

Mutations in crystallin genes result in congenital cataract associated with other ocular abnormalities

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Purpose: This study aims to describe the phenotypes and identify pathogenic mutations in Chinese patients who have congenital cataracts associated with other ocular abnormalities.

Methods: Eleven patients from four unrelated Chinese families plus two simplex cases were enrolled in this study. Detailed ophthalmic examinations were performed. DNA samples were isolated from peripheral blood collected from the patients. Next-generation sequencing of known ocular genes was applied to the proband of each family and two simplex cases to find pathogenic variances. PCR and Sanger sequencing were conducted for validation and segregation tests.

Results: All 13 patients had congenital cataracts, and other ocular abnormalities were found in some cases. Microcornea was found in 12 subjects, and ocular coloboma was observed in five. Various types of coloboma, including iris, choroid, macular, and optic disc, were described. Five mutations in crystallin genes were identified. Four of the mutations are novel: *CRYBB1*: p.(Arg230Cys), *CRYBB2*: p.(Gly149Val), *CRYGC*: p.(Met44CysfsTer59), and *CRYGC*: p.(Tyr144Ter). One mutation was reported previously: *CRYAA*: p.(Arg21Trp).

Conclusions: We examined a cohort of Chinese patients with congenital cataracts and studied the phenotypes and genotypes. Extralenticular abnormalities, such as microcornea and ocular coloboma, can also be found in patients with congenital cataracts. The phenotype of congenital cataracts associated with macular and optic disc coloboma was reported for the first time in this study. Four novel mutations and one previously reported mutation were identified. These data expand the mutation spectrum in crystallin genes and enhance our understanding of the phenotypes of congenital cataracts.

Congenital cataracts are a leading cause of childhood blindness with a prevalence of 0.63 to 9.74/10,000 around the world [1]. In China, the estimated prevalence is 4.24/10,000 [2]. Up to 25% of congenital cataracts are considered to be inherited. The most frequent mode of inheritance is autosomal dominant, but X-linked and autosomal recessive transmission modes have also been reported [3]. Congenital cataracts may occur alone (which accounts for approximately 70% of congenital cataract cases) or be accompanied by other ocular abnormalities (which accounts for approximately 15% of congenital cataracts), such as microcornea, microphthalmia, ocular coloboma, aniridia, retinal degeneration, and so on. In another 15% of cases, cataracts are one part of a multisystem genetic disorder [4].

To date, 45 genetic loci and 38 specific genes have been reported to be linked with non-syndromic congenital cataracts. These genes include lens-related crystallin, connexin, cytoskeleton-related genes, transcription factors, and a variety of other genes. Mutations in crystallin genes account for the majority of hereditary congenital cataracts

[5]. Because more than 40 loci can lead to congenital cataracts, high-throughput sequencing is an efficient method for detecting pathogenic genes and mutations. Next-generation sequencing (NGS) can save time and money, as well as offer adequate genetic information. We have successfully used NGS to investigate hereditary retinal diseases, such as retinitis pigmentosa [6], Leber congenital amaurosis [7], and Usher syndrome [8]. Therefore, we used NGS with targeted exon capture to explore the genetic defects in patients with congenital cataracts in this research study.

METHODS

Clinical evaluation: Eleven patients from four unrelated Chinese families and two simplex cases [8 males, 5 females; mean age \pm standard error of the mean (SEM): 40.4 \pm 15.6 years, range: 21–72 years] were recruited from the clinic at the Department of Ophthalmology at Peking Union Medical College Hospital (PUMCH). Ophthalmic examinations, including best-corrected visual acuity (BCVA), intra-ocular pressure (IOP), slit-lamp biomicroscopy, and indirect ophthalmoscopy were performed. Photographs of the anterior segment and fundus were taken if possible. Four patients underwent B-ultrasonography examination. The study adhered to the ARVO statement on human subjects and was

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approved by the Institutional Review Board of PUMCH. All research subjects provided written informed consent.

