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Mutation screening of crystallin genes in Chinese families with congenital cataracts

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Purpose: To identify mutations in crystallin genes in Chinese families with congenital cataracts.
Methods: Forty-two unrelated families with non-syndromic congenital cataracts were enrolled in this study. The coding exons and adjacent intronic regions of crystallin genes, including *CRYAA, CRYAB, CRYBA1, CRYBA4, CRYBB1, CRYBB2, CRYBB3, CRYGC, CRYGD* and *CRYGS*, were analyzed with Sanger sequencing. Novel variants were further evaluated in 112 ethnically matched controls. To confirm the novel mutations, short tandem repeat (STR) haplotypes were constructed to check the cosegregation with congenital cataract. The pathogenic potential of the novel mutations were assessed using bioinformatics tools, including Sorting Intolerant From Tolerant v5.1.1 (SIFT), Polymorphism Phenotyping v2 (PolyPhen-2), and Human Splicing Finder. The pathogenicity of all the mutations was evaluated according to the guidelines of the American College of Medical Genetics (ACMG) and InterVar software.
Results: Seven previously reported mutations in crystallin genes identified in ten unrelated families were associated with the congenital nuclear cataracts. Four novel mutations in crystallin genes, including c.35G>T (p.R12L) in *CRYAA*, c.463C>A (p.Q155K) in *CRYBB2*, IVS1 c.10–1G>A in *CRYGC*, and c.346delT (p.F116Sfsx29) in *CRYGD*, were identified in four unrelated families with congenital cataracts. These mutations cosegregated with all affected individuals in each family were not observed in the unaffected family members or in the 112 unrelated controls. All four novel mutations were categorized as disease "likely pathogenic" except IVS1 c.10–1G>A in *CRYGC* "pathogenic" using InterVar software in accordance with the ACMG standard. Mutations in crystallin genes were responsible for 33.33% of the Chinese families with congenital cataracts in this cohort.
Conclusions: In this study, we identified four novel mutations in crystallin genes in Chinese families with congenital cataracts. The results expand the mutational spectrum of crystallin genes, which may be helpful for the molecular diagnosis of congenital cataracts in the era of precision medicine.

BACKGROUND

Congenital cataracts (CCs) are defined as an opacity of the lens of the eyes at birth or during early childhood, which are the principal cause of treatable pediatric visual impairment [1]. The prevalence of congenital cataracts has been estimated between 1 and 15/10,000 children [2]. Approximately one third of congenital cataracts are genetically determined. The most frequent mode of inheritance is autosomal dominant (AD) although autosomal recessive (AR) and X-linked forms have also been reported. To date, more than 30 genes have been identified in congenital cataracts. These genes include crystallin genes, lens-specific connexins genes, major intrinsic protein or aquaporine genes, cytoskeletal structural proteins genes, paired like homeodomain transcription factor 3 (*PITX3*; Gene ID 5309,OMIM 602669) , V-MAF avian musculoaponeurotic fibrosarcoma oncogene homolog (*MAF*; Gene ID 4094, OMIM 177075) and heat shock transcription factor 4 (*HSF4*; Gene ID 3299, OMIM 602438) [3].

The water-soluble lens crystallins are divided into α, β, and γ according to the order of their elution on gel exclusion chromatography, which account for nearly 90% of the total lens proteins [4]. Our survey of subsets of these genes in the Chinese population suggests that more than 50% of the mutations in crystallin genes are associated with inherited AD and/or AR cataract, where *CRYAA* (Gene ID 1409, OMIM 123580), *CRYAB* (Gene ID 3316, OMIM 602179), *CRYBA1/3* (Gene ID 1411, OMIM 123610), *CRYBA2* (Gene ID 1412, OMIM 600836), *CRYBA4* (Gene ID 1413, OMIM 123631), *CRYBB1* (Gene ID 1414, OMIM 600929), *CRYBB2* (Gene

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ID: 1415, OMIM: 123620), *CRYBB3* (Gene ID 1417, OMIM 123630), *CRYGB* (Gene ID 1419, OMIM 123670), *CRYGC* (Gene ID 1420, OMIM 123680), *CRYGD* (Gene ID 1421, OMIM: 123690) and *CRYGS* (Gene ID 1427, OMIM 123730) were involved. In this study, 42 unrelated families with CCs were collected from southeast China. Four novel mutations and seven previously reported mutations in crystallin genes responsible for CCs were identified in the genetic study.

METHODS

Subjects and DNA specimens: The research followed the tenets of the Declaration of Helsinki. Informed consent was obtained from the subjects. If participants were under the age of 16, consent was obtained from their parents or legal guardians. The experiments were approved by the Ethics Committee of Fujian Medical University, and the study also adhered to the ARVO statement on human subjects.

