Genotype variant screening and phenotypic analysis of *FBN1* in Chinese patients with isolated ectopia lentis

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Abstract. Isolated ectopia lentis (IEL) can lead to blindness as result of severe complications, such as retinal detachment and secondary glaucoma. Pathogenic variants in the fibrillin 1 (*FBN1*) gene are a common cause of IEL. The aim of the present study was to investigate the frequency of pathogenic *FBN1* variants in twelve probands with IEL and to evaluate their associated phenotypes. Systemic clinical examination of the twelve probands indicated that all had bilateral EL with a median age at diagnosis of three years. High myopia was the most common feature among the probands (83.3%; 10/12 cases). No extraocular symptoms (either cardiovascular or skeletal) were observed among these patients. Genomic DNA was extracted from peripheral blood leukocytes from all patients for targeted exome sequencing. Seven heterozygous missense variants in *FBN1* were identified by bioinformatics analysis and further verified using Sanger sequencing. The seven variants were all classified as pathogenic after segregation analysis on available family members according to the American College of Medical Genetics and Genomics standards and guidelines. Of the seven variants, three were novel, namely c.2179T>C, c.2496T>G and c.3346G>C. The remaining four, namely c.184C>T, c.367T>C, c.1879C>T and c.4096G>A have been reported in previous studies. The seven pathogenic variants were identified in 8/12 (66.7%) probands with IEL. These results expand the variant spectrum of the *FBN1* gene as well as the understanding of the molecular pathogenesis of IEL.

Introduction

Congenital ectopia lentis (CEL) is characterized by displacement of the lens from its normal location (posterior to the iris and anterior to the vitreous body) as a result of congenital dysplasia of the suspensory ligament. It can lead to blindness due to complications, such as high myopia, irregular astigmatism, glaucoma, and retinal detachment (1,2). The estimated prevalence rate of CEL was 6.4/100,000 in Denmark in 1998 (3,4). CEL can occur in isolation; however, it is more common as a feature of disorders involving multiple systems (e.g., cardiovascular, skeletal, and nervous systems) and syndromic diseases, including Marfan syndrome [MFS; Online Mendelian Inheritance in Man (OMIM) no. 154700], homocystinuria (OMIM no. 236200), Weill-Marchesani syndrome (WMS; OMIM no. 277600), and Ehlers-Danlos syndrome (OMIM no. 225310) (5).

In a Danish study involving patients diagnosed with CEL, only 29.2% were diagnosed with isolated ectopia lentis (IEL) (3). Several studies have reported that CEL can not only be a monogenic disease but also an autosomal recessive inheritance (such as the ADAMTSL4 gene) (6-8). To our knowledge, IEL is most commonly caused by variants in the fillinbrin-1 (*FBN1*) gene. This gene is located on human chromosome 15q21 and contains 66 exons spanning 235 kb (9,10). In 1994, Lönnqvist *et al* first described the p.Glu2447Lys FBN1 variant in a family with autosomal dominant EL inheritance and no cardiovascular disorders characteristic of MFS (11). To date, there have been reports of only 16 *FBN1* variants causing IEL, of which 12 involved a cysteine substitution causing a missense variant and eight involved the EGF domain (12-18). Faivre *et al* (19) demonstrated that the cysteine substitution in the FBN1 protein was strongly associated with EL and that a missense variant in the 5'region of the *FBN1* gene may be more likely to lead to EL. Yang *et al* (18) found that a cysteine insertion in the LTBP motif of the FBN1 protein could cause IEL. Liang *et al* (20) suggested that the p.Cys587Arg variant could affect the function of ciliary zonules, causing IEL; however, it may not affect the conformation of FBN1 or elastin fibers, nor cause any syndromic features.

Although >2,500 variants have been identified in *FBN1*, few have been associated with IEL (21). Variant spectrum and potential genotype-phenotype correlation analyses of the *FBN1* gene in patients with IEL would be valuable for understanding the mechanism of high phenotypic heterogeneity in this gene. Therefore, in this study, variant screening of *FBN1* was carried out in twelve unrelated probands to investigate the genotype-phenotype association in patients with IEL.

