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Mutation analysis of 12 genes in Chinese families with congenital cataracts

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Purpose: To identify mutations in 12 genes in Chinese families with congenital cataracts.
Methods: Twenty five families with congenital cataracts involved in this study. The coding exons and adjacent intronic regions of 12 genes were analyzed by cycle sequencing, including the alpha A crystallin (*CRYAA*), alpha B crystallin (*CRYAB*), beta A1 crystallin (*CRYBA1*), beta A4 crystallin (*CRYBA4*), beta B1 crystallin (*CRYBB1*), beta B2 crystallin (*CRYBB2*), beta B3 crystallin (*CRYBB3*), gamma C crystallin (*CRYGC*), gamma D crystallin (*CRYGD*), gamma S crystallin (*CRYGS*), alpha 3 gap junction protein (*GJA3*), and alpha 8 gap junction protein (*GJA8*) genes. Novel variants were further evaluated in 96 normal controls.
Results: Nine mutations were identified in 10 of the 25 families (40%), including 5 novel (c.350_352delGCT in *CRYAA*, c.205C>T in *CRYAB*, c.106G>C in *CRYGD*, c.77A>G in *CRYGS*, c.1143_1165del23 in *GJA3*) and 4 known (c. 292G>A in *CRYAA*; c.215+1G>A and c.272_274delGAG in *CRYBA1*, and c.176C>T in *GJA3*). All novel mutations were predicted to be pathogenic and were not present in 96 controls.
Conclusions: Mutations in the 12 genes encoding crystallins and connexins were responsible for 40% Chinese families with congenital cataracts. Our results enriched our knowledge on the molecular basis of congenital cataracts in Chinese population.

Congenital cataract is a leading cause of childhood blindness, accounting for 10~38% of blindness in children. The prevalence of congenital cataract is estimated to be 0.6~6 per 10,000 live births [1,2]. Various etiological factors have been reported, including infection, neonatal asphyxia, malnutrition, and genetic defects. It was reported that 8.3%~25% of congenital cataracts were inherited [3]. Several genes have been identified to be associated with congenital cataracts [3,4], such as genes encoding crystallins, connexins and other membrane proteins, beaded filament proteins, growth and transcription factors, and others.

For cataract families with identified mutations, it has been suggested that about half had mutations in crystallins and a quarter in connexins (gap junction proteins) [3]. So far, mutations in 10 crystallin genes and 2 connexin genes have been identified to be responsible for congenital cataracts, including alpha A crystallin (*CRYAA*, OMIM 123580) [5-12], alpha B crystallin (*CRYAB*, OMIM 123590) [10, 13-15], beta A1 crystallin (*CRYBA1*, OMIM 123610) [16-23], beta A4 crystallin (*CRYBA4*, OMIM 123631) [24, 25], beta B1 crystallin (*CRYBB1*, OMIM 600929) [26-29], beta B2 crystallin (*CRYBB2*, OMIM 123620) [30-34], beta B3 crystallin (*CRYBB3*, OMIM 123630) [35,36], gamma C crystallin (*CRYGC*, OMIM 123680) [12,37-40], gamma D crystallin (*CRYGD*, OMIM 123690) [37,41-43], gamma S crystallin (*CRYGS*, OMIM 123730) [10,44,45], alpha 3 gap junction protein (*GJA3*, OMIM 121015) [34,46-50], and alpha 8 gap junction protein (*GJA8*, OMIM 600897) [40,51-56]. Several mutations in these genes have been identified but most reports are either based on one to a few gene(s) or one to a few family(ies). The exact mutation frequency of these genes in congenital cataract is unclear as comprehensive analysis of all the 12 genes in the same set of families is absent although a few studies reported analysis of some of them [10,29,36].

In this study, molecular genetic analysis was performed on all these 12 genes in 25 Chinese families with congenital cataract. Nine mutations, including 5 novel and 4 known, were identified in 10 of the 25 families (40%).