Forty-two probands with congenital cataracts and 225 related individuals from southeast China were enrolled in a genetic screening program. Clinical and ophthalmological examinations were performed on the affected individuals, as well as on the unaffected family members. Phenotype was documented with slit-lamp photography. One hundred twelve samples from ethnically matched control individuals were obtained before the study. 5 ml peripheral blood was drawn from each subjects and preserved at -20 °C prior to use. Genomic DNA was extracted from whole blood using the Wizard Genomic DNA Purification Kit (Promega, Beijing, China) according to the manufacturer's instructions [5,6].

Mutation screening: Mutation screening methods were based on the hot-spot regions of cataract-causing mutations that cover about 80% of mutations in inherited cataract the same as our previous study [5]. Briefly, all the probands of 42 families with CCs were tested for mutations in the common 18 genes that cause CC, including *CRYAA, CRYAB, CRYBA1, CRYBA4, CRYBB1, CRYBB2*, *CRYBB3*, *CRYGC*, *CRYGD*, *CRYGS*, *GJA8* (Gene ID 2703, OMIM 600897), *GJA3* (Gene ID 2700, OMIM 121015), *HSF4*, *MIP* (Gene ID 4284, OMIM 154050), *BFSP2* (Gene ID 8419, OMIM: 603212), *EPHA2* (Gene ID 1969, OMIM 176946), *FYCO1* (Gene ID 79443, OMIM 607182), and *PITX3*. The selected hot-spot coding exons and splice junctions of these genes were amplified with PCR from genomic DNA. The PCR primers and conditions for crystallin genes are listed in Table 1. Thermal cycling was performed with denaturing at 94 °C for 5 min, followed by 30 cycles of 94 °C for 30 s, 58-61 °C for 30 s and 72 °C for 45 s, with a final extension at 72 °C for 5 min and chilling to 4 °C. PCR products were purified and directly sequenced on an ABI 3730XL Automated Sequencer (PE Biosystems, Foster City, CA), using the same PCR primers. When mutations of crystallin genes were identified in probands, intrafamilial segregation analysis was performed. The identified novel mutations in crystallin genes were also tested in 112 normal unrelated individuals from the same ethnic background.

Haplotyping analysis: To further validate the present novel mutations, the genotyping was analyzed using two or three selected microsatellite markers flanking each corresponding pathogenic gene in available family members the same as in our previous study [5,6]. Briefly, PCR products from each DNA sample were separated with gel electrophoresis with a fluorescence-based on the ABI 3730 automated sequencer (Applied Biosystems) using ROX-500 as the internal lane size standard. The amplified DNA fragment lengths were assigned to allelic sizes with GeneMarker Version 2.4.0 software (Soft-Genetics, State College, PA). Pedigree and haplotype data were managed using Cyrillic (version 2.1) software.

Bioinformatics analysis: Mutations were described according to the recommendation of the Human Genomic Variation Society (HGVS). To predict the effects of novel mutations, Polymorphism Phenotyping v2 (PolyPhen-2) [7] and Sorting Intolerant From Tolerant v5.1.1 (SIFT) [8] were used for the missense mutations on the encoded proteins, and Human Splicing Finderv3.1 (HSF) [9] was employed for the intronic variants in the splicing site changes. InterVar from the standards and guidelines of the American College of Medical Genetics and Genomics (ACMG) was used to evaluate the pathogenicity of all the mutations [10,11].

RESULTS

Clinical finding: In the study, 42 probands were recruited from 30 autosomal dominant families, eight families without history, and four isolated cases. All the patients were diagnosed with bilateral cataracts in childhood. No other ophthalmic or systemic diseases presented in any of the participants. Of the probands, 33.33% (12 unrelated families and two isolated cases) were identified mutations in crystallin genes. Among them, four mutations were not previously reported in the CAT-11, CAT-27, CAT-33, and CAT-41 families (Figure 1). The inheritance pattern of the 12 families is autosomal dominant. Based on clinical descriptions provided by the referring clinician at the time of enrollment, the CAT-27 and CAT-41 families have a primary diagnosis of congenital nuclear cataracts, while the CAT-11 family has congenital cortical and coliform cataracts, and the CAT-33 family has congenital total cataracts.

Mutation analysis: Eleven mutations were identified in 14 unrelated families using Sanger sequencing of the coding region and the splicing sites of the crystallin genes (Figure

Table 1. The PCR primers and conditions for crystallin genes.

