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Identification and phenotypic analysis of novel LTBP2 mutations in a Chinese cohort with congenital ectopia lentis

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Purpose: To evaluate the frequency of LTBP2 mutations and to elaborate on LTBP2-related clinical phenotypes in a Chinese congenital ectopia lentis (CEL) cohort.
Methods: In total, 145 Chinese probands with CEL were recruited for this study and underwent ocular and systemic examinations. Whole-exome sequencing was used to identify mutations, and Sanger sequencing and bioinformatics analysis were further performed to verify pathogenic mutations.
Results: Overall, biallelic mutations in LTBP2 involving eight novel mutations (c.4370–7_4370–9delTCT, c.4370–5C>G, c.3452G>A, c.2253delG, c.4114T>C, c.1251G>A, c.4760G>A, and c.620G>A) were identified in four CEL probands (4/145, 2.76%). Patients with LTBP2 mutations were characterized by a megalocornea, spherophakia, high myopia, and glaucoma instead of a flat cornea, high corneal astigmatism, cardiovascular and skeletal abnormalities that were reported in other gene mutations. A novel homozygous frameshift mutation was detected, and this type of mutation was found to cause more complicated ocular symptoms than others, ranging from the anterior segment to the fundus.
Conclusion: This study reported the mutation frequency of the LTBP2 gene in a Chinese CEL cohort and provided novel insight into LTBP2-related genotype–phenotype associations in CEL.

Congenital ectopia lentis (CEL) is a rare but severe hereditary disease in which the lens zonules elongate or rupture due to congenital dysplasia, causing the lens to deviate from its normal position. This complex ocular disease has multiple pathogenic genes [1–3], and its ocular and systemic manifestations vary greatly. As previous studies have reported, different genes and mutations can greatly impact patient phenotypes [2,4].

The latent transforming growth factor-β-binding proteins (LTBPs) constituting the large latent complex that sequesters transforming growth factor β (TGF-β) are components of the extracellular matrix (ECM). LTBP genes encode the four human LTBPs associated with inherited connective tissue diseases. Among them, LTBP2 encodes for the second isoform of the LTBP fibrillin superfamily (LTBP-2), which is widely expressed, especially in the eye, cardiovascular system, skeletal muscles, lungs, liver, spleen, testes, and placenta [5,6]. In previous studies, mutations of LTBP2 were observed to cause a wide range of systemic manifestations, including congenital heart disease, idiopathic pulmonary fibrosis, short stature, and ocular manifestations such as lens dislocation, axial myopia, microspherophakia, megalocornea, and primary congenital glaucoma (PCG) [7–9]. Although several studies have mentioned the LTBP2 gene mutation instigates CEL, detailed reports are lacking, and the causative mechanism remains unknown [10–12].

Reports on LTBP2 as the cause of CEL are relatively rare, and the associations between the genotypes and phenotypes of LTBP2 and CEL are still unclear, as are their underlying mechanisms. This study aims to establish the gene mutation frequency of the LTBP2 gene and enrich the related mutation spectrum to help recognize LTBP2-related CEL clinical manifestations in the Chinese population.

METHODS

Patients: This study was conducted under the principles of the Declaration of Helsinki and was approved by the ethics committee of the Zhongshan Ophthalmic Center at Sun Yat-Sen University, China. Written informed consent was obtained from all participants or their guardians. CEL

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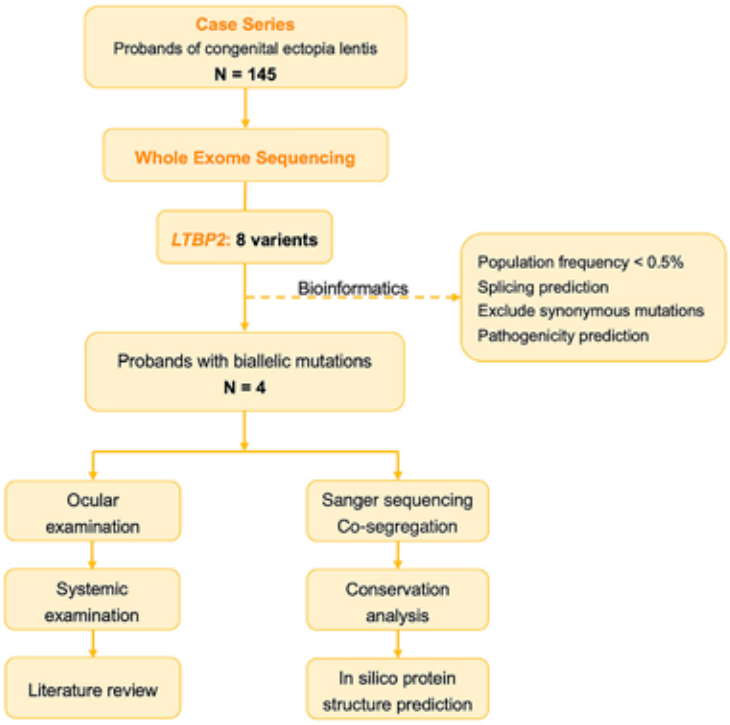

Figure 1. Workflow for the cohort study and whole-exome sequencing data processing.

patients were recruited from September 2018 to September 2021 from the Zhongshan Ophthalmic Center. Overall, 145 Chinese probands were included (Figure 1).

