• Basic Research •

Novel mutations in *CRYBB1/CRYBB2* identified by targeted exome sequencing in Chinese families with congenital cataract

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Abstract

• AIM: To summarize the phenotypes and identify the underlying genetic cause of the *CRYBB1* and *CRYBB2* gene responsible for congenital cataract in two Chinese families.

• METHODS: Detailed family histories and clinical data were collected from patients during an ophthalmologic examination. Of 523 inheritable genetic vision system-related genes were captured and sequenced by targeted next-generation sequencing, and the results were confirmed by Sanger sequencing. The possible functional impacts of an amino acid substitution were performed with PolyPhen-2 and SIFT predictions.

• RESULTS: The patients in the two families were affected with congenital cataract. Sixty-five (FAMILY-1) and sixty-two (FAMILY-2) single-nucleotide polymorphisms and indels were selected by recommended filtering criteria. Segregation was then analyzed by applying Sanger sequencing with the family members. A heterozygous *CRYBB1* mutation in exon 4 (c.347T>C, p.L116P) was identified in sixteen patients in FAMILY-1. A heterozygous *CRYBB2* mutation in exon 5 (c.355G>A, p.G119R) was identified in three patients in FAMILY-2. Each mutation co-segregated with the affected individuals and did not exist in unaffected family members and 200 unrelated normal controls. The mutation was predicted to be highly conservative and to be deleterious by both PolyPhen-2 and SIFT.

• CONCLUSION: The *CRYBB1* mutation (c.347T>C) and *CRYBB2* mutation (c.355G>A) are novel in patients with congenital cataract. We summarize the variable phenotypes among the patients, which expanded the phenotypic spectrum of congenital cataract in a different ethnic background.

• KEYWORDS: *CRYBB1*; *CRYBB2*; next-generation sequencing; congenital cataract
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INTRODUCTION

The majority of blindness cases in children result from congenital cataract (OMIM 601547). Congenital cataract refers to lens opacity that occurs in the first year of life, with an incidence of about 1/10 000 to 3/10 000[1]. Worldwide, more than 1 million children are blind because of cataracts[2]. Congenital cataracts occur independently or as part of multisystem abnormalities. Its most common pattern is autosomal dominance, but other genetic patterns have been reported[3]. Previous studies have reported a total of more than 200 genes or loci associated with cataract (Cat-Map)[4]. There are about 45 chromosome loci and 38 genes associated with non-syndromic cataracts[5]. About half of all inherited cataracts are caused by mutations in crystallins[3]. The order and stability of crystallin are key factors in maintaining lens transparency[6]. Some mutations in crystallin cause aggregation of mutant proteins that lead to congenital cataracts, which are usually monogenic diseases consistent with the Mendelian pattern. Other mutations, which increase the susceptibility of individuals to the environment, often contribute to the development of age-related cataracts, which are usually multifactorial[3]. Other pathogenic genes of cataract have been reported, *e.g.* lens specific connexins, major intrinsic protein, paired-like homeodomain transcription factor-3, aquaporin-0, lens intrinsic membrane protein 2, avian musculo-aponeurotic fibrosarcoma, and beaded filament structural proteins-2[6-13].

Compared with traditional approaches including direct sequencing and linkage disequilibrium, next-generation sequencing (NGS) has been demonstrated as a significant improvement that provides precise diagnostic information and extends the possibility of targeted treatments. In this study, a gene capture panel was used to encompass the exons and untranslated region of 523 genes related to inherited eye disorders. The capture probes were custom designed and produced by Joy Orient Translational Medicine Research (Beijing, China). Two new heterozygous mutations were identified in two congenital cataract families in China, including c.347T>C [p.L116P; exon 4 of *CRYBB1*, with co-segregation in 65 single-nucleotide polymorphisms (SNPs; FAMILY-1)] and c.355G>A [p.G119R; exon 5 of *CRYBB2,* with co-segregation in 62 SNPs (FAMILY-2)]. By functional prediction, we found that these two missense mutations were pathogenic, and may be the main cause of cataract formation in the two families. Our results demonstrated that the gene testing panel is a cost-effective and high-throughput method that could be applied in both research and clinically molecular diagnosis of genetic eye diseases.