Gene	Entrez Gene ID	#OMIM	Exon	Primer name	Primer sequence(5'-3')	Amplicon size (bp)	PCR condition*
CRYAA (NM_000394)	1409	123580	1	CRYAAe1F	CTTCTTCATGAGCTCACGCC	443	59 °C
				CRYAAe1R	TGACGGAGCAAGACCAGAGT		
CRYAA (NM_000394)	1409	123580	3	CRYAAe3F	CAGGCTGAGGTCCAGAGAAG	510	60 °C/GC Buffer1
				CRYAAe3R	GGGAAGCAAGGAAGACAGA		
CRYAB (NM_001289807)	1410	123590	1	CRYABe1F	AACCCCTGACATCACCATTC	352	58 °C
				CRYABe1R	AAGGACTCTCCCGTCCTAGC		
CRYAB (NM_001289807)	1410	123590	3	CRYABe3F	GTTGTCATGGCATTTGGTCTC	433	58 °C
				CRYABe3R	CTTGATAATTTGGGCCTGCC		
CRYBA1 (NM_005208)	1411	123610	3	CRYBA1e3F	ACTCTGGGCAAATGAACACC	399	58 °C
				CRYBA1e3R	TCCCCTATCCCCACTCTATG		
CRYBA1 (NM_005208)	1411	123610	4	CRYBA1e4F	CCTGTCAACTCATTCCTCAACTC	493	58 °C
				CRYBA1e4R	CACCTGGTGGAGAAAAATCAA		
CRYBA4 (NM_001886)	1413	123631	4	CRYBA4e4F	CTCCCCTAGTCGTGACAACC	394	58 °C
				CRYBA4e4R	TTTCAACTCTGGAACCTTTGA		
CRYBB1 (NM_001887)	1414	600929	6	CRYBB1e6F	GCACAGAGCAGGAAGGGATA	498	58 °C
				CRYBB1e6R	CGAGGAAGTCACATCCCAGT		
CRYBB2 (NM_000496)	1415	123620	2	CRYBB2e2F	CCTTCAGCATCCTTTGGGTTCTCT	597	58 °C
				CRYBB2e2R	GCAGTTCTAAAAGCTTCATCAGTC		
CRYBB2 (NM_000496)	1415	123620	5	CRYBB2e5F	TGGGTGCACTGGGAAGAGA	399	58 °C/GC Buffer1
				CRYBB2e5R	GAAGCCAGAGGTCAGCAGAG		
CRYBB2 (NM_000496)	1415	123620	6	CRYBB2e6F3	CTGACCCCAGTACAGTACAGT	661	61 °C
				CRYBB2e6R3	CATTTCTCTCTCGCTGT-CACTCTCTC		
CRYBB3 (NM_004076)	1417	123630	6	CRYBB3e6F	GAGGAATGTAGGCAGGCAGA	480	58 °C
				CRYBB3e6R	TCCTTCAGCACGCCTCTC		
CRYGC (NM_020989)	1420	123680	1, 2	CRYGCe1/2F	TGCATAAAATCCCCTTACCG	556	58 °C
				CRYGCe1/2R	CCTCCCTGTAACCCACATTG		
CRYGC (NM_020989)	1420	123680	3	CRYGCe3F	CGCAGCAACCACAGTAATCT	579	58 °C
				CRYGCe3R	CCCACCCCATTCACTTCTTA		
CRYGD (NM_006891)	1421	123690	1, 2	CRYGDe1/2F	AGAACACGAAAATGCCCTTG	579	58 °C/GC Buffer1
				CRYGDe1/2R	TGCTTGAAACCATCCAGTGA		
CRYGD (NM_006891)	1421	123690	3	CRYGDe3F	CCTCACCAAGCTGGACTGC	421	58 °C
				CRYGDe3R	GCCAGGAACACACAGAAAATATT		