Ocular examinations: All probands underwent comprehensive ophthalmic examinations, which included best-corrected visual acuity (BCVA), intraocular pressure measurement (IOP), slit-lamp biomicroscopy, anterior segment photography, fundus photography, and perimetry. Ophthalmic technicians or ophthalmologists examined the subjects. The values of the ocular biologic parameters, including axial length (AL), corneal simK, anterior chamber depth (ACD), length thickness (LT), and corneal diameter (WTW), were obtained with the IOL Master 700 (Software V5.4, Carl Zeiss Meditec, Inc., Dublin, CA). Optical coherence tomography (OCT; Carl Zeiss Meditec) was used to perform SD–OCT imaging and to analyze the retinal nerve fiber layer (RNFL).

Systemic examinations: Echocardiography was performed with a full ultrasonography system (Philips EnVisor C-HD; Philips Co., Best, the Netherlands). The internal diameter of the aortic root (AO) was measured following current guidelines [13]. The probands were X-rayed to evaluate their metacarpal index (MCI), a radiographic measure of the slenderness of the metacarpals. Such measurements are commonly used when screening for Marfan syndrome (MFS) [14].

The diagnosis of MFS was made according to the revised Ghent criteria [15]. The diagnosis of Weill–Marchesani syndrome (WMS) has been referred to in the previous literature [16].

Variant detection and mutation analysis: Genomic DNA samples from all probands were extracted from peripheral whole blood with a TIANamp Blood DNA Kit (Tiangen Biotech, Beijing, China) and verified using Nanodrop. Illumina paired-end libraries were prepared using the Kapa LTP library prep kit (Roche, Basel, Switzerland), and whole-exome sequencing (WES) was performed using the Agilent SSELXT Human All Exon V6. Raw reads were aligned to the human genome reference (hg19) using the BWA (Burrows Wheeler Aligner). Single-nucleotide variants (SNVs) and InDels (insertions and deletions) were called by Atlas-SNP2 and Atlas-Indel, respectively. The frequencies of all SNVs and InDels were annotated using the ExAC, gnomAD, CHARGE, 1000 Genome, UK10K databases, and the internal database of Clinbytes Inc. to filter common variants, with an allele frequency cutoff of 0.5% and 0.1% for recessive and dominant variants, respectively. Using the whole exome capture probe described in the experimental section, an average of 100X in the target region was achieved, the effective rate was 97%–98%, and 99% of the target region was covered 1X in the proband. The most frequently observed sequence

mean quality score, Q30, of each sample was over 90%. The mutations identified in the probands' family members were validated by Sanger sequencing. Suspected mutations were verified with a cosegregation analysis of available relatives. Copy number variations (CNVs) were analyzed using the SeqCNV method based on WES data. The prediction of the identified LTBP2 variants' pathogenicity was assessed using prediction tools, including Polymorphism Polyphen2, Mutation Taster, Sorting Intolerant from Tolerant (SIFT), the Berkeley Drosophila Genome Project (BDGP), and NetGene2. The Human Gene Mutation Database (HGMD), Human Genetics Knowledge for the World, and Clinical Genome Resource (ClinVar) were used to confirm whether an identified mutation was novel. Nucleotide conservation was evaluated with PhyloP, and BLAST was used to analyze the alteration of amino acids.

The structure, function, and potential pathogenic effects of the mutant LTBP2 proteins were predicted by in-silico protein modeling. Known protein domains in the full-length LTBP2 sequence were sought in the Pfam database, and the proteins of homologous templates were sought in the HHpred database. Based on the score of HHpred alignment, templates with more than 99% probability of being structurally connected to specific LTBP2 domain segments were selected. MODELER 9.23 was used for tertiary protein structure prediction and the homology modeling of the variants using the structures of previously identified templates. Five models were created, and the discrete optimized potential energy (DOPE) score was used to evaluate the best model for the study. PyMol was used to plot and analyze the chosen model.

RESULTS

Clinical features of the patients: In this study, four unrelated probands with novel causative LTBP2 variants were enrolled in a CEL cohort of 145 patients from a Chinese ophthalmic center. The proportion of LTBP2 variants within the CEL cohort indicates an overall mutational frequency of 2.76%. The demographic details and clinical data of the four probands are summarized in Table 1. Pedigree graphs of the four families are shown in Appendix 1.

One proband (ID 228) was a 43-year-old female with a long history of glaucoma and medicinally controlled IOP. She also had high myopia retinopathy with posterior staphyloma on her fundus and thinning of the retinal nerve fiber layer (RNFL) superior to the retina. The sibling of the proband (ID 229) underwent surgery at another hospital for ectopia lentis and was determined to have the same genotype as patient ID 228 when we performed Sanger sequencing and cosegregation testing.