SUBJECTS AND METHODS

Subject Recruitment and Clinical Examination Two families of autosomal dominant congenital nuclear cataracts were recruited at the Second People's Hospital of Jinan, China. Both families were from Linyi (Shandong, China). There were 28 people in FAMILY-1 (16 affected, 12 unaffected; 14 males and 14 females). There were 6 people in FAMILY-2 (3 affected, 3 unaffected; 4 males and 2 females). Members of both families had no other system anomalies. The study was conducted in accordance with the Helsinki Declaration and received informed consent from all participants. Diagnosis was confirmed by ophthalmologic examinations, including visual acuity, slit lamp examination, tonometer, corneal curvature measurement, corneal endothelium examination, ultrasound A/B scan or cataract extraction history. Eye photos were taken by slit lamp photography without pupil dilation. Totally 200 subjects from the same population without diagnostic features of congenital cataract were recruited as normal controls

Targeted Next-generation Sequencing Of 5 mL of venous blood was taken from each participant, and genomic DNA was extracted using a DNA isolation kit for mammalian blood (Tiangen, Beijing, China). Of 523 genetic vision system related genes were respectively sequenced for II:5 of FAMILY-1 and II:1 of FAMILY-2 (Joy orient translational medicine research, Beijing, China). Burrows Wheeler Aligner software was used to perform short read mapping and alignment. SOAPsnp software and GATK Indel Genotyper were used to test SNPs and insertions/deletions respectively.

Variant Analysis Data was provided as lists of sequence variants (SNPs and short indels), relative to the reference genome. Identified variants were filtered against the Single Nucleotide Polymorphism Database (dbSNP, http://www.ncbi.nlm.nih.gov/projects/SNP/snp_summary.cgi/), 1000 Genomes Project (http://www.1000genome.org/), HapMap 8 (http://hapmap.ncbi.nlm.nih.gov/) database, and YH database[14].

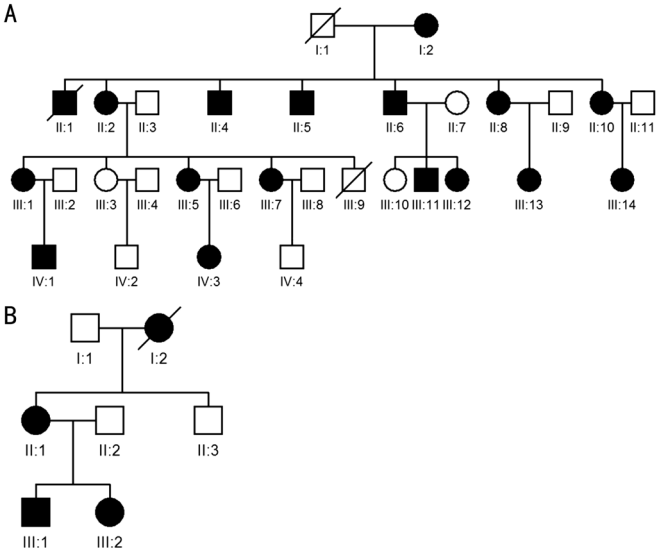

Figure 1 Pedigrees of two Chinese families with congenital cataract Filled square: Male patient; Filled circle: Female patient; Empty symbol: Normal healthy individual; "/": Deceased person.

Verification of Variants Sanger sequencing was carried out in two siblings and their children (II:2, II:6, III:3, III:7, III:10, III:12) to conform whether there were variants may participate in the disease process in FAMILY-1. DNA sequencing was further performed to screen the *CRYBB1* gene in all individuals in FAMILY-1. Sanger sequencing was also performed on all individuals to verify whether the potential candidate variants detected by targeted NGS were co-segregated with the disease phenotype in FAMILY-2.