Gene	Entrez Gene ID	#OMIM	Exon	Primer name	Primer sequence(5'-3')	Amplicon size (bp)	PCR condition*
CRYGS (NM_017541)	1427	123730	2	CRYGSe2F	GAAACCATCAATAGCGTCTAAATG	229	58 °C
				CRYGSe2R	TGAAAAGCGGGTAGGCTAAA		
Gene	Entrez Gene ID	#OMIM	Exon	Primer name	Primer sequence(**5'-3'**)	Amplicon size (bp)	PCR condition*
CRYAA (NM_000394)	1409	123580	1	CRYAAe1F	CTTCTTCATGAGCTCACGCC	443	59 °C
				CRYAAe1R	TGACGGAGCAAGACCAGAGT		
CRYAA (NM_000394)	1409	123580	3	CRYAAe3F	CAGGCTGAGGTCCAGAGAAG	510	60 °C/GC Buffer1
				CRYAAe3R	GGGAAGCAAAGGAAGACAGA		
CRYAB (NM_001289807)	1410	123590	1	CRYABe1F	AACCCCTGACATCACCATTC	352	58 °C
				CRYABe1R	AAGGACTCTCCCGTCCTAGC		
CRYAB (NM_001289807)	1410	123590	3	CRYABe3F	GTTGTCATGGCATTTGGTCTC	433	58 °C
				CRYABe3R	CTTGATAATTTGGGCCTGCC		
CRYBA1 (NM_005208)	1411	123610	3	CRYBA1e3F	ACTCTGGGCAAATGAACACC	399	58 °C
				CRYBA1e3R	TCCCCTATCCCCACTCTATG		
CRYBA1 (NM_005208)	1411	123610	4	CRYBA1e4F	CCTGTCAACTCATTCCTCAACTC	493	58 °C
				CRYBA1e4R	CACCTGGTGGAGAAAAATCAA		
CRYBA4 (NM_001886)	1413	123631	4	CRYBA4e4F	CTCCCCTAGTCGTGACAACC	394	58 °C
				CRYBA4e4R	TTTCAACTCTGGAACCTTTGA		
CRYBB1 (NM_001887)	1414	600929	6	CRYBB1e6F	GCACAGAGCAGGAAGGGATA	498	58 °C
				CRYBB1e6R	CGAGGAAGTCACATCCCAGT		
CRYBB2 (NM_000496)	1415	123620	2	CRYBB2e2F	CCTTCAGCATCCTTTGGGTTCTCT	597	58 °C
				CRYBB2e2R	GCAGTTCTAAAAGCTTCATCAGTC		
CRYBB2 (NM_000496)	1415	123620	5	CRYBB2e5F	TGGGTGCACTGGGAAGAGA	399	58 °C/GC Buffer1
				CRYBB2e5R	GAAGCCAGAGGTCAGCAGAG		
CRYBB2 (NM_000496)	1415	123620	6	CRYBB2e6F3	CTGACCCCAGTACAGTACAGT	661	61 °C
				CRYBB2e6R3	CATTTCTCTCTCGCTGT-CACTCTCTC		
CRYBB3 (NM_004076)	1417	123630	6	CRYBB3e6F	GAGGAATGTAGGCAGGCAGA	480	58 °C
				CRYBB3e6R	TCCTTCAGCACGCCTCTC		
CRYGC (NM_020989)	1420	123680	1, 2	CRYGCe1/2F	TGCATAAAATCCCCTTACCG	556	58 °C
				CRYGCe1/2R	CCTCCCTGTAACCCACATTG		
CRYGC (NM_020989)	1420	123680	3	CRYGCe3F	CGCAGCAACCACAGTAATCT	579	58 °C
				CRYGCe3R	CCCACCCCATTCACTTCTTA		
CRYGD (NM_006891)	1421	123690	1, 2	CRYGDe1/2F	AGAACACGAAAATGCCCTTG	579	58 °C/GC Buffer1
				CRYGDe1/2R	TGCTTGAAACCATCCAGTGA		

Gene	Entrez Gene ID	#OMIM	Exon	Primer name	Primer sequence(5'-3')	Amplicon size (bp)	PCR condition*
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				CRYGDe3R	GCCAGGAACACACAGAAAATATT		
CRYGS (NM_017541)	1427	123730	2	CRYGSe2F	GAAACCATCAATAGCGTCTAAATG	229	58 °C
				CRYGSe2R	TGAAAAGCGGGTAGGCTAAA		

*Note:GC buffer 1 is used for GC-rich templates in PCR.

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2 and Table 2). No variants in other screening regions of the 18 genes were detected in these families. The clinical significances of all the identified mutations were generated by InterVar software based on the criteria recommended by the American College of Medical Genetics and Genomics / Association for Molecular Pathology (ACMG/AMP) guidelines [11]. The process is automatically performed first, and then, manual adjustment to reclassify the mutations for the criteria that InterVar recommends follows. These 11 mutations match the criterion of pathogenic moderate 1 (PM1) as all are in hot-spot regions. All the mutations except a triple mutation (*CRYBB2* c.(433C>T; 440A>G; 449C>T) were found only in patients, not in healthy relatives or the 112 controls from the same ethnic background. The mutations were also