METHODS

Patients: Written informed consent conforming to the tenets of the Declaration of Helsinki and following the Guidance of Sample Collection of Human Genetic Diseases (863-plan) by the Ministry of Public Health of China were obtained from the participating individuals or their guardians before the study. Twenty five Chinese families with congenital cataracts were enrolled in this study. Genomic DNA was prepared from leukocytes of peripheral venous blood. This study was approved by the Institutional Review Board of the Zhongshan Ophthalmic Center, Sun Yat-sen University, Guangzhou, China.

Mutational screening: Bioinformation of the 12 genes was listed in Table 1, which was obtained from the National Center

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Table 1. Genomic information of the 12 genes referred in this study.

Gene	Genomic DNA	mRNA	Protein
CRYAA	NC_000021.8	NM_000394.2	NP_000385.1
CRYAB	NC_000011.9	NM_001885.1	NP_001876.1
CRYBA1	NC_000017.10	NM_005208.4	NP_005199.2
CRYBA4	NC_000022.10	NM_001886.2	NP_001877.1
CRYBB1	NC_000022.10	NM_001887.3	NP_001878.1
CRYBB2	NC_000022.10	NM_000496.2	NP_000487.1
CRYBB3	NC_000022.10	NM_004076.3	NP_004067.1
CRYGC	NC_000002.11	NM_020989.3	NP_066269.1
CRYGD	NC_000002.11	NM_006891.3	NP_008822.2
CRYGS	NC_000003.11	NM_017541.2	NP_060011.1
GJA3	NC_000013.10	NM_021954.3	NP_068773.2
GJA8	NC_000001.10	NM_005267.4	NP_005258.2

The information is based on human genome (Build 37.2).

for Biotechnology Information (NCBI). Polymerase chain reaction (PCR) was used to amplify the coding exons and adjacent intronic sequences of the 12 genes. The primer sequences used to amplify each coding exon and its adjacent regions of the 12 genes were referred to the previous publications [36,57-59] with modification for some primers (Appendix 1). The nucleotide sequences of PCR products were determined with the ABI BigDye Terminator cycle sequencing kit v3.1 on a genetic analyzer (ABI Applied Biosystems, Foster City, CA). Variations were identified by importing the sequencing results from patients and consensus sequences from the NCBI human genome database into the SeqManII program of the Lasergene package (DNAStar Inc., Madison, WI). Potential variants detected in patients were further evaluated in 96 normal controls by cycle sequencing.

Database and online tools: Mutation description followed the recommendation of the Human Genomic Variation Society (HGVS). The effects of novel missense mutations on the encoded proteins were further evaluated by Polymorphism Phenotyping (PolyPhen-2) [60,61] and Sorting Intolerant From Tolerant (SIFT) [62] at the protein level.

RESULTS

Based on complete analysis of the coding exons and their adjacent intronic regions in the 12 genes in 25 families, nine heterozygous mutations were detected in 6 genes in 10 families (Table 2, Figure 1). Of the nine mutations, five were novel (c.350_352delGCT in *CRYAA*, c.205C>T in *CRYAB*, c.106G>C in *CRYGD*, c.77A>G in *CRYGS*, c.1143_1165del23 in *GJA3*) and four were known (c.292G>A in *CRYAA*; c.215+1G>A and c.272_274delGAG in *CRYBA1*, and c.176C>T in *GJA3*). The c.272_274delGAG mutation in *CRYBA1* was present in two unrelated families. The pedigrees and cosegregation analyses of the 10 families with identified mutations were shown in Figure 2. All 5 novel mutations were not present in 96 normal controls.

Three of the five novel variants were missense mutations. Of the three, the c.205C>T (p.Arg69Cys) in *CRYAB* and the c.77A>G (p.Asp26Gly) in *CRYGS* involved highly conserved residues while the other one (p.Ala36Pro in *CRYGD*) replaced a nonconserved residue (Figure 3). The novel c.350_352delGCT mutation in *CRYAA* resulted in substitution of arginine at position 117 and deletion of tyrosine at position 118, where the two residues are highly conserved (Figure 3). The c.1143_1165del23 mutation resulted in frameshift with additional 48 new residues from residue 381.