Primers for flanking candidate loci were designed and synthesized. Each target fragment was amplified using Taq DNA polymerase (Takara, Dalian, China). Direct DNA sequencing was performed using the BigDyeTM Terminator v3.1 Cycle Sequencing Kit and the ABI PRISM 3730 Sequencer (Applied Biosystems Inc., USA). DNASTAR software package (DNASTAR Inc., USA) was used to align and analyze the sequences. The novel mutations of *CRYBB1/CRYBB2* were also genotyped in 200 unrelated healthy controls Sanger sequencing. The Sanger sequencing method is the same as the above description.

RESULTS

Clinical Assessment and Findings In this study, there were two Chinese pedigrees with an autosomal dominant inheritance pattern, both of which had congenital nuclear cataracts (Figure 1). FAMILY-1 was a four generation pedigree with 28 members, including 16 with congenital cataract (Figure 1A). FAMILY-2 was a three generation pedigree with 6 members, including 3 with congenital cataract (Figure 1B).

Table 1 Clinical phenotypes and findings of patients in the congenital cataract families

Patient	Age (y)/ Gender	Eye	Visual acuity	Best corrected visual acuity	Lens	Nystagmus	IOP (mm Hg)	Axial length (mm)	Endothelial cells density (mm)	B-scanning	Surgery and trauma history
FAMILY-1:II:2	58/F	OD	FC	FC	Cataract	Yes	16	27.13	2533	Posterior scleral staphyloma	No
		OS	FC	FC	Cataract	Yes	16	26.87	2681	Posterior scleral staphyloma	No
FAMILY-1:II:6	46/M	OD	0.02	0.02	IOL	Yes	14	26.37	3026	Posterior scleral staphyloma	Phacoemusification+IOL
		OS	0.02	0.02	IOL	Yes	12	26.77	2641	Posterior scleral staphyloma	Phacoemusification+IOL
FAMILY-1:III:5	28/F	OD	0.05	0.05	IOL	Yes	18	24.6	2490	NA	Phacoemusification+IOL
		OS	0.06	0.06	IOL	Yes	16	24.1	2615	NA	Phacoemusification+IOL
FAMILY-1:III:11	6/M	OD	FC	FC	IOL	Yes	15	25	NA	Posterior scleral staphyloma	Phacoemusification+IOL
		OS	0.01	0.01	IOL	Yes	15	25.1	NA	Posterior scleral staphyloma	Phacoemusification+IOL
FAMILY-1:III:12	5/F	OD	0.01	0.01	IOL	Yes	16	23.44	3037	Posterior scleral staphyloma	Phacoemusification+IOL
		OS	FC	FC	IOL	Yes	16	23.9	3119	Posterior scleral staphyloma	Phacoemusification+IOL
FAMILY-1:III:13	15/F	OD	0.1	0.1	IOL	Yes	20	24.97	3176	Vitreous bodies opaque, posterior scleral staphyloma	Phacoemusification+IOL
		OS	0.1	0.1	IOL	Yes	21	23.98	3005	Vitreous bodies opaque, posterior scleral staphyloma	Phacoemusification+IOL
FAMILY-1:III:14	10/F	OD	0.1	0.1	IOL	Yes	14	24.8	2963	Posterior scleral staphyloma	Phacoemusification+IOL
		OS	0.1	0.1	IOL	Yes	15	24.9	2912	Posterior scleral staphyloma	Phacoemusification+IOL
FAMILY-1:IV:1	9/M	OD	0.02	0.02	IOL	Yes	15	24.44	3092	Posterior scleral staphyloma	Phacoemusification+IOL
		OS	0.02	0.02	IOL	Yes	18	25.38	3247	Posterior scleral staphyloma	Phacoemusification+IOL
FAMILY-1:IV:3	6/F	OD	0.05	0.05	IOL	Yes	16	22.78	3350	Posterior scleral staphyloma	Phacoemusification+IOL
		OS	0.05	0.05	IOL	Yes	14	22.93	3287	Posterior scleral staphyloma	Phacoemusification+IOL
FAMILY-2:II:1	30/F	OD	0.02	0.05	Cataract	Yes	15	19.58	2980	NA	No
		OS	0.02	0.05	Cataract	Yes	17	19.89	2739	NA	No
FAMILY-2:III:1	7/M	OD	0.05	0.05	IOL	Yes	14	20.76	3500	Vitreous bodies opaque	Phacoemusification+IOL
		OS	0.05	0.05	IOL	Yes	16	20.35	3680	Vitreous bodies opaque	Phacoemusification+IOL
FAMILY-2:III:2	4/F	OD	NA	NA	Cataract	Yes	13	18.66	3697	Vitreous bodies opaque	No
		OS	NA	NA	Cataract	Yes	15	19.06	3819	Vitreous bodies opaque	No