The second proband (ID 294), a 5-year-old girl from consanguineous parents, carried a homozygous mutation. She was referred to our clinic for very low vision and high intraocular pressure (31 mmHg OD and 31 mmHg OS). In the anterior segment, we discovered a bilateral megalocornea, corneal haze, angle closure, ectopia lentis, and spherophakia (Figure 2D) after dilation. The fundus examination indicated glaucomatous optic nerve atrophy and a very thin RNFL. The patient's cup/disc (C/D) values were found to be 0.96/0.95 (OD/OS; Figure 2E).

The third proband (ID 382), a 33-year-old female, showed bilateral EL, lenticular coloboma (Figure 2C), and spherophakia. The fundus examination revealed retinal degeneration and a shallow optic disk with a blurred border, resulting in an undefined C/D ratio (Figure 2F).

The last proband (ID 431), a 4-year-old girl, was diagnosed with bilateral EL (Figure 2B) and had a megalocornea (Figure 2A), spherophakia, and angle closure but did not have a high IOP.

All the probands had AO diameters that were within the normal range, with no abnormalities that could be detected by electrocardiogram or Doppler echocardiography. No obvious deformity of the skeletal system was observed; the MCI in all patients exceeded the normal range, but did not reach the abnormal range of MFS.

As summarized in Table 1, bilateral ectopia lentis was observed in all patients, while "Marfan-like" or "Weill–Marchesani" systemic abnormalities were not found. Spherophakia was found in three patients (3/4), and in two patients, it was accompanied by a megalocornea and angle closure (2/4). Glaucoma was found in two patients (2/4), and three patients had fundus abnormalities (3/4). Refractive errors were common to all the patients, and a long axial length was witnessed in one patient (ID 228). None of the four patients manifested systemic abnormalities. None of the probands' family members with heterozygous mutations was diagnosed with ectopia lentis, glaucoma, or other ocular or systemic disorders.

Treatment and prognoses: Except for the third proband (ID 382), the patients underwent the transscleral suture fixation of the posterior chamber of the intraocular lens, and their BCVAs after surgery ranged from 0.1 to 1.0. There were four eyes with postoperative IOP elevation, and any IOP could be controlled by antiglaucoma eye drops (1~3 medicines). To observe disease progression and prognosis, the median follow-up time was 23 months (ranging from 19 to 28

Table 1. Genotypes and clinical features of CEL patients included in this study.

Patients (ID)	228	294	382	431
Genotype	c.3452G>A(;) 4370-7_4370-9del(;) 4370-5C>G	c.[2253del]; [2253del]	c.[1251G>A]; [4114T>C]	c.[620G>A]; [4760G>A]
Gender, Age (years)	F, 43	F, 5	F, 33	F, 4
Family history	+	Consanguineous marriage	-	-
Height (cm)/Weight (Kg)	167/61	105/18	170/72	108/18.1
Ocular features (OD/OS)				
Bilateral/Unilateral	Bilateral	Bilateral	Bilateral	Bilateral
EL direction				
OD	Inferotemporal	Inferotemporal	Inferotemporal	Inferonasal
OS	Inferonasal	Inferotemporal	Inferotemporal	Inferonasal
Refraction*	-24.0Ds/-23.0Ds	-30.0Ds/-31.0Ds	-6.25Ds/-5.25Ds	-20.0Ds/-20.0Ds
BCVA	0.4/0.3	0.1/0.05	0.7/0.7	0.1/0.1
IOP (mmHg)	12.7/14.3#	31/31	17/18	16/12
Average C/D ratio	0.66/0.53	0.96/0.95	0.0/0.0	0.65/0.62
Average RNFL (μm)	97/80	36/38	111/112	123/126
Axial Length (mm)	28.46/28.20	23.72/23.82	23.02/22.92	21.48/21.57
Corneal simK (Diopters)	45.27/44.37	42.80/42.72	44.96/44.28	43.13/42.85
ACD (mm)	4.06/3.62	2.62/2.31	3.42/3.43	2.39/2.64
LT (mm)	4.48/4.74	4.78/5.01	5.19/5.19	5.18/5.27
Corneal diameter (mm)	12.0/12.2	13.3/13.5	12.7/12.8	13.1/13.4
Cataract	+/-	-/-	+/+	-/-
Ocular Complications	high myopia glaucoma posterior staphyloma thinner RNFL layer of superior retina	megalocornea corneal haze spherophakia closed angle glaucoma optic atrophy	spherophakia retinal degeneration optic disc abnormality	megalocornea spherophakia closed angle
Metacarpophalangeal index (right/left)	7.7/7.7	8.2/8.1	8.3/8.3	7.8/7.9
Diameter of aorta sinus (cm)	22	20	26	19

Abbreviations: OD, right eye; OS, left eye; EL, ectopia lentis; BCVA, best corrected visual acuity; RNFL, retinal nerve fiber layer; simK, average simulated corneal keratometry reading. * Spherical equivalent; # Under medication control; -, Negative; +, Positive.