The clinical data of the available patients with mutations were listed in Table 3 and cataract phenotypes for some patients were shown in Figure 4.

DISCUSSION

In this study, nine heterozygous mutations in the 12 genes encoding crystallins and connexins were identified in 10 out of 25 Chinese families (40%) with congenital cataract. Eight of the 25 families (32%) had mutations in crystallin genes and two of them (8%) had mutations in connexin genes.

For the congenital cataract families with identified mutations reported before, about three-fourths of the families had mutations in the 10 crystallin and 2 connexin genes, which was mostly based on studies of individual gene [3]. However, this mutation proportion may not reflect the actual frequency of these genes in congenital cataract, since the genes encoding crystallins and connexins might be more frequently analyzed. In fact, varied frequencies of mutation detection, mostly lower, were reported in several studies involving analysis of multiple genes. Devi et al. found crystallin mutations in 16.6% (10 of 60) Indian families by analyzing the 10 crystallin genes [10]. Burdon et al. [63] detected crystallin mutations in 5.3%

Table 2. Summary of mutations detected in patients with congenital cataracts in this study.

Gene	Nucleotide change	Amino acid change	Effect prediction		Frequency in		Note	References
			PolyPhen-2	SIFT	patients	controls		
CRYAA	c.292G>A	p.Gly98Arg	probably damaging	damaging	1/25	N/A	reported*	[7]
CRYAA	c.350_352 delGCT	p.[Arg117His, Tyr118del]	N/A	N/A	1/25	0/96	novel	
CRYAB	c.205 C>T	p.Arg69Cys	probably damaging	damaging	1/25	0/96	novel	
CRYBA1	c.215+1G>A	splicing donor abolished	N/A	N/A	1/25	N/A	reported	[10,16,19,20,63]
CRYBA1	c.272_274 delGAG	p.Gly91del	N/A	N/A	2/25	N/A	reported	[18,21-23]
CRYGD	c.106G>C	p.Ala36Pro	benign	damaging	1/25	0/96	novel	
CRYGS	c.77 A>G	p.Asp26Gly	probably damaging	damaging	1/25	0/96	novel	
GJA3	c.1143_1165del23	p.381fs*48	N/A	N/A	1/25	0/96	novel	
GJA3	c.176 C>T	p.Pro59Leu	probably damaging	damaging	1/25	N/A	reported	[36,50]

*Reported indicates that these known mutations have been reported previously in other families.

Table 3. The clinical information of the patients with congenital cataracts and identified mutations.

ID	Gene	Mutation	Gender	Age (yrs) at		Inheritance	Visual acuity (right;left)	Cataract types
				exam	onset			
QT237	*CRYAA*	c.292G>A	male	10	7	AD	0.2; 0.5	lamellar, punctate
QT237 II:1	*CRYAA*	c.292G>A	female	N/A	N/A	AD	0.6; 0.7	lamellar, Y-suture
QT261	*CRYAA*	c.350_352delGCT	male	5	at birth	AD	N/A; 0.2	N/A
QT192	*CRYAB*	c.205 C>T	male	N/A	N/A	AD	N/A	N/A
QT286	*CRYBA1*	c.215+1G>A	male	6	at birth	AD	0.3; 0.1	lamellar
QT456	*CRYBA1*	c.272_274delGAG	male	19	at birth	AD	0.1; 0.1	nuclear
QT174	*CRYBA1*	c.272_274delGAG	female	49	at birth	AD	FC; FC	nuclear
QT268	*CRYGD*	c.106G>C	male	40	at birth	AD	0.3; 0.2	nuclear
QT427	*CRYGS*	c.77 A>G	female	27	at birth	sporadic	N/A	coppock
QT206	*GJA3*	c.176 C>T	female	26	at birth	AD	0.6; 0.5	N/A
QT260	*GJA3*	c.1143_1165del23	female	17	at birth	AD	0.4; 0.2	punctate nuclear

FC: Finger counting. N/A: not available. AD: autosomal dominant.