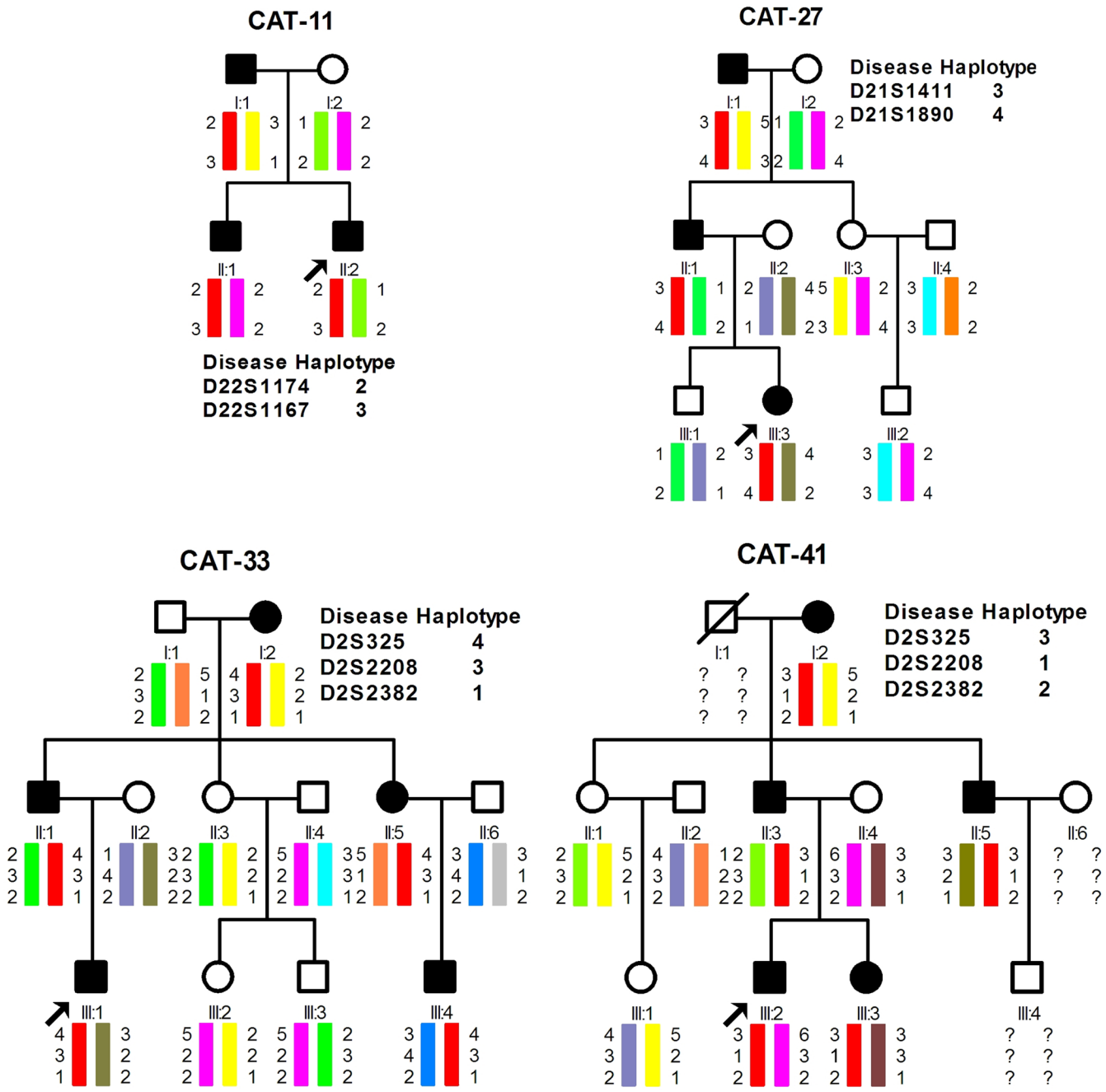

Figure 1. Haplotypes of crystallin genes in each family. All the pathogenic haplotypes (red) indicate segregation of the haplotypes in affected individuals in each family but not in the unaffected family members. The pathogenic haplotypes are annotated beside the pedigrees.

absent in databases of probably benign variation, indicating that they match the criterion of PM2 (absent from controls in this study; Exome Sequencing Project, 1000 Genomes, or ExAC). Ten mutations were cosegregated with CCs in each affected family member (Figure 1 and Figure 2 show the four novel mutations), indicating that they match the criterion for PP1 (cosegregation with disease in multiple affected family members in a gene definitively known to cause the disease). All the missense and splice site mutations were predicted to satisfy the criteria of PP3 (multiple lines of computational evidence support a deleterious effect on the gene or gene product (conservation, evolutionary, splicing impact, etc.)) by SIFT, PolyPhen-2, and InterVar software. All the previous reported mutations match the criterion of PP5 (reputable source recently reports variant as pathogenic, but the evidence is not available to the laboratory to perform an independent evaluation).

Two splice site mutations, including IVS3 c.215+1G>A in *CRYBA1* (the CAT-14 family and the CAT-32 family) and IVS1 c.10–1G>A in *CRYGC* (the CAT-33 family), alter the wild-type (WT) donor site, and most likely affect splicing, which make these mutations match the criterion of PVS1 (null variant (nonsense, frameshift, canonical ± two splice sites, initiation codon, or single or multiexon deletion) in a gene where loss of function (LOF) is a known mechanism of disease). All the splice site mutations were categorized as disease "pathogenic" based on the criteria PS1, PM1, PM2, PP1, and PP3.