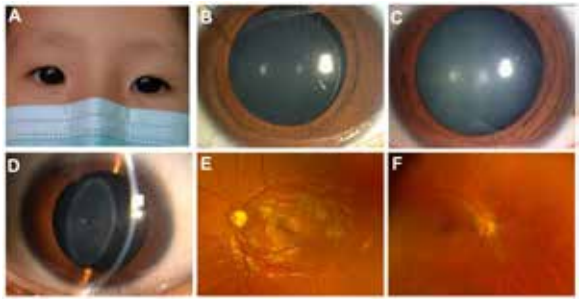

Figure 2. Representative ocular clinical manifestations and clinical examinations of patients with LTBP2 mutations. A: The eye appearance of Patient ID431 reveals a megalocornea feature. B: The slit-lamp photography of Patient ID431 illustrating an ectopia lentis (EL). C: EL, coloboma lentis, and persistent pupillary membrane of Patient ID382. D: Anterior segment photography of Patient ID294 depicts spherophakia and segmental iris atrophy (right eye). E: A fundus examination of patient ID382 shows a high cup/disc ratio; 0.95 in the left eye. F: Fundus photography of Patient ID382 hints of optic disk abnormality.

months). None of the pedigrees was found to have systemic illnesses during the follow-up.

Genetic findings: In the current study, eight novel LTBP2 variants were detected in CEL patients from four unrelated Chinese families using WES (Table 2 and Appendix 1). All were negative for FBN1 or ADAMTSL4 mutations. Among them, three patients had compound heterozygous mutations, and the other had a homozygous deletion mutation. The novel variants, including missense (3/8), splicing (2/8), frameshift (1/8), and nonsense mutations (2/8), were distributed in the exon or exon–intron boundaries of LTBP2.

Patient ID 228 and her sibling (ID 229) carried three of the same heterozygous mutations, including c.4370–7_4370–9delTCT, c.4370–5C>G, and c.3452G>A. Their parents did not present signs of lens dislocation and glaucoma and had passed away; therefore, the parents did not take the WES test. Therefore, we could not determine the source of these three mutations (Appendix 2). The only homozygous mutation of LTBP2 found in our study was c.2253delG (p.R751fs) in exon 12, which causes amino acid 751–829 substitutions and premature translation termination. This frameshift mutation was detected in patient ID 294, whose parents were in a consanguineous marriage. In addition, we found that this patient with a frameshift mutation had more severe ocular phenotypes than the other three probands, which was similar to previous reports (Table 3 and Appendix 3), suggesting that frameshift mutations of LTBP2 would result in more varied and severe ocular phenotypes. Additionally, the nonsense mutation c.1251G>A (p.W417X) in exon 6 was detected

Table 2. Novel *LTBP2* mutations identified in CEL patients in this study.

Position at Chr	Exon	cDNA Change	Amino acid Change	Mutation type	Mutation Taster	SIFT	Polyphen2	BDGP	NetGene2
14	Exon 30	c.4370-7_4370-9delTCT	-	Splicing	-	-	-	LAS	LAS
14	Exon 30	c.4370-5C>G	-	Splicing	-	-	-	LAS	LAS
14	Exon 23	c.3452G>A	p.C1151Y	Missense	Disease causing	Damaging	Probably damaging	-	-
14	Exon 12	c.2253delG	p.R751fs	Frame-shift	-	-	-	-	-
14	Exon 28	c.4114T>C	p.C1372R	Missense	Disease causing	Damaging	Probably damaging	-	-
14	Exon 6	c.1251G>A	p.W417X	Nonsense	Disease causing	-	-	-	-
14	Exon 33	c.4760G>A	p.W1587X	Nonsense	Disease causing	-	-	-	-
14	Exon 3	c.620G>A	p.C207Y	Missense	Disease causing	Damaging	Probably damaging	-	-

LAS, loss of acceptor site.

Table 3. Summary of clinical features among patients with *LTBP2* frameshift mutations.