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Materials and methods

Study participants. A total of twelve unrelated probands with IEL participated in this study. Detailed medical histories were obtained, and physical examinations were performed on each proband, including evaluation of the ophthalmic, cardiovascular, and skeletal systems. The ophthalmic examination included vision, intraocular pressure, dilated optometry results, slit lamp inspection, Pentacam, and Ultrasound Biomicroscope. In addition, a cardiac color Doppler ultrasound was performed to assess the aortic diameter and heart valve. Height, fingers and sternum were examined to evaluate the skeletal system. Patients were included if they had EL in one or both eyes and if their clinical features satisfied the 2010 Ghent criteria (22). Patients with a history of trauma (such as contusion of the eyeball) were excluded. This study was performed in accordance with The Declaration of Helsinki and was approved by The Institutional Review Board of the Zhongshan Ophthalmic Center, Sun Yat-sen University of Medicine. Written informed consent was obtained from all 12 participants (including the probands, their parents and the legal guardians of participants <18 years old) in accordance with The Declaration of Helsinki.

Variant detection. Genomic DNA was extracted from peripheral blood leucocytes of the 12 probands with IEL and their relatives using Maxwell 16 DNA Purification kit (Promega Corporation) according to the manufacturer's protocol. Targeted exome sequencing (TES) involving 126 genes associated with inherited eye diseases, including *FBN1*, was performed on DNA samples from all probands, as described previously (23). Briefly, coding sequences of the 126 genes were captured using the SeqCap EZ Library SR V5 kit (cat. no. 7145594001; Roche NimbleGen, Inc.). A paired library was generated using a KAPA HTP Library Preparation kit (cat. no. 07961901001, Roche), then sequenced on the Illumina Miseq platform (Illumina, Inc.) using an Illumina Miseq v2 kit (cat. no. MS-102-2002, Roche; 300 cycles; paired-end). The average sequencing coverage was ≥125x. The raw sequencing data in fastq format were exported to the Strand NGS software (v2.6; Strand Life Sciences Pvt. Ltd.) to call single nucleotide variants and insertion-deletion variants. All small insertions, deletions indels and single-nucleotide variants (SNVs) were annotated and filtered using dbSNP138 InDels, NCBI dbSNP 146, NCBI RefSeq Gene (https://www.ncbi.nlm.nih.gov/refseq/rsg/), Human Genome Mutation Database (HGMD; Professional Version; Qiagen GmbH), and 1000 Genomes Project, ExAC database (https://console.cloud.google.com/storage/browser/gnomad-public/legacy).

Pathogenic variants in FBN1 gene (GRCh37, accession number NC_000015.9). Variants in FBN1 were selected with a position ranging from 48700503 to 48937985 at chromosome 15 (Human GRCh37/hg19) from the TES data from the twelve probands. Common variants with an allelic frequency of ≥0.01 according to the non-coding region or synonymous variations not affecting splicing were excluded. The pathogenicity of missense variants was predicted using several online tools, including Polyphen-2 (score 0-1, http://genetics.bwh.harvard.edu/pph2/), Combined Annotation Dependent Depletion (CADD; https://cadd.gs.washington.edu/), Rare Exome Variant Ensemble learner (REVEL score 0-1; https://sites.

google.com/site/revelgenomics/), and ClinPred (score 0-1; release 2018.10; https://sites.google.com/site/clinpred/). The PhyloP software (https://ccg.epfl.ch//mga/hg19/phylop/phylop.html) and GERP++_RS software (http://mendel.stanford.edu/SidowLab/downloads/gerp/index.html) were used to analyze the conservation of variations. A missense variant would be classified as potentially pathogenic if it was predicted to be deleterious by at least two of the four prediction tools. The sequences of potential pathogenic variants were validated using Sanger sequencing. Protein structures were predicted using wild-type and mutant amino acid sequences using Swiss-model software (https://swissmodel.expasy.org/). All potential variants were classified manually according to the guidelines of the American College of Medical Genetics and Genomics (24). Comparison between the results of the present study with the main available variants in FBN1 reported by previous literatures was also performed (25,26).