FC: Finger counting; F: Female; M: Male; IOL: Intraocular lens; NA: Not available; OD: Right eye; OS: Left eye; IOP: Intraocular pressure.

The patients in these two families had similar clinical phenotypes, such as congenital nuclear cataract and nystagmus. None of the two families had any other systemic diseases. The detailed clinical and ophthalmological findings of the patients of both families are shown in Table 1.

Identification of *CRYBB1/CRYBB2* as a Candidate Gene Next, we aimed to identify pathogenic genes among 523 inheritable vision system-related genes by targeted exome sequencing (II:5 and II:1 in FAMILY-1 and 2, respectively). BWA was used for data analysis as proposed previously[15]. The average sequencing depth of II:5 (FAMILY-1) was 58.34; 72.21% of exon sequences were sequenced at least 10 times. The average sequencing depth of II:1 (FAMILY-2) was 60.89, and 84.33% of exon sequences were sequenced at least 10 times. NGS data are summarized in Table 2.

SNPs and indels were annotated, and filtered against 1000 Genome Project, the HapMap 8 database, the Single Nucleotide Polymorphism database, and the YH database. Since synonymous variants are unlikely to be pathogenic, we screened out non-synonymous mutations (nonsense, missense and read-through), coding indels, and variants of splice donor and acceptor sites. The filtered data are shown in Table 3.

Table 2 Coverage parameters of targeted exome sequencing in the affected individuals, namely II:5 and II:1 in FAMILY-1 and 2, respectively

Patient	II:5 of FAMILY-1	II:1 of FAMILY-2
Average sequencing depth on target	58.34	60.89
Fraction of target covered with		
at least 4×	89.48%	91.45%
at least 10×	72.21%	84.33%
at least 20×	53.9%	76.57%

Table 3 SNPs and indels identified in the patients (II:5 and II:1 in FAMILY-1 and 2, respectively) by targeted NGS

Filter[a]	Genetic variants			
	SNP exon	SNP: splicing site	Indel	Total
II:5 (FAMILY-1)	62	2	1	65
II:1 (FAMILY-2)	57	4	1	62

SNP: Single-nucleotide polymorphism; [a]Not in 1000 Genomes Project, the dbSNP, HapMap 8, or YH database.

Sanger Sequencing to Verify the Candidate Gene *CRYBB1/CRYBB2* Sixty-two non-synonymous SNPs, 2 splicing sites, and 1 indel were selected by recommended filtering criteria

Table 4 SNPs and indels identified in the two congenital cataract families

Patient	Chromosome/ Position/Gene name	dbSNP rs# cluster ID	Mutation type	Codons	Substitution	Prediction from SIFT	Prediction from PolyPhen-2	Score of prediction from PolyPhen-2
FAMILY-1	Chr22/26607978/*CRYBB1*	Novel	Missense	T347C	L116P	DAMAGING	POSSIBLY DAMAGING	0.518
FAMILY-2	Chr22/2562541/*CRYBB2*	Novel	Missense	G355A	G119R	DAMAGING	PROBABLY DAMAGING	1.0

SNP: Single-nucleotide polymorphism.