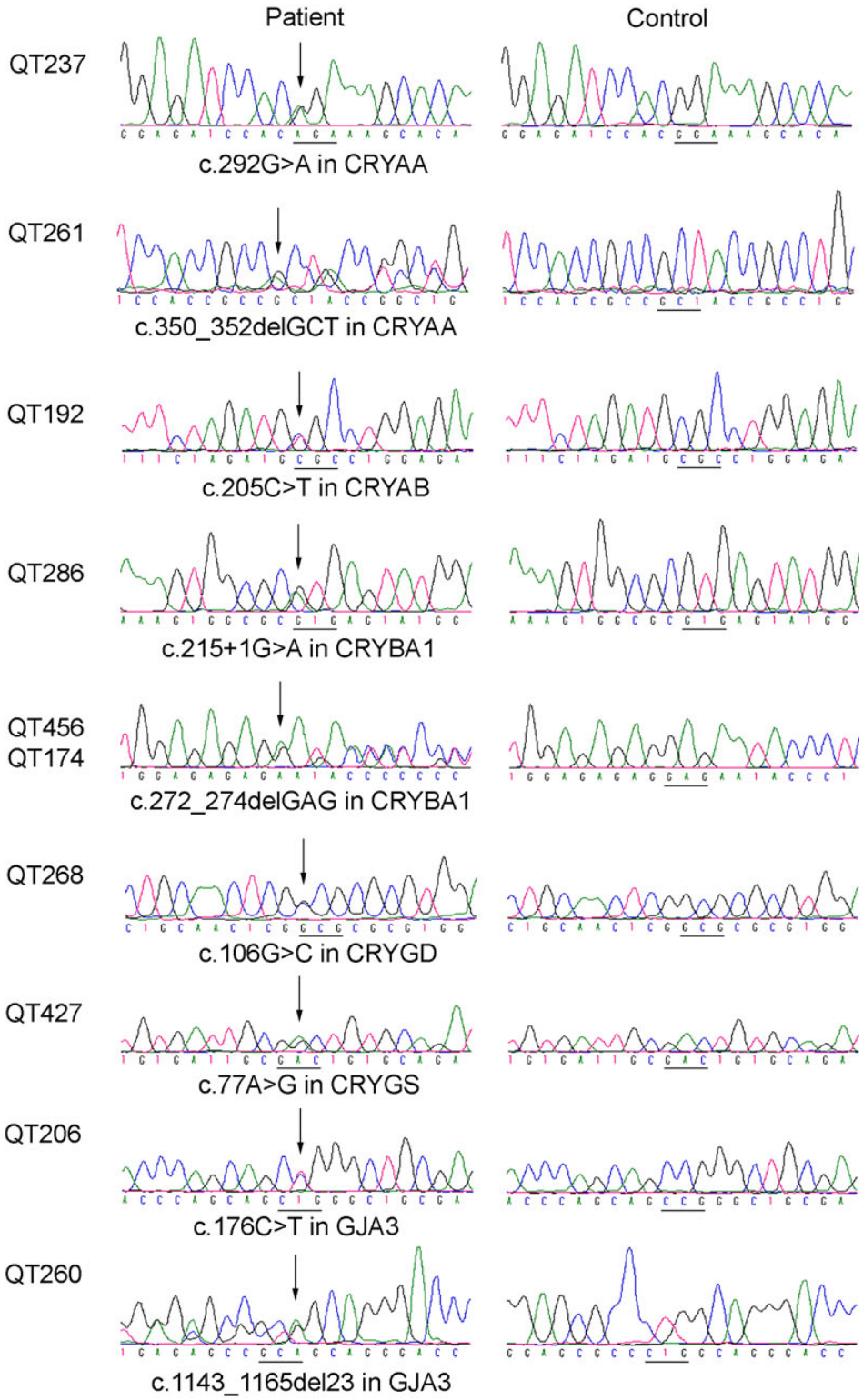

Figure 1. Sequence chromatography. The family number of each proband was shown in the left column. Sequences with mutations from probands were shown in the middle and those from normal controls were aligned on the right column. For families QT456 and QT174, only the mutant sequence of the proband from family QT456 was shown