Genomic DNA preparation: Peripheral blood from all patients and any available unaffected family members (including patient II:3, patient II:8, and patient III:4 from family A and patient I:2 and patient II:3 from family D) was collected. 5 ml peripheral blood was drawn from elbow vein of each subjects and was preserved at 4 °C prior to use. Genomic DNA was extracted from peripheral leukocytes using a commercial kit (QIAamp Blood Midi Kit; Qiagen, Hilden, Germany) according to the manufacturer's protocol. The brief procedures were as follows: 200 µl QIAGEN Protease, 2 ml blood sample and 2.4 ml Buffer AL were mixed then incubated at 70 °C for 10 min. 2 ml 100% ethanol was added then the mixture was added into the QIAamp Midi spin column and centrifuged at 1207 xg for 3 min. 2 ml Buffer AW1 was added into column then centrifuged at 2717 xg for 1 min and 2 ml Buffer AW2 was added then centrifuged at 2717 xg for 15 min. 300 µl Buffer AE was added into the column, incubated at room temperature for 5 min, then centrifuged at 2717 xg for 2 min. The eluate was reloaded into the column and the procedure was repeated then DNA was got.

Library preparation and targeted sequencing: For sequencing, 1 µg of the DNA sample from the proband of each family was sheared into fragments that were 200–500 bp long. The sheared fragments received blunt-end repair, and Klenow exonuclease was used to add a single-adenine base to the 3' ends. Then, adapters (Illumina, San Diego, CA) were ligated to the repaired ends, and the DNA fragments were amplified in a PCR after ligation. PCR conditions were: denaturing at 98 °C for 2 min followed by 8 cycles of 98 °C for 30 s, 65 °C for 30 s and 72 °C for 1 min, then a final extension step at 72 °C for 10 min. The targeted DNA was captured using a customized panel of 762 genes, which included all the known genes related to eye diseases and sequenced by the Illumina HiSeq X Ten machine [9]. All the genes we detected are listed in Appendix 1.

Bioinformatics analysis: Sequencing data were analyzed with NextGene V2.3.4 software (Softgenetics, State College, PA), and the reads were compared to the reference sequence of hg19 from the University of California Santa Cruz (UCSC) Genome Browser. A comparison was conducted in the [1000 Genomes Project](#) database, the Single Nucleotide Polymorphism database ([dbSNP](#)), and the Exome Aggregation Consortium database ([ExAC](#)) to exclude nonpathogenic polymorphisms. [Polyphen2](#), [Sorting Intolerant From Tolerant \(SIFT\)](#), and [Mutation Taster](#) were used to predict damaging missense mutations. We also analyzed the results in the Human Gene Mutation Database (HGMD) to confirm

whether the pathogenic mutations we found had been reported before. The [ProtScale](#) program was used to predict protein hydrophobicity.

PCR and Sanger sequencing: PCR and Sanger sequencing were conducted for all patients and available unaffected subjects to determine segregation. The primer sequences are listed in Appendix 2. The final volume of 50 µl for each reaction contained 25 µl 2× Taq PCR master mix, 21 µl double-distilled water (ddH₂O), 40 ng genomic DNA, and 10 pmol of primer. PCR reactions were performed with denaturing at 94 °C for 5 min, followed by 30 cycles 94 °C for 30 s, 60 °C for 30 s and 72 °C for 45 s. A final extension step at 72 °C was performed for 7 min. After purification, the amplicons were sequenced using forward primers. The sequences were assembled and analyzed using Lasergene SeqMan software (DNASTAR, Madison, WI) and were compared to reference sequences.

RESULTS

Clinical findings: We investigated 11 patients with congenital cataracts from four Chinese families and some of their unaffected family members, as well as two simplex cases. Bilateral cataracts were revealed in 11 patients, and unilateral cataracts were found in the other two. Extralenticular abnormalities can also be found in patients with congenital cataracts. Microcornea, which occurred in 12 of 13 patients, was the most frequent concomitant disorder. Ocular colobomas involving different structures, including the iris, choroid, macular, and optic disc, were found in five subjects. The concise clinical findings for the patients are displayed in Table 1.

In family A, patient II:2 developed disciform and granular pacification in the center and inferior region of the lens nucleus. A fundus photograph revealed optic disc and macular colobomas. We could see an enlarged optic disc with a bowl-shaped excavation in the center and preserved neural tissue in the surrounding area for both eyes. Additionally, there was a round coloboma of the choroid in the macular region with some irregular pigments for the right eye (Figure 2). Microcornea was found in all patients in family A, and nystagmus and blepharoptosis were observed for patient II:2 and patient III:1.