Mutation type	DNA location	cDNA change	Clinical features
Frameshift mutation	Exon 1	c.412delG	PCG
	Exon 4	c.1012delT	Megalocornea, lens dislocation, secondary glaucoma
	Exon 6	c.1243-1256 del114	PCG, megalocornea, corneal haze, lens dislocation
	Exon 7	c.1415delC	PCG, megalocornea, corneal haze, lens dislocation
	Exon 12	c.2253delG*	Megalocornea, corneal haze, spherophakia, lens dislocation, closed angle glaucoma, optic atrophy
	Exon 23	c.3427delC	PCG, buphthalmia, high C/D ratio
	Exon 24	c.3542delT	High myopia, lens dislocation, microspherophakia, secondary glaucoma, nystagmus
	Exon 25	c.3672delC	
	Exon 36	c.5376delC	Lens dislocation
	Exon 9	c.1796_1797insC	Spherophakia, lens dislocation, megalocornea, axial myopia, retinal detachment and high IOP (4-5 years after onset)
	Exon 24	c.3614_3618dupCTGGC	Microspherophakia, lens dislocation, secondary glaucoma, optic disc atrophy, high C/D ratio
	Exon 27	c.4031_4032insA	Lens dislocation, myopia, glaucoma, high C/D ratio
	Exon 36	c.5439_5440insA	Microspherophakia, nuclear cataract, high myopia
	Exon 36	c.5446dupC	Microspherophakia, lens dislocation, secondary glaucoma

* Novel mutation in current study. **Abbreviations:** PCG, primary congenital ectopia; C/D ratio, cup/disc ratio; IOP, intraocular pressure.

in patient ID 382. Patient ID 431 also carried compound heterozygous mutations, the missense mutation c.620G>A (p.C207Y) in exon 3, which was the first mutated site identified on this exon, and the nonsense mutation c.4760G>A (p.W1587X) in exon 33. Two nonsense mutations were in this cohort, c.1251G>A (p.W417X) and c.620G>A (p.C207Y); these caused stop codes and generated truncated proteins.

All six exonic mutated amino acids in the identified pathogenic variants were highly conserved in the homologs of LTBP2 in other species (Appendix 2). Two of the novel mutants were in the calcium-binding epidermal growth factor-like domain (EGF–CA), two were in the epidermal growth factor-like domain (EGF-like), one was in the transforming growth factor beta (TGFβ)-binding protein-like domain (TB), and one was outside the important domains but caused truncated proteins. The details of the novel causative mutations identified in LTBP2 that are present with CEL are summarized in Table 2 and in Figure 3.

In-silico, 3D protein modeling (Figure 4) was used to present the potential pathogenic effects of partial exonic variants on the structures and functions of LTBP2 proteins. It was indicated that p.C1151Y (Patient ID 228 and 229), p.C1372R (Patient ID 382), and p.C207Y (Patient ID 431) are expected to result in broken disulfide bonds and the substitution of macrocyclic aromatic side chains for the cysteineamino acids. Without the covalent interactions, protein stability decreased. Both p.W417X (ID 382) and p.W1587X (ID 431) showed a large piece of truncated protein.

DISCUSSION

LTBP2 mutations are widely reported to cause PCG; however, LTBP2 has rarely been mentioned as a pathogenic gene for CEL. To date, only 32 LTBP2 mutations have been identified as causing congenital ocular disease (Appendix 3), and only eight have been reported in the Chinese population. In this study, eight novel mutations of the LTBP2 gene were detected in 4 out of 145 Chinese pedigrees with CEL. More strikingly, a variety of ocular phenotypic presentations were observed in the four probands, including a megalocornea, angle closure, ectopia lentis, spherophakia, long axial, retinal degeneration, high myopia, and glaucoma. This study provides further insights into genetic defects and LTBP2-related phenotypes in CEL.

In the current study, the percentage of CEL patients with an LTBP2 mutation was 2.76%. Similarly, another study of the genetic testing of CEL patients found that the percentage of patients with LTBP2 mutations was 1.71% (3/175) [17], which was relatively close to our result. As previous studies reported [1,2,17], FBN1 mutations, which mainly cause MFS, account for a large portion of mutations in CEL patients (approximately 70%–83.43%). Meanwhile, other gene mutations only cover a small portion of that, such