as both probands had the same mutation. Each mutation was described under the corresponding sequence.

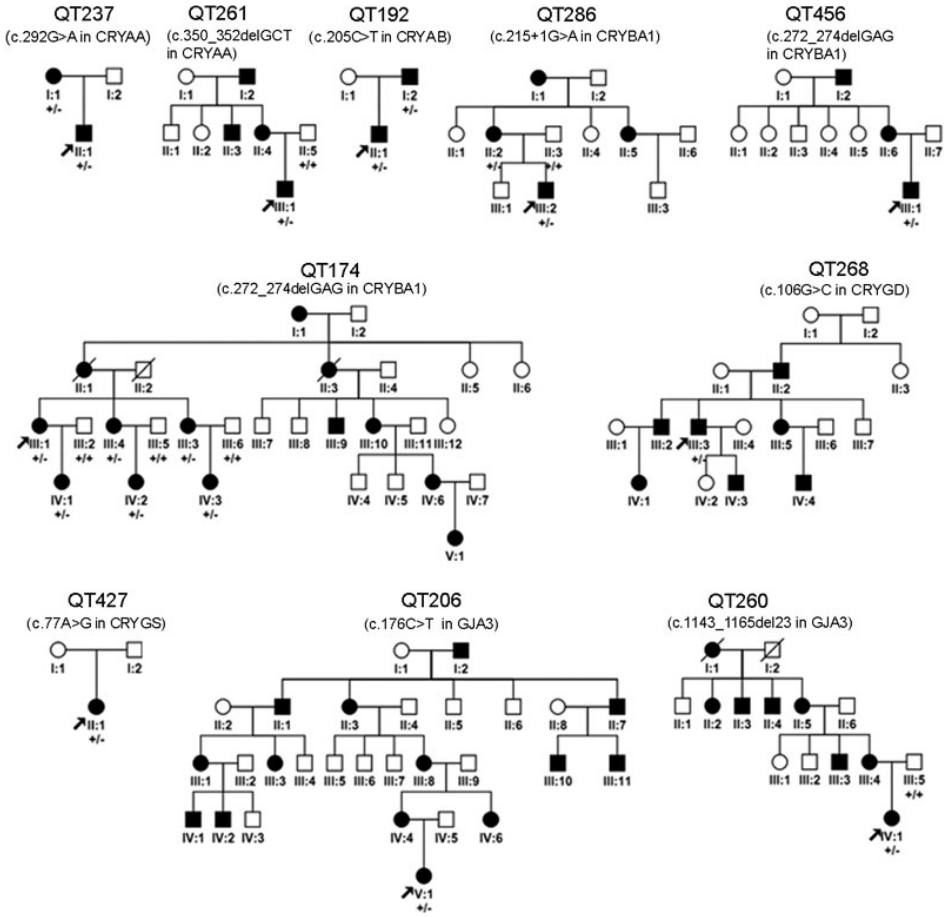

Figure 2. Pedigrees of the ten families with mutations. The family numbers and their corresponding mutations were shown just above the pedigree. The +/− indicated heterozygous mutation and the +/+ indicated wild type.