Statistical analysis. The differences in the genomic position of the *FBN1* variants and in the age at diagnosis between patients with MFS or IEL were analyzed using Mann-Whitney U test. Data analysis was performed using the SPSS statistical package (version 26.0 for Windows; IBM Corp.) P<0.05 was considered to indicate a statistically significant difference.

Results

Clinical findings. A total of twelve probands participated in the study, including nine male and three female patients. Clinical information of all patients is displayed in Table I. The age at examination ranged from 3 to 27 years old, with a mean age of diagnosis of 9.91 years (±8.85 years). All subjects had symptoms of poor vision and were diagnosed with IEL. Eleven were first diagnosed with IEL before six years of age, while the remaining proband was first diagnosed at 20 years of age. All patients with IEL in the present study presented lens subluxation or luxation in both eyes and none showed extraocular (cardiovascular or skeletal) features. High myopia was a common feature of these probands (10/12 cases; 83.33%). The other two patients had hyperopia. Except for probands 4 and 9, who were not eligible for surgery due to their young age, all other probands underwent transscleral-fixated intraocular lens implantation, which effectively improved their vision.

Variants in FBN1 gene. In total, seven missense variants in *FBN1* were identified in eight of the 12 probands with EL, and no variant was detected in the other four probands. The variant frequency was 66.67%. All eight variants of *FBN1* were *de novo*, since they were not detected in the parents of the eight probands. The pedigrees of eight families are displayed in Fig. 1. In total, three (c.2179T>C, c.2496T>G and c.3346G>C) of the eight variants were novel variants. The other four (c.184C>T, c.367T>C, c.1879C>T and c.4096G>A) had been reported previously (Table II; Fig. 2) (27-29). All eight variants were located at the calcium-binding epithermal growth factor (cbEGF)-like domain (Fig. 3) and were predicted to be deleterious by all four prediction tools (Table II).

The lens dislocation of a patient with a heterozygous c.367T>C (p.Cys123Arg) variant in *FBN1* is shown in Fig. 4.

Table I. Characteristics of patients with IEL in the present study.

| Patient | Age, years | Sex | Age at, diagnosis years | Ocular examination | | | | | Extraocular system | | |
				VA, (logMAR) OD/OS	Preoperative Refraction, OD/OS (D)	Surgery for IEL	Postoperative BCVA, (logMAR) OD/OS	Skeletal anomaly	AO diameter, mm	Cardiovascular symptom
1	6	M	2	0.5/1.3	-13.5/-9.5	Yes	0.09/0.3	None	22	None
2	5	M	4	1.3/1.0	+14.0/+14.75	Yes	0.3/0.0	None	18	None
3	4	M	3	1.2/1.0	-10.0/-12.0	Yes	0.15/0.0	None	23	None
4	3	M	3	NA	-7.75/-8.0	No	NA	None	18	None
5	3	M	3	1.3/1.5	-24.0/-23.0	Yes	0.15/0.3	None	23	None
6	4	M	3	1.3/0.6	-14.0/-18.0	Yes	0.15/0.3	None	17	None
7	11	F	5	1.3/1.5	-8.0/-7.0	Yes	0.15/0.09	None	29	None
8	27	F	5	0.7/0.9	-10.0/-14.0	Yes	0.04/0.00	None	29	None
9	4	M	3	0.6/1.0	+2/+2	No	NA	None	18	None
10	26	F	6	2.0/1.3	-18.0/-14.0	Yes	NA/0.00	None	27	None
11	21	M	20	0.3/1.3	-17.0/-8.5	Yes	0.15/0.00	None	NA	None
12	5	M	3	1.1/0.9	-7.5/-5.5	Yes	0.15/0.00	None	16	None