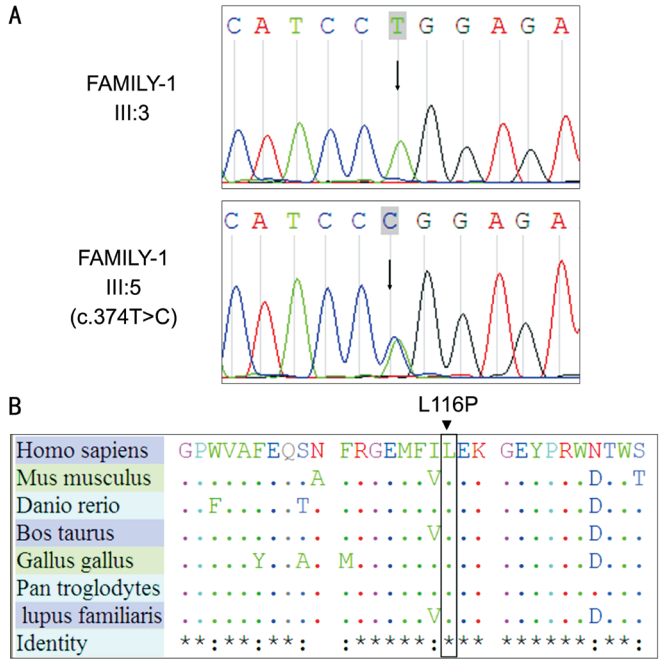

Figure 2 Chromatograms depicting the *CRYBB1* mutations of FAMILY-1 A: First and second chromatograms were obtained from samples of diseased and healthy individuals, respectively; arrow, location of the mutation; B: Sequence alignment of the genetic region around the L116P mutations in *Homo sapiens* (NP_001878.1), *Mus musculus* (NP_076184.1), *Danio rerio* (NP_775338.2), *Bos Taurus* (NP_776951.1), *Gallus gallus* (NP_989511.1), *Pan troglodytes* (XP_001172375.2), and *Canis lupus familiaris* (XP_543459.2). Leucine at the 116th position in *CRYBB1* is highly conserved among the above species.

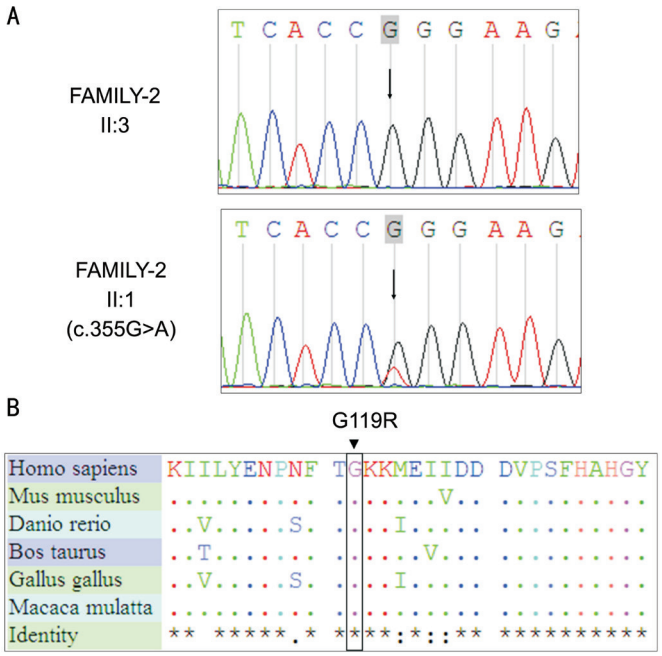

Figure 3 Chromatograms depicting the *CRYBB2* mutations of FAMILY-2 A: First and second chromatograms were obtained from samples of diseased and healthy individuals, respectively; arrow, location of the mutation; B: Sequence alignment of the genetic region around the G119R mutations in *Homo sapiens* (NP_000487.1), *Mus musculus* (NP_031799.1), *Danio rerio* (NP_001018138.1), *Bos Taurus* (NP_777232.1), *Gallus gallus* (NP_990506.2), and *Macaca mulatta* (NP_001116366.2). Glycine at the 119th position in *CRYBB2* is highly conserved among the above species.

in FAMILY-1. Only one SNP (Chr22:26607978) was co-segregated in FAMILY-1, in exon 4 of *CRYBB1*; this was a T>C change (c.347T>C, p.L116P; Table 4, Figure 2A). This mutation was not found in 200 control individuals.