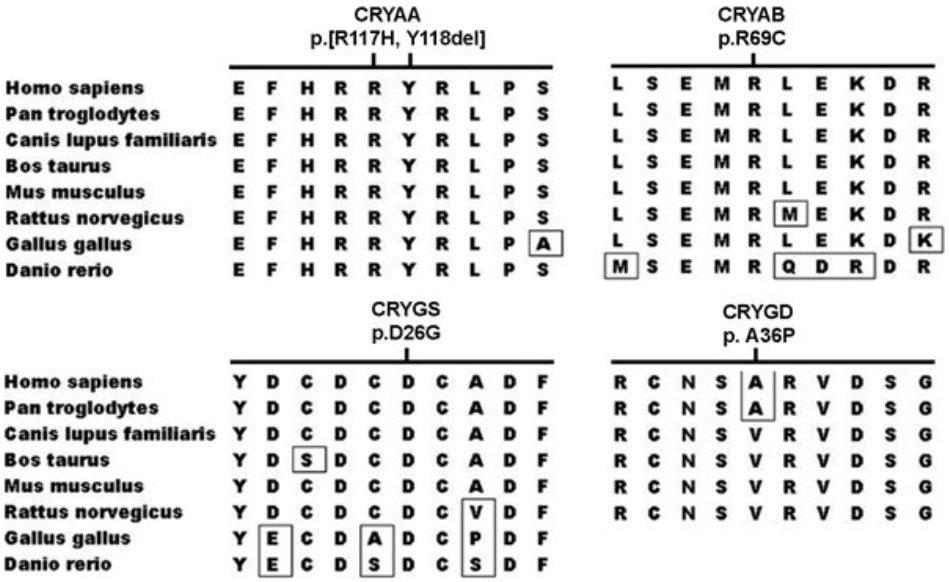

Figure 3. Conservation alignments of protein orthologs for 4 of the 5 novel mutations. The regions with p.[R117H,Y118del] in *CRYAA*, p.R69C in *CRYAB*, and p.D26G in *CRYGS* are highly conserved, while the p.A36P in *CRYGD* is not conserved (only 6 of the 8 orthologs available for *CRYGD*).