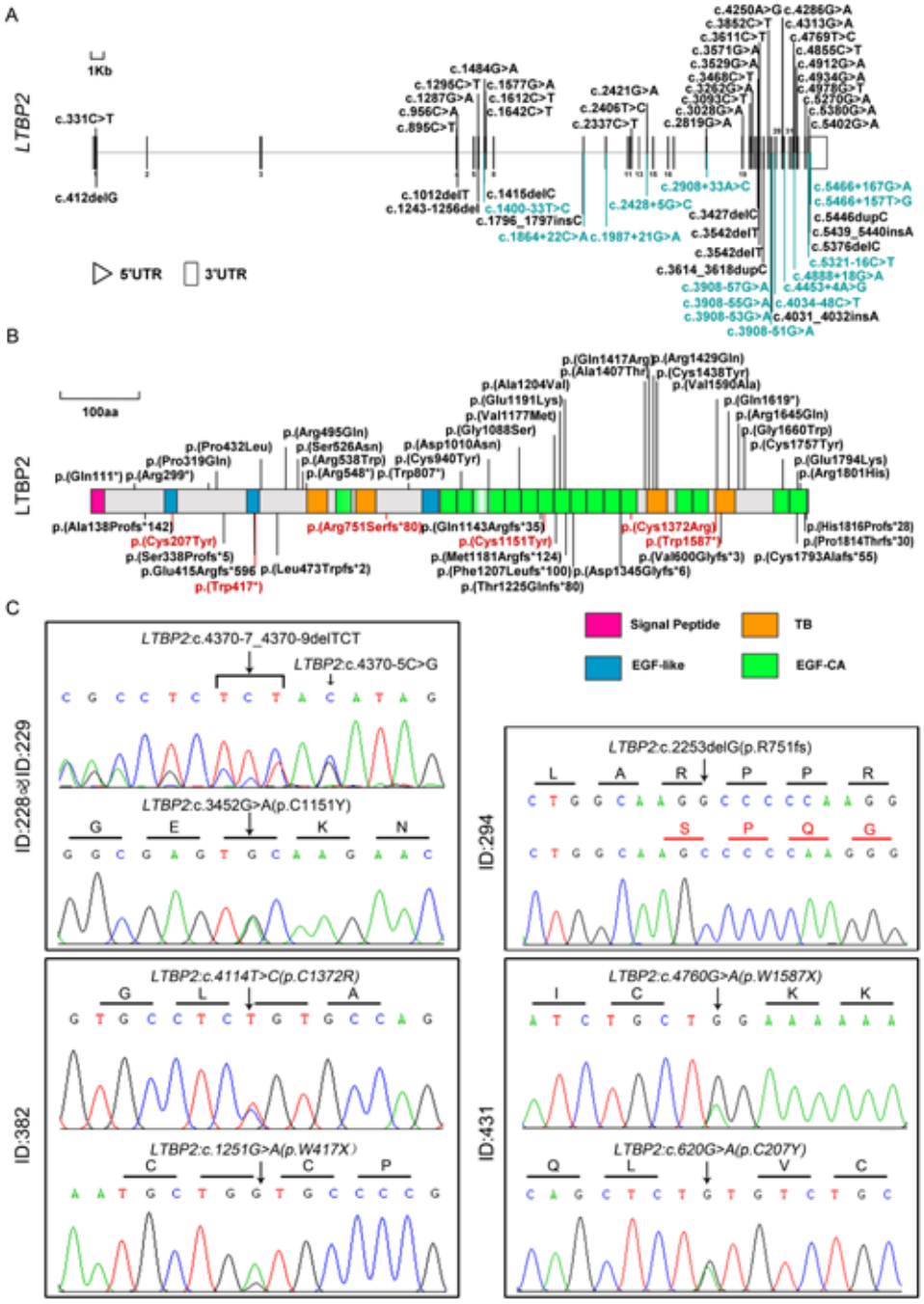

Figure 3. Schematic representation of LTBP2 genetic analysis. A: A diagrammatic representation of the LTBP2 gene structure is shown. Partial exon numbers are displayed in the scheme. Variants in previous reports are depicted in black (except for the intronic variants in blue), and the novel variants identified in this study are in red. B: The position of LTBP2 mutations identified in the study and in previous reports along the protein structure. The novel mutations detected in our study are characterized in red. The domains in the protein are also portrayed. SP: Signal peptide; TB: Transforming growth factor beta binding protein-like motif; EGF-like: Epidermal growth factor-like domains; and EGF-CA: Epidermal growth factor-like domains with calcium binding motifs. C: Sanger sequencing in four families of probands carrying novel LTBP2 mutations. Black arrows indicate the mutation loci.