In family B, the affected members were diagnosed with congenital cataracts, microcornea, and posterior segment coloboma. Due to the invisible fundus, we used B-ultrasonography to assess the posterior segment of the eye. B-ultrasonography exhibited a focal and irregular introcession in the eyeball wall for both eyes of patient II:2 and the right eye of patient III:1, which probably conformed to posterior segment coloboma (Figure 3). Patient II:2 developed glaucoma with

TABLE 1. CLINICAL FEATURES AND MUTATIONS OF PATIENTS.

Patient	Eye	BCVA	Microcornea (corneal diameter)	Lens	Coloboma	Other ocular abnormality	Mutation	Genetic testing method
Family A I:2	OD OS	NA	Yes	Cataract	No	No	CRYAA p.(Arg21Trp)	Sanger
Family A II:2	OD	0.1+	Yes (9mm)	Aphakia	optic disc and macular coloboma	nystagmus and blepharoptosis	CRYAA p.(Arg21Trp)	Sanger
Family A II:4	OS	0.08	Yes	Cataract	optic disc coloboma	No	CRYAA p.(Arg21Trp)	Sanger
Family A III:2	OD OS	0.02 0.08	Yes (9mm)	Aphakia	No	nystagmus and blepharoptosis	CRYAA p.(Arg21Trp)	NGS
Family B II:2	OD OS	HM 0.03	Yes (8.5mm)	Cataract	posterior segment coloboma	high intraocular pressure of 29 mmHg high intraocular pressure of 53 mmHg	CRYBB2 p.(Gly149Val)	Sanger
Family B III:1	OD	0.1	Yes(9.5mm)	Cataract	posterior segment coloboma	No	CRYBB2 p.(Gly149Val)	NGS
Family C I:1	OS OD	HM NA	Yes	Aphakia Aphakia	No No	corneal degeneration and retinal detachment	CRYGC p.(Tyr144Ter)	Sanger
Family C II:1	OD OS	NA	Yes	Aphakia	No	high intraocular pressure of 34 mmHg high intraocular pressure of 39 mmHg	CRYGC p.(Tyr144Ter)	NGS
Family D I:1	OD OS	0.2 NLP	Yes	pseudophakia	No	No	CRYGC p.(Met44CysfsTer59)	Sanger
Family D II:1	OD OS FC	0.15 0.3 0.1	Yes (6.5 mm) Yes (7 mm)	pseudophakia pseudophakia	No No	retinal detachment	CRYGC p.(Met44CysfsTer59)	NGS
Family D II:5	OD OS	0.3 0.1	Yes No	pseudophakia	No	No	CRYGC p.(Met44CysfsTer59)	Sanger
Simplex case 1	OD	0.1+	No	Cataract	inferior iris and chorioid coloboma	No	CRYBB1 p.(Arg230Cys)	NGS
Simplex case 2	OS OD OS	0.1 HM 0.4	Yes No	Normal Cataract Normal	optic disc coloboma No	No	CRYGC p.(Arg48His)	NGS

NA, not available; OD, right eye; OS, left eye ; HM, hand motion; NLP, no light perception; FC, finger count.

an intraocular pressure of 29 mmHg for the right eye and 53 mmHg for the left eye.

All patients from family C and family D had congenital cataracts and microcornea. Complications of cataract extraction surgery, such as retinal detachment and corneal degeneration, were observed in two patients. Patient II:1 from family C had bilateral glaucoma with an intraocular pressure of 34 mmHg for the right eye and 39 mmHg for the left eye. Unilateral mild to moderate cataracts and significantly ocular coloboma were revealed in two simplex cases. Simplex case 1 had an inferior iris and choroid coloboma for both eyes. The appearance of coloboma was observed in the right eye of simplex case 2 (Figure 4). We could see an enlarged, vertically oval, and excavated optic disc of the right eye, which meant there was an optic disc coloboma. Two focal irregular-shaped colobomas of the retina and the choroid were also observed near the vascular arcade. The retinal vessels in the posterior pole were disordered, and a normal macular structure was not observed. No other ocular abnormalities were found in the left eye of simplex case 2, except a relatively small and crowded optic disc.

