysteine residue at position 102 is highly conserved in the species listed (Figure 4). SIFT, which assigns scores from 0 to 1, predicts that the substitutions with scores below 0.05 are deleterious, whereas those with scores above or equal to 0.05 are tolerable [10]. PolyPhen takes into account the evolutionary conservation and physicochemical characteristics of the amino acid subjected to the mutation [11]. Substitution at position 102

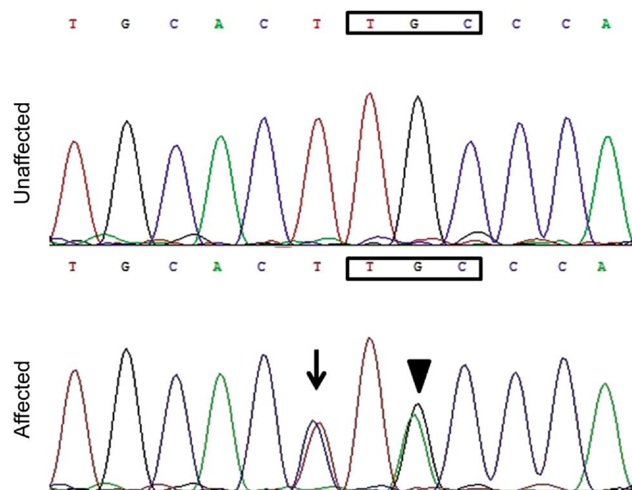

Figure 3 Sequence analysis of the affected and unaffected individuals.

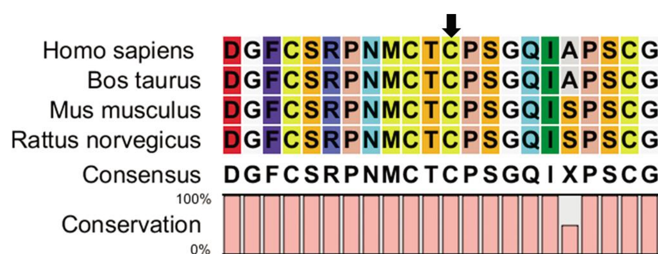

Figure 4 Multiple-sequence alignment in the FBN1.

(from C to Y) is predicted to affect protein function with a SIFT score of 0.03 (SIFT predicts substitutions with scores less than 0.05 as deleterious). PolyPhen analysis produced a score of 0.995, which is predicted to be "probably damaging".

DISCUSSION

In this study, we report a novel missense mutation (c.305G>A) in exon 3 of the FBN1 gene, which caused ELS in a Chinese family. This mutation cosegregated with affected individuals, and was not detected in either healthy family members or 100 ethnically matched controls. As a result of this mutation, a highly conserved cysteine residue was replaced by a tyrosine residue in the first EGF-like domain encoded by the FBN1 exon 3.

MFS is characterized by high clinical heterogeneity. The patients in this family had ectopia lentis without aortic root aneurysm/dissection, and the FBN1 mutation we detected (p.C102Y) has not previously been associated with aortic root aneurysm/dissection. According to the revised Ghent nosology, the diagnosis should be ELS. Chandra *et al* [12] indicated that ectopia lentis caused by mutations in FBN1 is actually part of a spectrum of fibrillinopathies with MFS. They reanalyzed the diagnosis of isolated ectopia lentis in 198 patients published in the last 20y, and found 46.3% of probands fulfilling the revised Ghent criteria for MFS based on the FBN1 mutation the probands carried. They suggested the term "isolate ectopia lentis" should be avoided in such cases[12]. As two young patients in our tested family are under the age of 20, their diagnoses are still undetermined since cardiovascular development is incomplete before the age of 20. ELS, the new designation of diagnosis proposed in the revised Ghent nosology, is in the correspondence with their clinical situation. Thus, an annually cardiovascular follow-up by imaging should be maintained throughout their life. The presence of a personal or family history of aortic root dissection, or the link of their FBN1 mutation to aortic aneurysm in the future, would be sufficient to transition their diagnosis to MFS [2]. Besides, ectopia lentis among MFS-related patients is more likely to be a subluxation, and only 2%-3% of them appear with complete luxation of the lens [13]. Interestingly, all of the patients in the family had ocular features of complete luxation of the lens, which is very rare.

FBN1, a 350 kDa glycoprotein, was discovered in 1986 and characterized as the main component of extracellular microfibrils [14], which are thought to play a role in tropoelastin deposition and elastic fiber formation, in addition to possessing an anchoring function in some tissues[15]. At present, more than 1200 mutations in the FBN1 gene have been identified in patients affected by Marfan and Marfan-related disorders[4,16-21].

We investigated the causative mutation of ELS without aortic dilatation on the UMD-FBN1, the public Human Genome Mutation Database (HGMD), and National Center for Biotechnology Information (NCBI)/ Pubmed, to explore underlying genotype-phenotype correlations. Unlike MFS which links to more than one disease-causing genes, ELS is only associated with FBN1 mutations. Schrijver *et al* [22] indicated that the cysteine substitution mutations in EGF-like domains were associated with a significantly greater incidence of ectopia lentis. However, no other genotype-phenotype correlations of ELS have been established.

FBN1 contains 47 EGF-like modules, and 43 of them are cbEGF-like modules. Each EGF-like domain of FBN1 contains six highly conserved cysteine residues that form three intra-domain disulfide bonds (C1-C3, C2-C4, and C5-C6) [23]. Since three disulfide bonds are required to maintain an EGF-like module-fold, the loss or addition of cysteine residues would result in module misfolding[24]. Some studies suggest that calcium ions binding to cbEGF-like modules play a functional role in protein-protein interactions, and that mutations alter calcium binding affinities, which could result in MFS [25]. Our study, however, reported a cysteine residue mutation in the first EGF-like module which resulted in the marfanoid phenotype without disturbing the calcium-binding domain. According to the UMD website, four cysteine residue mutations in the first EGF-like domain

were published, and none of these mutations affected codon 102.

We have identified a distinct phenotype of ELS (including hypoplasia of the iris and anisometropia) associated with the c.305G>A mutation of FBN1 gene in a Chinese family. This is the first report to relate this mutation to ELS. Further follow-up is needed for the patients in this family, as it could reveal the potential link between this mutation and MFS.

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