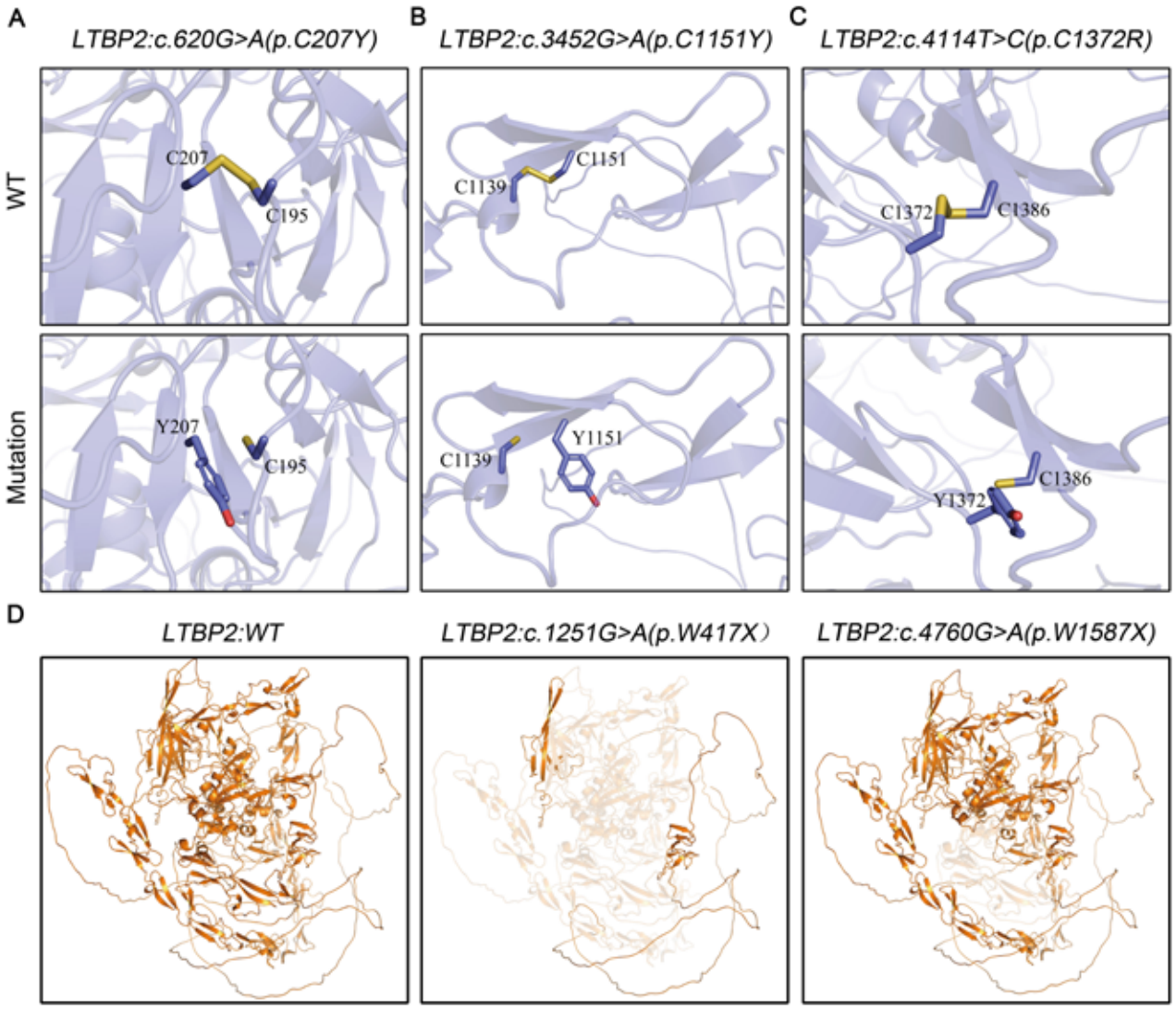

Figure 4. Predicted protein effect of LTBP2 variants on structure–function properties. A–C (first column) show the wild-type in p.C207, p.C1151, and p.C1372. A–C (second column) correspond to the variants of the wild-type described above. The disulfide bond is indicated by the yellow line. The aromatic nucleus is blue. D indicates the predicted overall protein structure of wild-type, p.W417X, and p.W1587X in brown. The transparent part indicates large fragment truncation.

as ADAMTSL4 (3.43%–3.94%), CBS (2.29%), ADAMTS17 (2.16%), CPAMD8 (1.71%), and COL4A5 (0.57%). In different ethnic groups, the distribution of gene mutations in CEL may be different, and in remote rural areas, a consanguineous marriage may increase the probability of LTBP2-related CEL.

In this study, patients with novel mutations in LTBP2 had variable and complicated ocular manifestations from the anterior segment to the fundus. In addition, 50% of the patients with LTBP2 mutations had megalocornea, a clinical feature commonly reported in patients with LTBP2 mutations [16]. Previous reports have suggested that LTBP2 mutations typically result in spherophakia with an increased lens thickness (4.06–6.75 mm) [18,19]. Spherophakia also occurred in three of our four probands. Furthermore, the RNFL was markedly thin in both patients with glaucoma, which has been similarly reported in juvenile glaucoma [20]. Evidence also illustrated that, in feline congenital glaucoma (FCG) caused by the LTBP2 mutation, optic nerve axon counts were significantly lower [21,22]. Furthermore, the second proband's (ID 294) optic nerves had significantly atrophied, which was rarely mentioned in previous studies. This might be because previous LTBP2 mutations were mainly detected and reported in infants and younger children with congenital glaucoma whose optic nerve and retinal damage had not fully

developed to reveal manifestations. These findings indicate that LTBP2 plays a critical role in retina development, and patients should be informed of the risk of fundus impairment.

Moreover, LTBP2 mutations could result in distinctive clinical characteristics in CEL patients. Our previous studies have reported that pathogenic mutations in CEL, including FBN1, ADAMTSL4, and LTBP2, were associated with distinct clinical manifestations in CEL patients. We found that CEL patients with FBN1 mutations usually presented with a long axial, high corneal astigmatism, flat cornea, cardiovascular abnormalities, and skeletal malformations [1]. However, patients with ADAMTSL4 mutations usually showed isolated lens dislocation without extraocular abnormalities, and ocular phenotypes include a long axial, ectopic pupil, pupil residual membrane, and congenital cataract [2]. In contrast, LTBP2 mutations were associated with more complex ocular phenotypes such as a megalocornea, corneal haze, angle closure, spherophakia, high myopia, glaucoma, thinning RNFL, and optic nerve atrophy or optic disk dysplasia. Hence, the LTBP2 mutation should be highly suspected when a large cornea and spherical lens are observed in a CEL patient. The IOP should be monitored regularly to prevent the occurrence and progression of glaucoma. In this study, we did not find systemic diseases in patients with LTBP2 mutations, which was consistent with previous studies concerning Chinese patients with LTBP2 mutations [23,24]. However, LTBP2 has been found to cause WMS in Iranians and Saudi Arabians. In addition to ocular disease, they presented marked short stature, brachydactyly, and joint stiffness. This suggests that there may be ethnic disparities in the clinical manifestations caused by LTBP2 mutations.