Mutation analysis: Using next-generation sequencing, we identified five mutations in crystallin genes, including one nonsense mutation, one frame-shift mutation, and three missense mutations that were predicted by three software programs to be pathogenic. These mutations included four novel mutations: *CRYBB2* (ID: 1415, OMIM: 123620): c.446G>T, p.(Gly149Val); *CRYBB1* (ID: 1414, OMIM: 600929): c.688C>T, p.(Arg230Cys); *CRYGC* (ID: 1420, OMIM: 123680): c.432C>G, p.(Tyr144Ter); and *CRYGC*: c.130delA, p.(Met44CysfsTer59). We also identified one previously reported mutation: *CRYAA* (ID: 1409, OMIM: 123580): c.61C>T, p.(Arg21Trp). We found a variance in the *CRYGC* gene: c.G143A, p.(Arg48His), which was considered to be an SNP in the dbSNP. However, the variance was thought to be a pathogenic mutation in two previous studies [10,11]. The variances and the results of the predictive programs are listed in Table 2. We confirmed that the affected family members carrying the same mutations and the unaffected family members are wild-types using Sanger sequencing (Figure 1). Sequence tracings of the proband of each family are shown in Figure 5. We also detected several variants for other genes associated with eye disease. Detailed information about these mutations is listed in Appendix 3. We

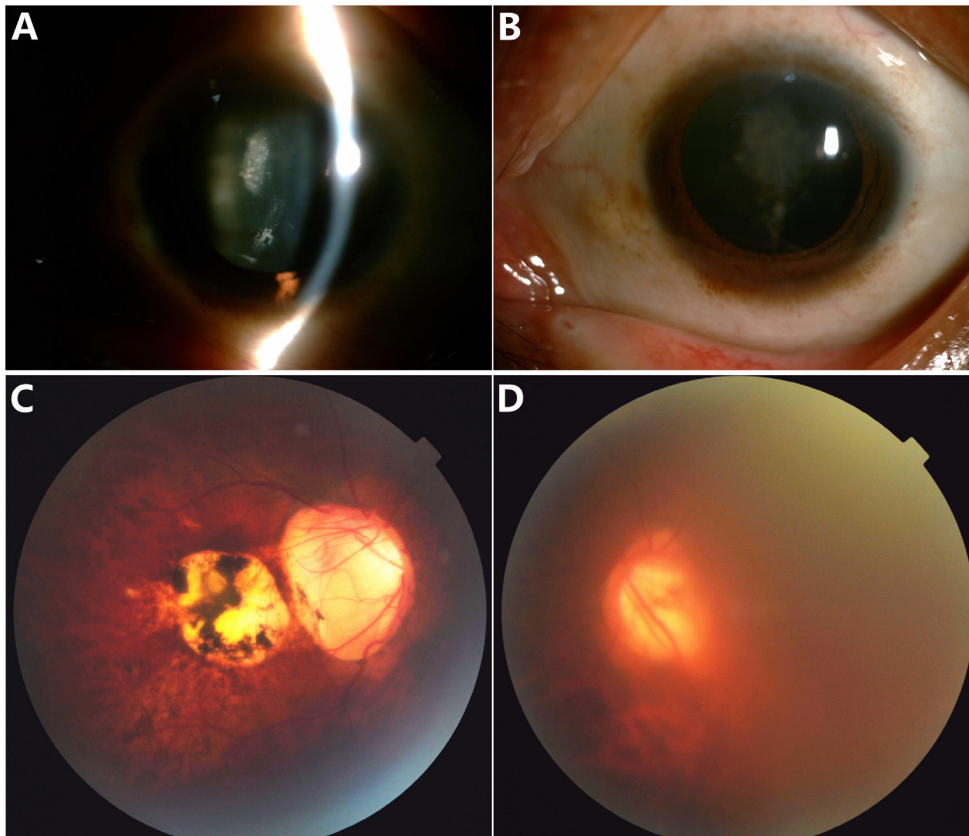


Figure 2. Ocular photographs of patient II:1 from family A. A and B: Photographs of the anterior segment of the left eye, showing a congenital nuclear cataract. C and D: Fundus photographs of the right and left eyes, respectively, showing optic disc coloboma for both eyes and macular coloboma for the right eye.