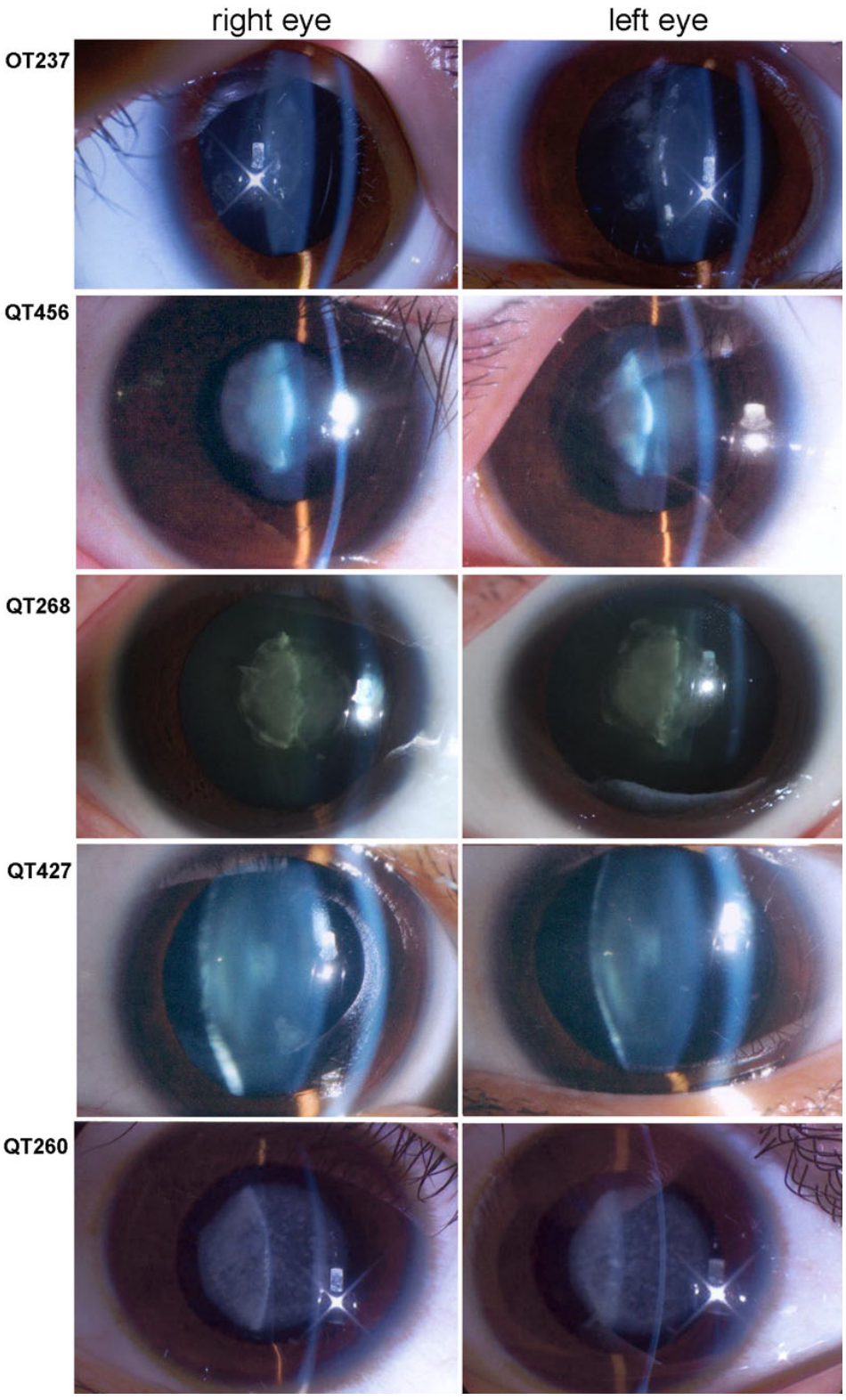

Figure 4. Lens photos showing cataract phenotypes in probands with identified mutations. Family number of each proband was listed in left column. The proband from family QT237 with the c. 292G>A mutation in *CRYAA* had bilateral lamellar and punctate cataract. The proband from QT456 with the c. 272–274delGAG mutation in *CRYBA1* had bilateral nuclear cataract. The proband from QT268 with the c. 106G>C mutation in *CRYGD* had bilateral nuclear cataract. The proband from QT427 with the c.77 A>G in *CRYGS* showed bilateral coppock cataract. The proband from QT260 with the c.1143–1165del23 mutation in *GJA3* had bilateral punctate nuclear cataract.

(2/38) Australian families by analyzing 7 crystallin genes. Hansen et al. [36] detected crystallin and connexin mutations in 35.7% (10/28) and 21.4% (6/28) Danish families, respectively. Kumar et al. [40] detected mutations in 20% (6/30) Indian families by analyzing 4 of the 12 genes. Wang et al. [29] identified mutations in 15% (3/20) Chinese families by analyzing 10 of the 12 genes. None of these studies performed analysis of all the 12 genes in the same set of families. In this study, we detected mutations in 40% families by analyzing all of the 12 genes. Overall, the frequencies of mutations in the 12 genes varied significantly in different ethnic populations but were more likely to be less than three fourth of families with congenital cataracts. The over-representation of mutations in these 12 genes is more likely due to more frequent studies on these genes.

Although several mutations in the 12 genes have been identified so far, we still identified five novel mutations in the Chinese families with congenital cataracts. Our results expand the mutation spectrum of these genes in Chinese population. The families without identified mutations will be good candidates for future study in screening for additional causative genes.

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Appendix 1

Primers used to amplify the coding and adjacent regions of the 12 genes. To access the data, click or select the words "Appendix 1." This will initiate the download of a Microsoft Excel (.xml) file that contains the data.

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