VA, visual acuity; OD, oculus dexter; OS, oculus sinister; D, degree; BCVA, best corrected visual acuity; logMAR, logarithm of the minimum angle of resolution; NA, not available; IEL, isolated ectopia lentis; AO, aortic.
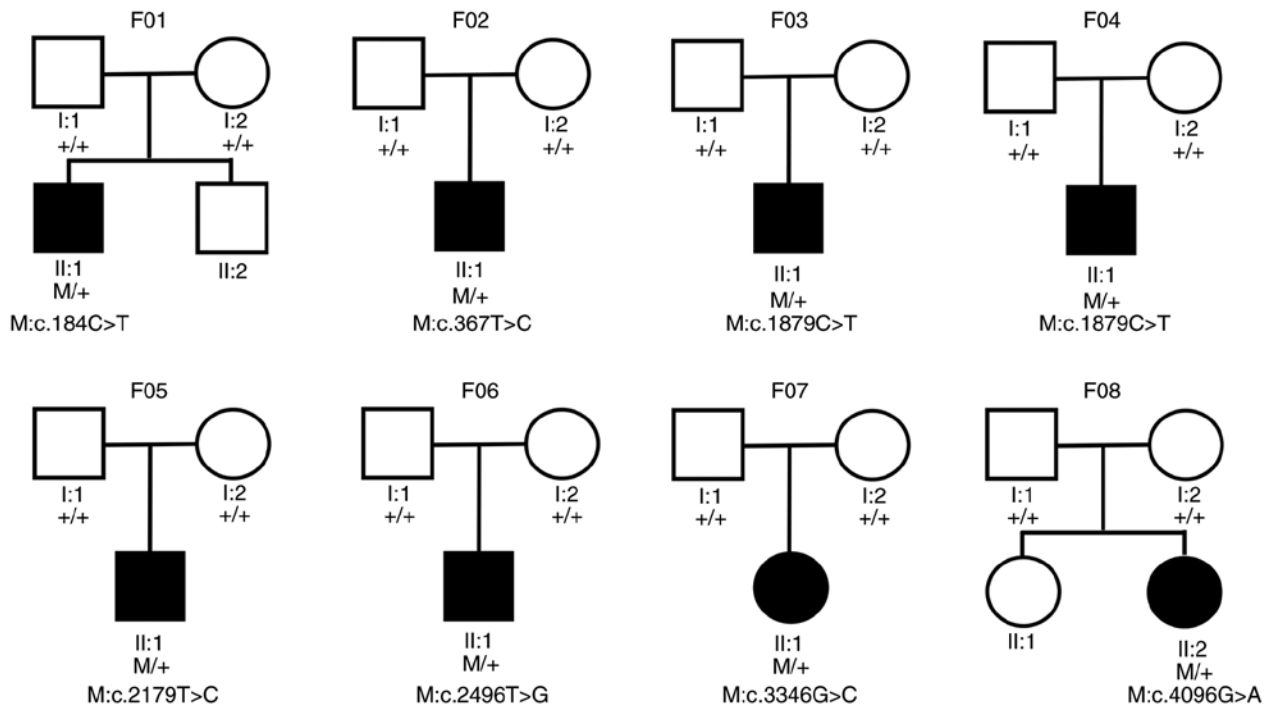

Figure 1. Pedigrees of eight families with fibrillin-1 variants. The filled squares (male) and circles (female) are the affected individuals. The + symbol indicates carriers of the wild-type gene. I and II indicate the generation. F, family; M, mutant.

The main reported variants in FBN1 gene were collected and shown in Table SI, the average age of patients is 24.6 years old, and there is no obvious trend in the mutation sites of the FBN1 gene. Compared with patients with MFS, patients with IEL were diagnosed at younger ages (Fig. 5A; P<0.001). Moreover, the variants identified in the probands with IEL in the present study were located in upstream coding regions of the gene (from exon 2 to exon 33; Table II), whereas variants associated with MFS were located throughout the whole region of the gene as reported by previous studies (25,26). The genomic positions of the *FBN1* gene variants were in the upstream regions of chromosome 15 (P=0.04; Fig. 5B).