The p.L116P missense mutation was generated by c.347T>C. Homology analysis of leu116 loci of CRYBB1 proteins in different species indicated that these loci were highly conserved (Figure 2B). SIFT forecasted the p.L116P missense mutation to cause a deleterious change in CRYBB1 function (Table 4).

Fifty-seven non-synonymous SNPs, 4 Splicing sites, and 1 indel were selected by recommended filtering criteria in FAMILY-2. Only one SNP (Chr22:2562541) was co-segregated in FAMILY-2, in exon 5 of *CRYBB2*, a G>A change (c.355G>A, p.G119R; Table 4, Figure 3A). The mutation was not found in 200 control individuals.

The p.G119R missense mutation was generated by c.355G>A. Homology analysis of Gly119 loci of *CRYBB2* proteins in different species indicated that these loci were highly conserved (Figure 3B). SIFT forcasted the p.G119R missense mutation to cause a deleterious change in *CRYBB2* function (Table 4).

DISCUSSION

The lens is a vascular organ whose transparency is important for maintaining normal function. Transparent lenses allow light to pass through and focus on the retina. The lens consists of two types of cells, including epithelial and fibrous cells. Lens fiber cells are differentiated from epithelial cells and contain high concentrations of soluble crystallin. The metabolic activity of mature fibroblasts is very limited, and most of the metabolism, biosynthesis, and active transport are accomplished by surface cells. Crystallins and a wide range of cell-cell communication systems are key factors in establishing and maintaining lens transparency. Abnormalities in lens cells

and/or crystallin may cause lens opacity, resulting in decreased vision and severe blindness[16].

In the vertebrate lens, β-crystallins are found as homomers or heteromers, which are thought to be important in maintaining the optical properties of the lens throughout an individual's life[17-20].

The new heterozygous c.347T>C mutation found in *CRYBB1* (FAMILY-1), co-occurred with autosomal dominant cataract. *CRYBB1* represents the main β-crystallin subunit, and accounts for about a tenth of human non-membrane crystallins[21]. The β-crystallin structure is considered to be critical in protein aggregation and orientation, with terminal arm loss altering their dimerization and causing cataract development[22]. Mackay *et al*[23] demonstrated that the G220X mutation in *CRYBB1* is related to autosomal dominant cataract, likely disrupting the 4th Greek key motif and destabilizing *CRYBB1*. Meanwhile, 3 *CRYBB1* mutations related to autosomal dominance and/or autosomal recessive congenital cataracts were identified[24-26].

The new *CRYBB2* (FAMILY-2) mutation found in exon 5 (c.355G>A, p.G119R) co-occurred with autosomal dominant cataract. A total of 3 additional *CRYBB2* mutations were described, namely Q155X, D128V, and W151C, also destabilizing *CRYBB*[27].

Reports describing gene mutations related to congenital cataracts often use whole exome sequencing with linkage assessment among relatives[28-33]; alternatively, a specific gene is screened in more individuals[34]. A total of 100 patients with congenital cataract were assessed by exon sequencing of fourteen genes, and 18 variant genes were detected, including 14 involving crystallins[35]. In another previous study of 61 genes in 74 non-related patients, 32 sporadic congenital cataract specific SNPs were obtained in 29 cases, including 31 with a heterozygous pattern[36].

The NGS approach was employed in the present work for the assessment of 523 documented inheritable vision system-related genes in two families. Heterozygous mutations were found in pedigrees 1 (*CRYBB1,* exon 4; c.347T>C, p.L116P) and 2 (*CRYBB2,* exon 5; c.355G>A, p.G119R), exclusively co-segregated with diseased family members, and not found in 200 control Chinese individuals.

The *CRYBB1* (c.347T>C) and *CRYBB2* (c.355G>A) mutations in this study are not found in previous reports. In comparison with whole exome sequencing, target region sequencing presents several advantages in assessing SNPs in sporadic cases due to low cost and easy analysis. It should be noted that the present sequencing strategy cannot identify unknown genes. Overall, these findings provide new insights regarding the etiological features of congenital cataract.

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