In the CAT-21 family, the affected individuals carry a heterozygous c.272–274GAGdel deletion in *CRYBA1*, which results in the glycine deletion at position 91. In the CAT-41 family, affected individuals carry heterozygous single base-pair deletion exon 3 causing a frame shift (c.346delT, p.F116Sfsx29) and a stop codon 29 amino acids downstream if a mutant CRYGD protein can be produced. The frame-shift mutations (CRYBA1:p.G91del, and CRYGD:p.F116Sfsx29) belong to PM4 (protein length changes due to in-frame deletions/insertions in a non-repeat region or stop-loss variants) according to the ACMG guidelines.

A complex three missense mutation in *CRYBB2* previously reported twice was identified in the CAT-24 and CAT-54 families and a sporadic case in the CAT-53 family. This complex mutation was first reported by Hansen [12]. All the other six missense mutations here were predicted to be "probably damaging" by PolyPhen-2 and "deleterious" by

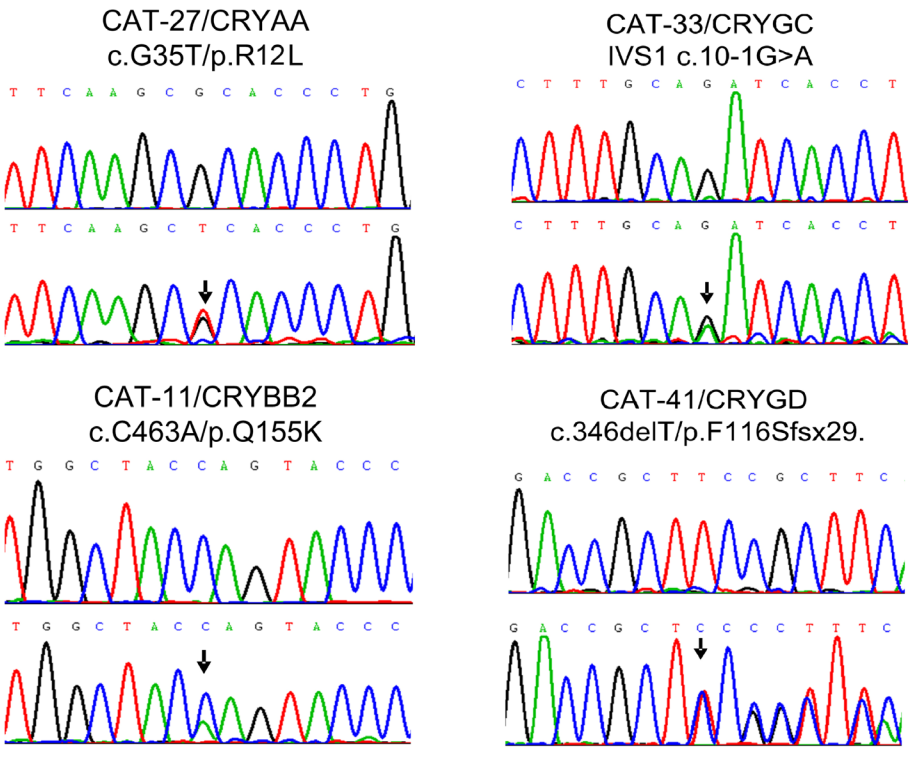

Figure 2. The four novel mutations in crystallin genes identified with direct sequencing. The black arrows indicate the mutations in the probands, and the wild-type can be seen in the corresponding sequences from the normal control.

Table 2. Classification of 12 mutations in crystallin genes in this study according to ACMG guideline.