Table II. The seven *FBN1* variants identified in patients with isolated ectopia lentis.

First author, year	Patient	Exon	Nucleotide variant	Protein variant	Domain	PolyPhen-2	REVEL	CADD	ClinPred	HGMD	ACMG	(Refs.)
Körkkö *et al*, 2002	1	2	c.184C>T	p.Arg62Cys	4-Cys motif LTBP-like	1.0	0.601	28.6	0.994	DM	PS1, PS2, PM2, PP3	(27)
Arbustini *et al*, 2005	2	4	c.367T>C	p.Cys123Arg	ECF-like #02	0.998	0.991	28.0	0.999	DM	PS1, PS2, PM2, PP3	(28)
Hayward *et al*, 1994	3 and 4	15	c.1879C>T	p.Arg627Cys	cbEGF-like #06	1.0	0.684	22.9	0.768	DM	PS1, PS2, PM2, PP3	(44)
Present study	5	18	c.2179T>C	p.Cys727Arg	cbEGF-like #07	0.997	0.977	27.6	0.999	NA	PS2, PM2, PP3	-
Present study	6	20	c.2496T>G	p.Cys832Trp	cbEGF-like #09	0.999	0.914	12.56	0.999	NA	PS2, PM2, PP3	-
Present study	7	27	c.3346G>C	p.Glu1116Gln	cbEGF-like #13	0.997	0.899	28.0	0.979	NA	PS2, PM2, PP3	-
Uyeda *et al*, 2004	8	33	c.4096G>A	p.Glu1366Lys	cbEGF-like #19	0.999	0.950	41	0.999	DM	PS1, PM2, PP3	(46)

LTBP, latent transforming growth factor-beta binding proteins; EGF, epidermal growth factor; cbEGF, calcium-binding epidermal growth factor; HGMD, Human Genome Mutation Database; DM, disease-causing mutation; NA, not available; PS, pathogenic strong; PM, pathogenic moderate; PP, pathogenic supporting. The number behind # indicates the number of domains in the entire FBN1 gene. Polyphen-2 and REVEL (P<10⁻¹²) are scored on a range from 0-1, and a score of 1 is a likelihood of that variant being disease-causing. CADD provides a rank of genome wide scores and higher scores are more likely to be deleterious. The variants get scores >30, it is more likely to be considered as a harmful substitution. ClinPred are on score range from 0-1. When the score is >0.5, it is pathogenic, and <0.5 is neutral. The higher the score, the higher the chance of the disease.

Discussion

CEL is an autosomal dominant connective tissue disorder. Several *FBN1* variants have been reported to be pathogenic for CEL, the majority of which are associated with syndromic CEL (including MFS) (19). However, only a few studies have examined *FBN1* variants in patients with IEL (30). In the present study, seven *FBN1* variants in eight patients with IEL, including three novel variants and four previously reported variants.

The *FBN1* gene encodes the fibrillin 1 protein, which is an essential element of microfibrils found in elastic and nonelastic tissues, including ciliary bodies, blood vessels, lungs, and skin (5). In patients with MFS, WMS or IEL (31), >2,500 variants have been identified in this gene, most of which were identified in patients with MFS. It has been reported that variants in *FBN1* accounted for ~90% of patients with MFS (32). However, only 16 identified variants in the *FBN1* are associated with IEL (30), consistent with the variant frequency in the present study. Missense variants account for two-thirds of the 2,500 known variants in *FBN1* (19). Most missense variants are located at the cbEGF domain, and ~70% of these are conserved cysteine substitutions (33). Cysteine plays an important role in the EGF-like domain of the FBN1 protein. Indeed, substitutions of cysteine residues can disrupt one of three disulfide bridges that covalently connect three pairs of cysteine residues that are highly conserved in EGF-like domains, thereby affecting the structural stability of the domain itself and neighboring domains as well (34,35). Previous studies have suggested that missense variants in *FBN1* are associated with EL phenotype (19,36). EL is more likely when the missense variants involve cysteine residues (37). In the present study, missense variants were detected in eight probands, of which six were located in the cbEGF domain, and five involved a cysteine residue. The present findings are consistent with those of the previous study in which EL was associated with missense mutations and cysteine residue change (37).