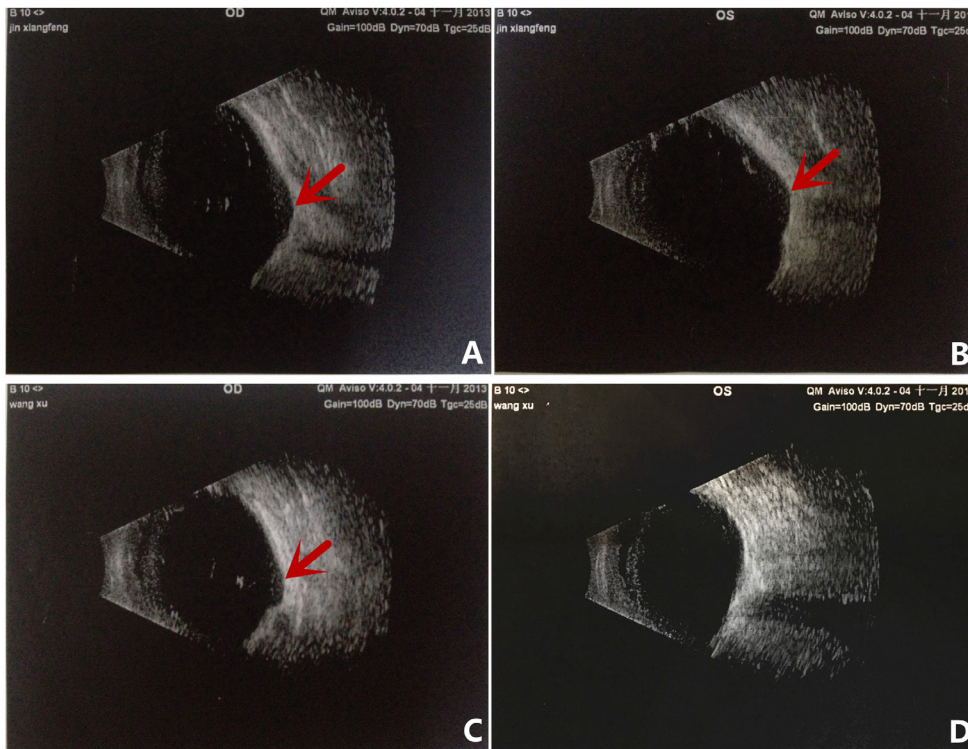


Figure 3. B-ultrasonography images of patients from family B. **A** and **B**: Images of the right and left eyes, respectively, of patient II:2. **C** and **D**: Images of the right and left eyes, respectively, of patient III:1. The B-ultrasonography images show posterior segment coloboma for both eyes of patient II:2 and the right eye of patient III:1. Focal and irregular introcessions in the eyeball wall are marked with red arrows.

used the ProtScale program to predict the protein hydrophobicity for these mutants in the study. The prediction results of the ProtScale program showed that the two missense mutations (*CRYBB2*: p.(Gly149Val) and *CRYBB1*: p.(Arg230Cys)) have substantially higher hydrophobicity compared to the wild-type genes (Appendix 4).

DISCUSSION

In this study, we summarized a cohort of Chinese patients with congenital cataracts and studied the phenotypes and genotypes. To find the underlying genetic defects, NGS was

applied to search for pathogenic variations in known pathogenic genes.

The phenotypes of the patients were relatively complicated because other ocular abnormalities, such as microcornea and ocular coloboma, were observed. Microcornea is possibly caused by an arrest in growth of the cornea after the fifth gestational month [12] and is not rare in patients with congenital cataracts with mutations in crystallin genes [13-21]. Coloboma is an ocular abnormality that can affect many structures, such as the iris, choroid, retina, and optic nerve. Typical colobomas result from defective closure of the fetal optic fissure and are located in the inferior and

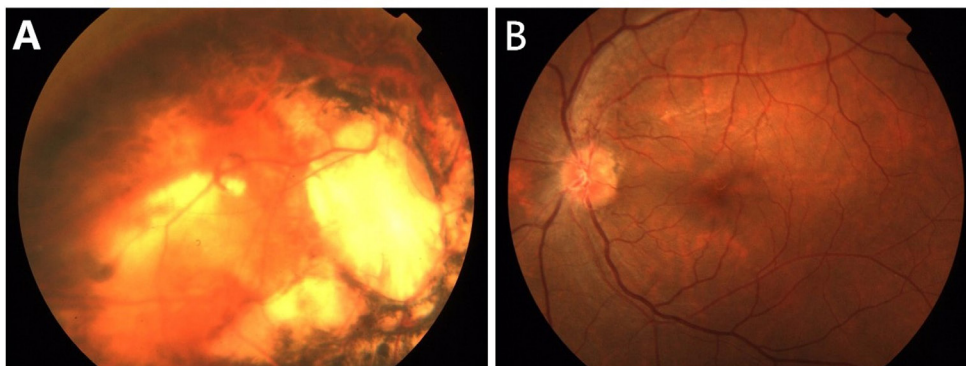


Figure 4. Fundus photographs of simplex case 2. **A**: Right eye. **B**: Left eye. The fundus photographs of simplex case 2 show optic disc coloboma for the right eye and a relatively normal phenotype for the left eye.