Family ID	Inheritance	Gene	cDNA change	p.change	Status	Type	PVS1	PM1	PM2	PM4	PP1	PP2	PP3	PP5	Classification	Reference
CAT-27	AD	CRYAA	c.35G>T	p.R12L	Hetero	missense		Y	Y		Y	Y	Y		Likely pathogenic	Novel
CAT-40	AD	CRYAA	c.346C>T	p.R116C	Hetero	missense		Y	Y		Y	Y	Y	Y	Likely pathogenic	[17-20]
CAT-42	AD	CRYAA	c.61C>T	p.R21W	Hetero	missense		Y	Y		Y	Y	Y	Y	Likely pathogenic	[12,21-24]
CAT-14	AD	CRYBA1	IVS3 c.215+1G>A	/	Hetero	canonical splice site	Y		Y		Y	Y	Y		Pathogenic	[25-28]
CAT-21	Sporadiccase	CRYBA1	c.272-274GAG del	p.G91del	Hetero	frameshift		Y	Y	Y				Y	Likely pathogenic	[29,30]
CAT-32	AD	CRYBA1	IVS3 c.215+1G>A	/	Hetero	canonical splice site	Y		Y		Y		Y		Pathogenic	[25-28]
CAT-11	AD	CRYBB2	c.463C>A	p.Q155K	Hetero	missense		Y	Y		Y	Y	Y		Likely pathogenic	Novel
CAT-44	AD	CRYBB2	c.355G>A	p.G119R	Hetero	missense		Y	Y		Y	Y	Y	Y	Likely pathogenic	[24]
CAT-24	AD	CRYBB2	c.(433C>T; 440A>G; 449C>T)	p.(R145W; Q147R; T150M)	Hetero	Complex missense		Y				Y	Y	Y	Likely pathogenic	[12,31]
CAT-53	Sporadiccase	CRYBB2	c.(433C>T; 440A>G; 449C>T)	p.(R145W; Q147R; T150M)	Hetero	Complex missense		Y				Y	Y	Y	Likely pathogenic	[12,31]
CAT-54	AD	CRYBB2	c.(433C>T; 440A>G; 449C>T)	p.(R145W; Q147R; T150M)	Hetero	Complex missense		Y				Y	Y	Y	Likely pathogenic	[12,31]
CAT-33	AD	CRYGC	IVS1 c.10-1G>A	/	Hetero	canonical splice site	Y		Y		Y	Y	Y		Pathogenic	Novel
CAT-41	AD	CRYGD	c.346delT	p.F116Sfsx29	Hetero	frameshift		Y	Y	Y	Y				Likely pathogenic	Novel
CAT-46	AD	CRYGD	c.70C>A	p.P24T	Hetero	missense		Y	Y		Y	Y		Y	Likely pathogenic	[24,31,31]

Note: AD=autosomal dominant; Hetero=heterozygosity; PVS1=null variant (nonsense, frameshift, canonical +- 2 splice sites, initiation codon, single or multiexon deletion) in a gene where LOF is a known mechanism of disease PM1=Located in a mutational hot spot and/or critical and well established functional domain (e.g., active site of an enzyme) without benign variation; PM2=Absent from controls (or at extremely low frequency if recessive) in Exome Sequencing Project, 1000 Genomes Project, or Exome Aggregation Consortium; PM4=Protein length changes as a result of in-frame deletions/insertions in a nonrepeat region or stop-loss variants; PP1=Cosegregation with disease in multiple affected family members in a gene definitively known to cause the disease; PP2=Missense variant in a gene that has a low rate of benign missense variation and in which missense variants are a common mechanism of disease; PP3=Multiple lines of computational evidence support a deleterious effect on the gene or gene product (conservation, evolutionary, splicing impact, etc.) PP5=Reputable source recently reports variant as pathogenic, but the evidence is not available to the laboratory to perform an independent evaluation; PP6=The user has additional 2 supporting pathogenic evidences.

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SIFT. These predictions indicated that the six missense variants perhaps impair protein function.

According to the ACMG guidelines and InterVar software [11], all 11 mutations in the crystallin genes were categorized as disease "likely pathogenic," except two splice site mutations were "pathogenic." More details can be seen in Table 2.