A total of three novel heterozygous variants (c.2179T>C/p. Cys727Arg; c.2496T>G/p. Cys832Trp; c.3346G>C/p. Glu1116Gln) in *FBN1* were identified in three probands with IEL. The initial symptoms experienced by the three probands included blurred vision. Only high myopia and EL were present in these three probands without extraocular features. All three novel variants are *de novo* and were predicted to have a possible pathogenic effect according to PolyPhen-2 and SIFT. In addition, the two novel variants, p.Cys727Arg and p.Cys832Trp, occurred at the same positions as the known p.Cys727Tyr and p.Cys832Tyr respectively. These two variants may abolish a disulfide bond and affect the first (C1) and sixth (C6) conserved cysteine of the cbEGF domain that are essential for correct EGF-like domain structure (38,39). The variant c.3346G>C is located in cbEGF domain and changes the conserved glutamic acid residue. It is suggested that the change of glutamic acid may affect Ca²⁺ binding of cbEGF domain and destructs the structure of FBN1 gene (35).

The variant c.184C>T carried by proband 1 has previously been associated with late-onset IEL (38,40). In a Chinese family, the age of diagnosis ranged between 41 and 65 years (41). In contrast with these previous findings, in the present study, the patient with this variant developed EL symptoms at age 3, however in this study it was found that it could develop at 2 years old. The c.367T>C variant is associated with classical MSF,
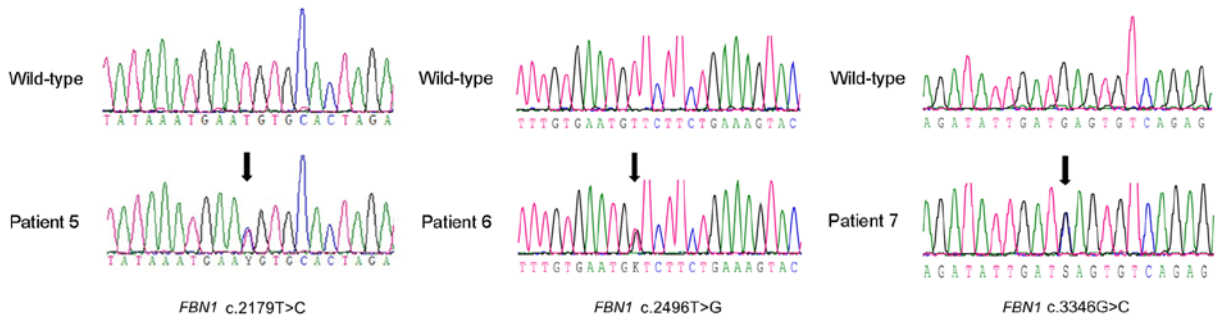
Figure 2. Fragment of *FBN1* cDNA sequence in probands 5, 6 and 7. Sequencing traces from probands 5, 6 and 7 with the c.2179T>C, c.2496T>G and c.3346G>C variants, respectively. *FBN1*, fibrillin 1.
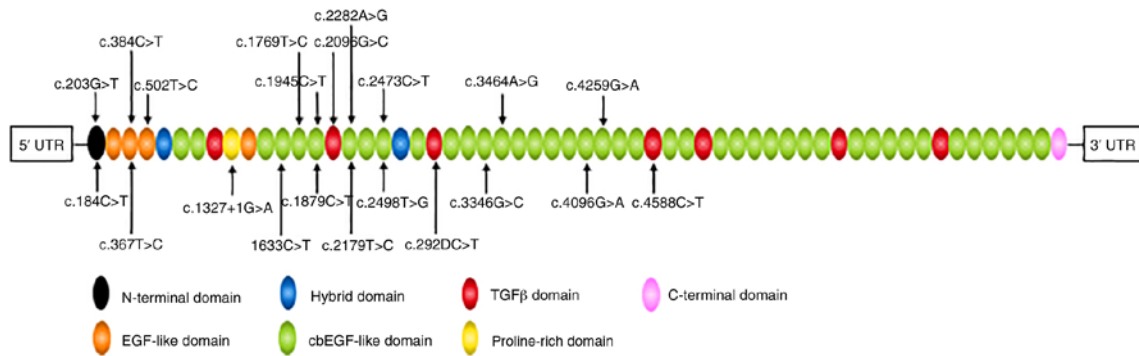