TABLE 2. MUTATIONS IDENTIFIED IN OUR STUDY AND RESULTS OF PREDICTIVE PROGRAMS.

Patient	Gene	Base change	Amino acid change	Allele		Polyphen2	SIFT	Mutation Taster
				Frequency(ExAC)	Frequency			
Family A	CRYAA	c.61C>T	p.(Arg21Trp)	—	—	Probably damaging	Affect protein function	Disease causing
Family B	CRYBB2	c.446G>T	p.(Gly149Val)	—	—	Probably damaging	Affect protein function	Disease causing
Family C	CRYGC	c.432C>G	p.(Tyr144Ter)	—	—	—	—	—
Family D	CRYGC	c.130delA	p.(Met44CysfsTer59)	—	—	—	—	—
Simplex 1	CRYBB1	c.688C>T	p.(Arg230Cys)	—	8.29E-06	Probably damaging	Affect protein function	Disease causing
Simplex 2	CRYGC	c.143G>A	p.(Arg48His)	—	0.01742	Benign	Tolerated	Disease causing

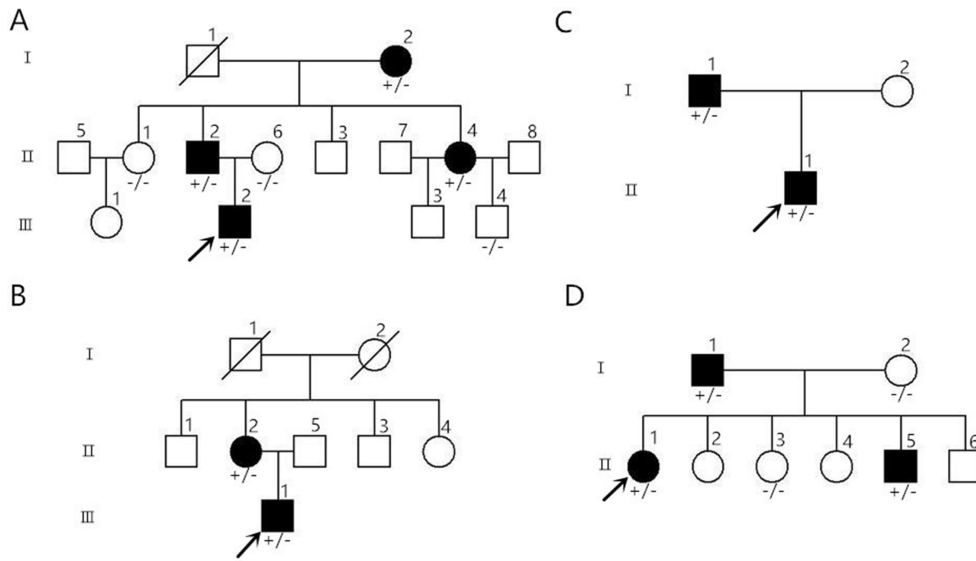


Figure 1. Pedigrees of four Chinese families. A–D: Pedigrees of family A through family D, respectively. Squares and circles indicate men and women, respectively. Filled and empty symbols indicate affected and unaffected members, respectively. Deceased individuals are indicated with slashes. Probands are indicated with arrows.

infranasal areas of the globe. Atypical coloboma is located elsewhere and has an unclear mechanism [22,23]. We can infer that defective closure of the optic fissure may not to be the reason colobomas arose in these patients because only one of the patients had typical coloboma, whereas the other four were atypical. Apart from colobomas, two patients had glaucoma. Patient II:1 from family C developed high IOP after cataract surgery, so we think the high intraocular pressure is

a complication of cataract surgery. Patient II:2 from family B had had poor vision since childhood and occasional mild distending pain in both eyes since she turned 50 years old, but she did not visit a doctor until 2013. Therefore, we did not know exactly when her IOP increased, and the type of glaucoma could not be confirmed. Either primary open angle glaucoma (POAG) or high IOP related to congenital cataracts is possible.