In this study, we found that frameshift mutations may cause more complicated ocular symptoms. In two reports of Chinese patients affected by LTBP2 frameshift mutations, the patients had ectopia lentis, spherophakia, low vision, high myopia, and secondary glaucoma [23,25]. Similarly, the ocular phenotype was relatively more severe in our pediatric patient (ID 294) with the novel homozygous LTBP2 frameshift mutation c.2253delG (p.R751fs). She had difficult-to-control secondary glaucoma and rapidly progressing retinal damage, with a C/D ratio of 0.95/0.96 (OD/OS), optic atrophy, and thinning of the retina. She also had a megalocornea, angle closure, ectopia lentis, spherophakia, and high myopia, which were similar to previous studies [8,19]. In particular, systemic manifestations were also rarely mentioned in frameshift mutations of LTBP2, while missense or nonsense mutations have occasionally been reported in previous studies (Appendix 3**)**. Frameshift mutations usually cause a series of code changes downstream and produce truncated proteins, which may account for more complex ocular phenotypes. However, due to the few reported frameshift mutations in the Chinese population, a larger sample size study with phenotypic and mechanism validation is required.

Mutations in the other three subtypes (LTBP1/3/4) rarely cause ocular disease [26–28], suggesting the importance of the LTBP2 gene for maintaining ocular development. Thus, it is crucial to elucidate the function of the LTBP2 gene and the impact of its mutations. FBN1 and LTBP2 encode the two major components of zonules [29]. LTBPs are microfibril-associated proteins essential for sequestering TGF-β in the ECM and for the proper assembly of ECM components [30]. The protein prediction model of this study indicates that the variation disrupts the disulfide bond of the LTBP2 protein, reduces protein stability, and may thus disrupt the anchoring of microfibrils. A misaligned microfibril structure may affect the level of TGF-β in the ECM [31,32]. In the study of MFS aortic aneurysms, TGF-β was activated in the aortic tissue due to the FBN1 mutation, increasing the expression of matrix metalloproteinases (MMPs), which in turn affected the degradation and remodeling of aorta tissue [33,34]. This mechanism may also underlie the degradation of the zonules and the eventual occurrence of CEL caused by the LTBP2 mutation. Furthermore, spherophakia may be caused by a lack of tension in weak or ruptured zonule fibers. This weakening can hinder the development of the lens and keep the lens spherical instead of a normal double convex shape [35].

The strength of this study is that we identified eight novel LTBP2 mutations, reported LTBP2-related phenotypes in a Chinese CEL cohort, and elaborated on the distinction of clinical phenotypes in CEL pathogenic genes. Several limitations should be emphasized. First, the sample size was not large enough, as CEL is a rare disease. Second, despite the absence of a syndromic disorder during the follow-up period, there remains a possibility that systemic symptoms have not fully developed in the young probands. Therefore, further studies with longer follow-ups and larger samples are needed.

In conclusion, this study was based on the whole-exome sequencing of 145 CEL patients and detected eight novel mutations in the LTBP2 gene among four Chinese families. The results summarized the discriminative clinical features of LTBP2-related CEL in the Chinese population. We proposed that the biallelic homozygous frameshift mutation of LTBP2 may be associated with a more severe ocular phenotype, and retinal damage should be noticed in these patients.

APPENDIX 1.

To access the data, click or select the words "Appendix 1." Schematic pedigrees of families with causative LTBP2 mutations. Black arrows indicate probands affected by CEL. Open symbols: Clinically unaffected patients; Squares: Males; Circles: Females; Half-filled shaded areas: Heterozygous individuals; Solid symbols with both halves filled: Compound heterozygous or homozygous figures.

APPENDIX 2.

To access the data, click or select the words "Appendix 2." The amino acid sequence alignments of the LTBP2 gene in five species where novel mutations were located. The results of the amino acid sequences of LTBP2 from five species reported in the NCBI database are shown: Homo sapiens, Macaca mulatta, Bos taurus, Mus musculus, and Rattus norvegicus. Amino acid residues of C207, W417, R751, C1151, C1732, and W1587 in humans are indicated.

APPENDIX 3.

To access the data, click or select the words "Appendix 3." Summary of Reported LTBP2 Mutations in Patients with Congenital Ocular Disease

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