Figure 3. Schematic presentation of *FBN1* variants. The position of reported *FBN1* variants associated with isolated ectopia lentis from the present study and the literature (29). *FBN1*, fibrillin 1; EGF, epidermal growth factor; cbEGF, calcium-binding epidermal growth factor; UTR, untranslated region.
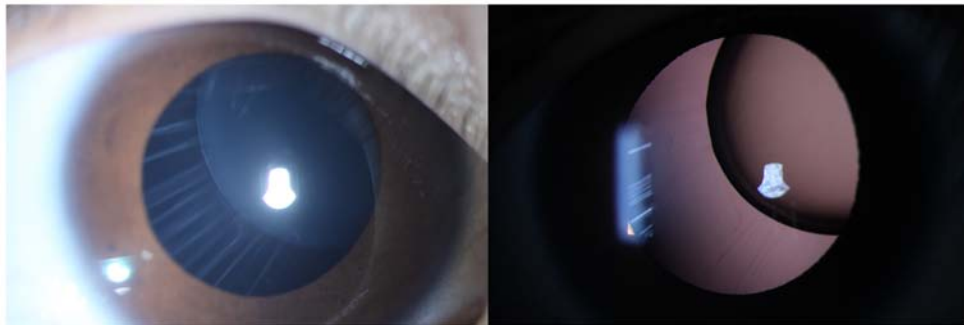

Figure 4. The Anterior segment photography of proband 2 with a heterozygous c. 367T>C (p.Cys123Arg) fibrillin 1 variant. The left panel shows a clear dislocation of the lens, while the right panel shows the elongated lens zonule using slit lamp photography.
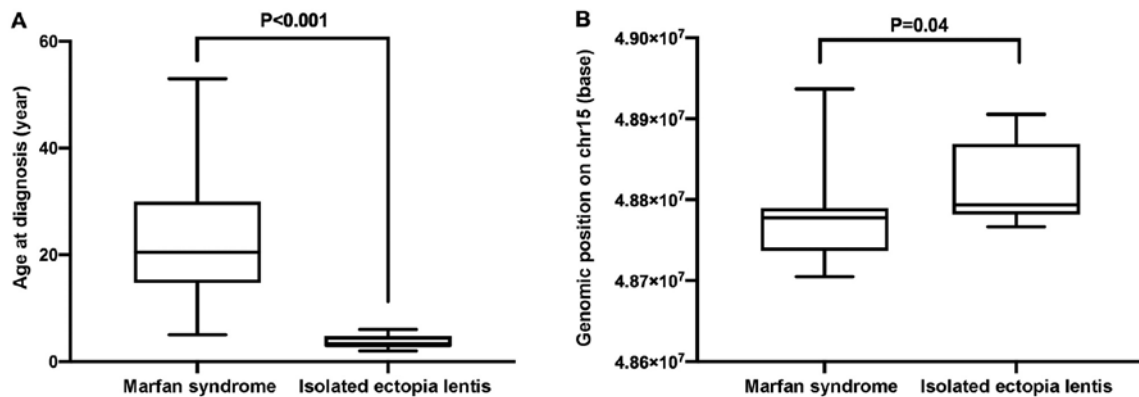

Figure 5. The mutation distributions from different diagnosis ages of MFS and IES patients. Comparison of age at diagnosis (A) and genomic position of the fibrillin 1 variants (B) in patients with Marfan syndrome or isolated ectopia lentis. chr15, chromosome 15.

was identified in a Frenchman in 2005 (28). The phenotype of the patients in this study only involved the ocular system. The c.1879C>T variant was reported in 1994 and causes manifestations in the ocular and skeletal systems, with mild aortic involvement (29), but the present study did not find any system disorder in probands. The c.4096G>A variant was identified in 2004 in two patients, one with cardiovascular manifestations but no ocular signs, and the other with MFS (36) and no cardiovascular system signs were found in any of the probands in the present study.