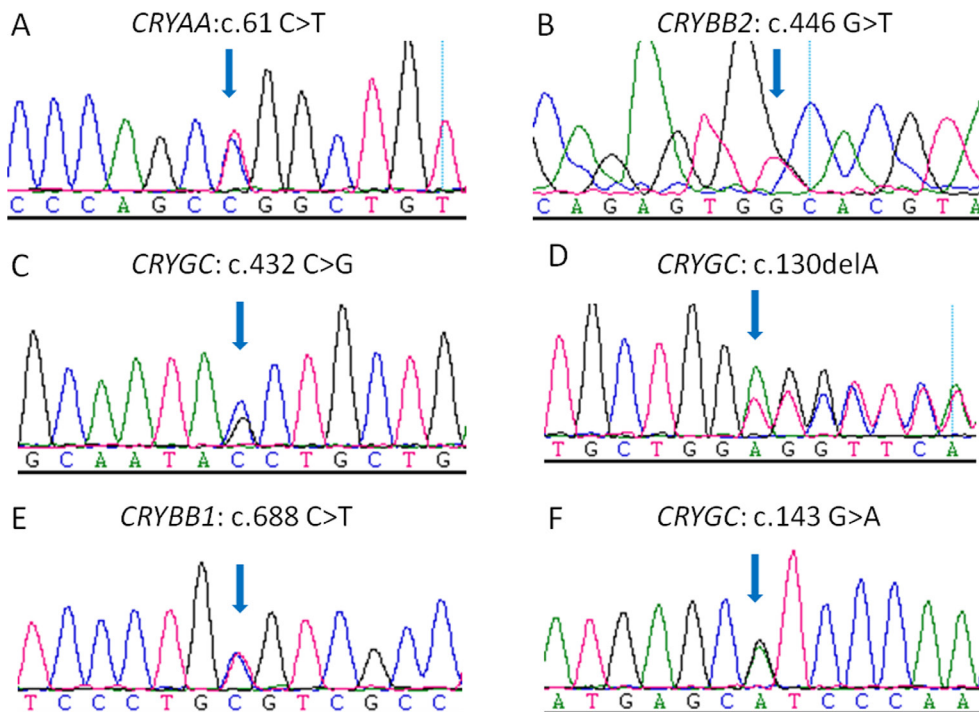


Figure 5. Chromatograms of all detected mutations. A: Sequence for patient III:2 from family A. B: Sequence for patient III:1 from family B. C: Sequence for patient II:1 from family C. D: Sequence for patient II:1 from family D. E: Sequence for simplex case 1. F: Sequence for simplex case 2. The arrow indicates the mutation.

Using NGS, we identified five pathogenic mutations and one variation in crystallin genes. Crystallin is a major component of lens proteins that constitutes more than 90% of water-soluble lens proteins. There are mainly three types of crystallins in human lenses, including α -, β -, and γ -crystallin, which are encoded by 11 crystallin genes [24]. Destruction or structural abnormalities of crystallins will result in irregular arrangement of lens fibers and lead to opacity in the lens [25]. The p.(Arg21Trp) mutation of the *CRYAA* gene is a hotspot mutation that can give rise to congenital cataracts, and phenotypic heterogeneity was found in these mutants [13,14,19,26]. Except this one mutation, the other four mutations have not been reported before. A nonsense mutation and a frame-shift mutation of the *CRYGC* gene (p.(Tyr144Ter) and c.130delA) were identified in family C and family D. When considering the severity of these two kinds of mutations, we have confidence that they are pathogenic. Two missense mutations were identified in family B (*CRYBB2*: p.(Gly149Val)) and simplex case 1 (*CRYBB1*: p.(Arg230Cys)). The prediction results for hydrophobicity show that both mutants have higher hydrophobicity that may influence the structure and function of the protein and lead to opacity in the optic lens.

The *CRYGC* variation Arg48His had an allele frequency of 1.74% (shown in ExAC). However, this variation has been reported twice as a pathogenic mutation in previous studies [10,11]. Manoj Kumar et al. suggested that the p.(Arg48His) mutant changes the hydrogen bonds between Arg48 with several other amino acids and leads to an increase in

hydrophobicity, which influences the solubility and stability of the γ -C crystallin [10]. There are different prediction outcomes for different software programs. Polyphen2 and SIFT showed that the mutation is nonpathogenic, but Mutation Taster predicted that the mutation causes disease. Family members of this patient were not available for testing; thus, we could not collect clinical and genetic information. Therefore, the pathogenicity of this variance is not clear. Perhaps more experiments should be conducted to explore the relationship between the p.(Arg48His) variant and disease.