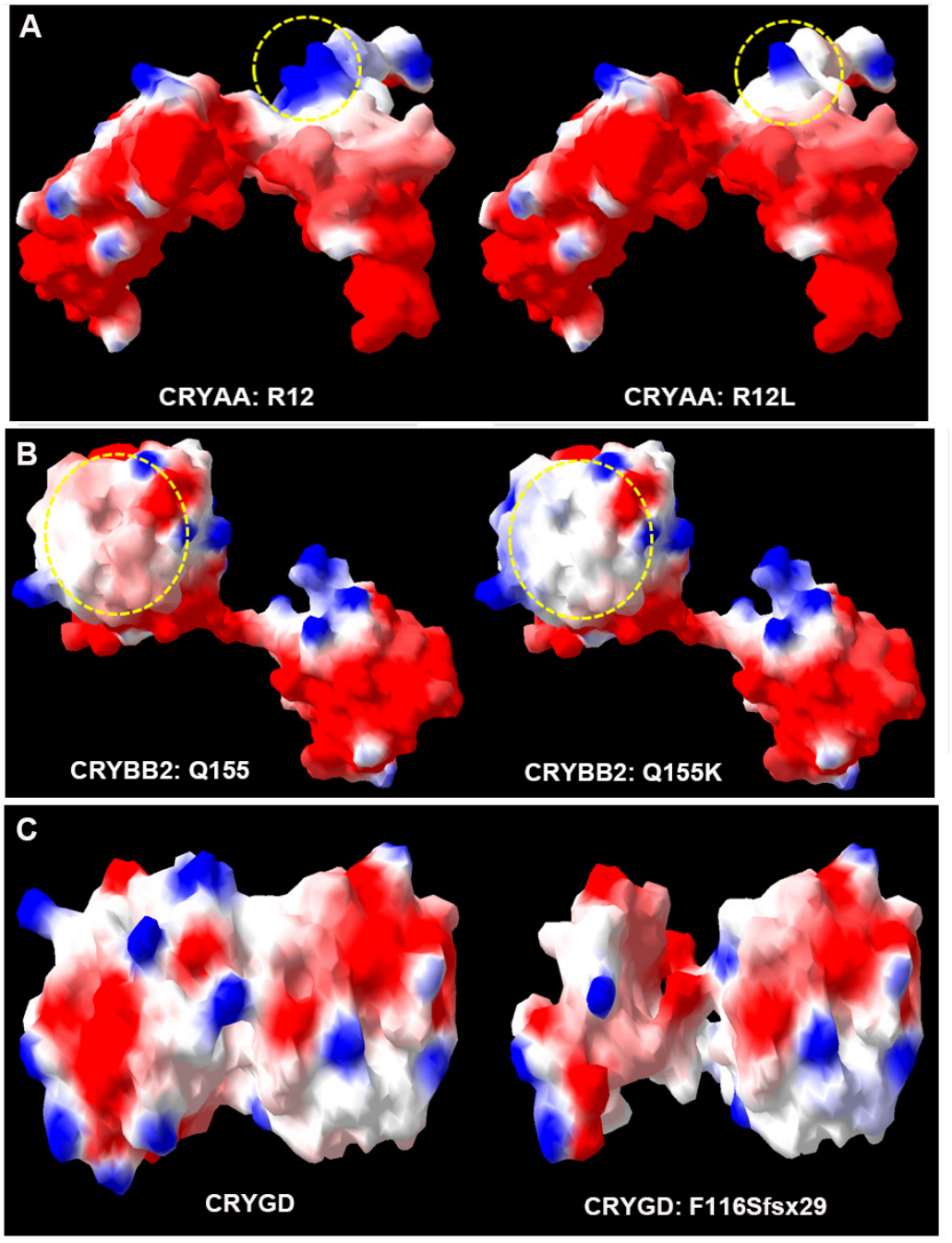

Figure 3. The structural changes in the novel mutations. The molecular surface is colored according to the electrostatic potential with Swiss-PdbViewer, with red-white-blue corresponding to acidic-neutral-basic potential. **A**: Surface change in CRYAA:p.R12L. **B**: Surface change in CRYBB2:Q155K. **C**: Surface change in CRYGD:p.F116Sfsx29. The yellow dotted circle represents the region of significant alteration.

DISCUSSION

In this study, we conducted a mutational screening of known pathogenic genes in a cohort of Chinese patients with congenital cataracts. Moreover, the causative mutations in crystallin genes were identified and summarized. Eleven mutations, including four novel mutations and seven previously reported mutations in crystallin genes, in 14 probands were identified and categorized as "likely pathogenic" except two splice site mutations that were "pathogenic" according to the ACMG guidelines, which contributed to 33.33% of the genetic etiology of congenital cataracts. This result is consistent with another study in the Chinese population, which was eight of the 25 families (32%) [13].

Mutations in crystallin genes account for the majority of hereditary congenital cataracts [14]. Three major classes of crystallins have been found in the vertebrate eye lens. In the molecular structure of crystallin proteins, there are α-crystallin protein (40%), β-crystallin protein (35%), and γ-crystallin protein (25%). The three novel mutations, c.35G>T (p. R12L) in *CRYAA*, c.463C>A (p. Q155K) in *CRYBB2*, and c.346delT (p. F116Sfsx29) in *CRYGD*), cause changes in amino acids, which may eventually result in structural changes in the α-crystallin, β-crystallin, and gamma-crystallin D proteins, respectively. Swiss-PdbViewer predicted that the molecular surface may be changed by the mutations (Figure 3). The ratio of the crystallin protein composition and its spatial sequence in the maintenance of crystallin transparency is very important. The mutations in crystallin genes may not only affect the protein structure, which is closely packed, but also reduce the solubility of crystallin proteins to form opacities [15,16]. Thus, using the primers of crystallin genes in Table 1 followed by intrafamilial cosegregation, bioinformatics analyses, and interpretation of the variants according to the ACMG guidelines might be a cost-effective paradigm in the genetic diagnosis of congenital cataracts in Chinese.

In summary, we identified four novel mutations and seven previously reported mutations in crystallin genes associated with congenital cataracts in a cohort of Chinese families, exhibiting that the mutations in crystallin genes are responsible for 33.33% (14 out of 42 families with CCs) of families with CCs in this cohort. This report extends the mutation spectrum of crystallin genes in the Chinese population, which may be helpful for the molecular diagnosis of CCs in the era of precision medicine.

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