Mutations in the *FBN1* gene are commonly associated with MFS. However, in the present study, the eight probands with *FBN1* variants, did not have any systemic disorders except for IEL. The examination of aortic diameter indicated that these patients did not meet the standards for abnormal aortic roots, nor did they meet the revised Ghent criteria for MFS (22). Nevertheless, it remains possible that the patients could develop aortic root dilation or other manifestations of MFS in the future. The present findings also suggested that ocular disorders might occur in isolation or may be the first sign of the syndrome. Currently, it remains difficult to diagnose MFS in young patients with EL; as a result, these pediatric patients still require long-term annual cardiovascular imaging.

In the present study, the *FBN1* variant identified in the eight probands were located in the first 33 exons, and some involved arginine-to-cysteine substitutions. Faivre *et al* (19) also noted that EL was more likely when missense changes occurred in the exon 1-21. Compared with patients with MFS, the average genomic positions of the variants carried by the eight probands with IEL were located more upstream of the *FBN1* gene. Moreover, IEL was diagnosed at a younger age, compared with MFS. This result is consistent with those of previous studies describing EL as the first symptom of MFS, occurring before the age of 10 (42). Most probands with *FBN1* gene variant suffered from poor visual acuity, significant EL, and high myopia. Most also underwent surgical treatment, except for proband 11 who was older than 20 years, and proband 4, who was not eligible for surgery due to parents' disagreement. Therefore, it may be hypothesized that these variants lead to earlier ocular symptoms.

The limitations of the current study were that the patients were young; patients may not have developed other clinical manifestations at the time of the present study. In addition to MFS and EL, the *FBN1* gene has also been implicated in conditions such as WMS, isolated ascending aorta, mitral valve prolapse, aortic root dilatation without dissection (43), and familial arachnodactyly (44). Associations between genotype and phenotype have not been determined. In addition to the *FBN1* gene, IEL can also be inherited in an autosomal recessive fashion because of *ADAMTS4*, identified in 2009; variants in this gene can cause earlier ocular phenotypes than those associated with *FBN1* in Caucasian populations (16,45). Altogether, the evidence suggests a need for the identification of other target genes and examination of larger patient cohorts in future studies. Further study is required to examine associations between genotype and phenotype in the *FBN1* gene. Early genetic screening contributes to early disease prediction and management for patients and their families.

In summary, three novel variants and four reported variants were identified in the *FBN1* gene from eight Chinese probands with IEL. These findings further enrich the *FBN1* variant spectrum and may provide insight into the clinical diagnosis and management of IEL.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request. The sequencing datasets are also available from the Sequence Read Archive under BioProject no. PRJNA668437.

Authors' contributions

YZ, GJ and DZ conceived and designed the experiments. YZ performed the experiments. DG, QAC and XZ analyzed the data. YZ, DG, QAC and XZ contributed reagents/materials/analysis tools. YZ, GJ and DG wrote the manuscript. GJ and DZ confirmed the authenticity of the data in the present study. All authors read and approved the final manuscript.

Ethics approval and consent to participate

This study was performed in accordance with The Declaration of Helsinki and was approved by The Institutional Review Board at the Zhongshan Ophthalmic Center, Sun Yat-sen University of Medicine. Written informed consent was obtained from all participants and from the legal guardians of participants <18 years old, in accordance with The Declaration of Helsinki.

Patient consent for publication

All participants provided informed consent (including the probands, their parents and the legal guardians of participants under 18 years old) for the publication of their data, including images and examination results.

Competing interests

The authors declare that they have no competing interests.

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