The correlation between mutations in crystallin genes and extralenticular signs, especially coloboma, is quite confusing. As we know, crystallin genes are mainly expressed in lenses. Therefore, whether these mutations are associated with complicated extralens abnormalities is unknown. However, in several previous studies, crystallin genes were slightly detected in non-lens tissues, especially in mammal retinas [27-30], and α -crystallin was suggested to play a role in antiapoptosis [31,32]. Perhaps these findings may help explain the appearance of the extralens phenotypes in this study. As shown in Table 3, some mutations in crystallin genes with rare extralenticular abnormalities have been reported previously [19,26,33-36]. These cases suggest the complicated phenotypes are related to congenital cataracts but are not an isolated disease. However, we could not rule out the possibility that additional phenotypes are caused by novel genes or even have nothing to do with heredity, especially when some of these abnormalities are variable within a

TABLE 3. MUTATIONS IDENTIFIED IN CRYSTALLIN GENES LEADING TO CONGENITAL CATARACT ACCOMPANIED WITH OTHER OCULAR ABNORMALITIES.

Gene	Mutation	Ocular abnormalities	Area	Reference
CRYAA	p.(Arg12Cys)	Congenital cataract, microcornea, macrocephaly; <i>coloboma, glaucoma</i>	Canada	[33]
CRYAA	p.(Arg21Trp)	Congenital cataract; <i>microphthalmia, glaucoma</i>	Korea	[26]
CRYAA	p.(Arg21Trp)	Congenital cataract; <i>microcornea(1/9), inferior iris coloboma(1/9)</i>	Denmark	[19]
CRYAA	p.(Arg116Cys)	Congenital cataract, iris coloboma; <i>microphthalmia(2/12)</i>	France	[34]
CRYBA2	p.(Val50Met)	Congenital cataract; <i>myopia, glaucoma</i>	Uruguay	[33]
CRYBA4	p.(Leu69Prp)	Congenital cataract, microphthalmous	India	[35]
CRYBB1	p.(Val96Phe)	Congenital cataract, glaucoma; <i>microcornea</i>	USA	[33]
CRYBB2	p.(Arg145Trp), p.(Gln147Arg) and p.(Thr150Met)	Congenital cataract, microphthalmia; <i>glaucoma(6/10)</i>	Jewish	[36]
CRYBB3	p.(Val194Gly)	Congenital cataract; <i>glaucoma(2/5)</i>	Italy	[33]
CRYGD	p.(Arg140Ter)	Congenital cataract, hyperopia, strabismus	Jewish	[33]
CRYGC	p.(Tyr139Ter)	Congenital cataract; <i>microphthalmia/microcornea, corneal opacity, glaucoma</i>	USA	[33]

Variable features within the family are noted in italics and the proportion is shown in brackets if there is a description in the literature.

family. Rare variants in other ocular disease genes were also identified which may offer some clues for the noncataractous phenotype; however, whether these variants are associated with the noncataractous phenotype is difficult to confirm. In conclusion, the relationship between complicated phenotypes and mutations in crystallin genes are not explicit. Thus, more cases should be included, and more experiments should be performed to verify this connection.

In summary, we examined a cohort of Chinese patients with congenital cataracts and studied the phenotypes and genotypes. We described a special phenotype of congenital cataracts associated with macular and optic disc colobomas. Four novel mutations and one reported mutation were identified in these patients. Although the pathogenic mechanism of crystallin gene mutants is not clear, the findings in this study may provide clues for further research to verify the exact role of crystallin genes in the formation of congenital cataracts and development of the eye.

APPENDIX 1. GENES ASSOCIATED WITH INHERITED EYE DISEASES.

To access the data, click or select the words “[Appendix 1.](#)”

APPENDIX 2. PRIMERS USED FOR POLYMERASE CHAIN REACTION.

To access the data, click or select the words “[Appendix 2.](#)”

APPENDIX 3. VARIANTS OF THE PROBANDS DETECTED BY NGS

To access the data, click or select the words “[Appendix 3.](#)”

APPENDIX 4. PREDICTING RESULTS OF PROTSCALE

The predicting results of ProtScale showed that the mutant had a higher hydrophobicity compared with the wild-type; A, hydrophobicity of wild-type (right) and *CRYBB2*: p.(Gly149Val) mutant (left); B, hydrophobicity of wild-type (right) and *CRYBB1*: p.(Arg230Cys) mutant (left). To access the data, click or select the words “[Appendix 4.](#)”

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