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# Identification and characterization of six β-crystallin gene mutations associated with congenital cataract in Chinese families

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## Abstract

**Background:** This study aims to identify the underlying genetic defects of β-crystallin (*CRYB*) genes responsible for congenital cataracts in a group of Chinese families.

**Methods:** Detailed family history and clinical data of six Chinese families with autosomal dominant congenital cataracts were recorded. Targeted exome sequencing was applied to detect the underlying genetic defects for the families. Generated variants were confirmed by PCR and sanger sequencing. Afterward, bioinformatic analysis through several computational predictive programs was performed to assess impacts of mutations on protein structure and function.

**Results:** A total of 53 participants (23 affected and 30 unaffected) from six unrelated Chinese families were recruited. Cataract phenotypes covered nuclear, total, posterior polar, pulverulent, snowflake-like, and zonular. Through targeted exome sequencing, six mutations in four β-crystallin genes were revealed which included five missense mutations *CRYBB1* p.Q70P, *CRYBB2* p.E23Q, *CRYBB2* p.A49V, *CRYBB2* R188C, *CRYBA4* p.M14K and one splice mutation *CRYBB3* c.75+1 G>A. In silico results predicted pathogenic for all four missense variants except variant *CRYBB2*-p.A49V yielded results as tolerant. The *CRYBB3* c.75+1 G>A splice site mutation was predicted to be deleterious by leading to a broken splice site, a premature stop codon, and subsequently resulting in a short peptide of 113 amino acids, which may affect protein features.

**Conclusion:** The obtained results expanded mutational and phenotype spectrum of β-crystallin genes and offer clues for pathogenesis of congenital cataracts. The data also demonstrated that targeted exome sequencing is valuable for providing molecular diagnostic information for congenital cataract patients.

**KEYWORDS**

autosomal dominant congenital cataract, gene mutation, genetic diagnosis, targeted exome sequencing, β-crystallin

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# 1 | INTRODUCTION

Congenital cataract, a major cause of childhood blindness, is opacity of the lens resulting from breakdown or aggregation of lens protein. Approximately one-third of congenital cataract cases are inherited either in isolation or as part of an ocular syndrome or systemic abnormalities, with autosomal dominance the major mode of inheritance (Berry et al., 2020). Among the aetiologies of hereditary cataract, genetic mutation, especially missense mutation, is likely the most common cause (Chan et al., 2012; Li et al., 2020). To date, over 50 candidate genes have been identified associated with isolated autosome dominant congenital cataract (https://cat-map.wustl.edu/), and almost half show mutations in genes encoding for crystallins (Pichi et al., 2016; Shiels & Hejtmancik, 2017). The crystallins constitute up to 90% of water-soluble proteins in human lens and play critical roles in maintenance of lens transparency (Ghosh & Chauhan, 2019). Mutations in crystalline genes would result in protein aggregation and consequently, opacity of the lens (Xi et al., 2014; Xu et al., 2018; Zhu et al., 2018). Among the crystalline-associated genes, β-crystallin is the most common reported gene cluster which includes six genes CRYBA1/A3 (OMIM 123610), CRYBA2 (OMIM 600836), CRYBA4 (OMIM 123631), CRYBB1 (OMIM 600929), CRYBB2 (OMIM 123620), and CRYBB3 (OMIM 123630) (Wistow, 2012). According to previously reported data, approximately 75 mutations in β-crystallin genes have been identified in sporadic and familial cataract cases and a long list of mutations remain to be characterized.

Despite remarkable improvements in cataract surgery, there are still limitations in eye surgery for infants (Louison et al., 2019). The research of non-surgical treatment of congenital cataract is expected to make a breakthrough. Very recently, findings of crystallin aggregation reversed by lanosterol and regeneration of human lens using endogenous stem cells of infants provide new insights to non-surgical treatment of congenital cataracts (Lin et al., 2017; Zhao et al., 2015). It is noteworthy that they are all based on crystallin gene mutation study. Identification of causative mutations in hereditary cataract patients should help clarify the pathology of congenital cataract formation and has significant relevance for developing new strategies for cataract prevention and early treatment.

Our study group has previously applied high-throughput targeted exome sequencing (TES) for mutation detection in congenital cataract patients and achieved a detection rate of approximately 60% (Zhai et al., 2017). TES, which can screen a set of candidate genes at each time, has been proved to be efficient for precise molecular diagnosis of congenital cataracts in several recent studies (Astiazaran et al., 2018; Gillespie et al., 2016; Ma et al., 2016). In the present study, using the same strategy as described, we successfully detected six mutations involving β-crystallin genes in a cohort of Chinese families. The findings in our study expand the mutational spectrum of β-crystallin genes in congenital cataracts and may offer clues for research of crystallin genes in pathogenesis of congenital cataracts.

# 2 | MATERIALS AND METHODS

## 2.1 | Ethical compliance

This study was approved by the Institutional Review Board of Second Affiliated Hospital of Zhejiang Medicine University and all study procedures conformed to the tenets of the Declaration of Helsinki.

## 2.2 | Patients and sample collection

Families with a positive history of inherited cataracts were recruited at Eye Center, the Second Affiliated Hospital, Zhejiang University School of Medicine, Hangzhou, China. Written informed consent was obtained from all participants or their guardians prior to the study. Detailed family history and medical records were collected. Presence and types of cataract phenotype in both affected and unaffected individuals were confirmed by slit-lamp bio-microscopy. 4 ml of peripheral venous blood samples were collected for genomic DNA extraction. One hundred unrelated individuals without eye diseases were recruited as normal controls.

## 2.3 | TES and validation

TES strategies were followed the same as described previously by our group (Zhai et al., 2017). DNA samples to be tested were prepared using Truseq DNA Sample preparation Kit (Illumina, Inc). A panel of amplicons targeting the coding exons, flanking regions, and promotor regions were designed to include 54 known cataract-related genes. After hybridization and enrichment, the custom amplicon samples were sequenced on the Illumina HiSeq2000 Sequencer with an average sequencing depth of 500-fold. Afterward, to confirm the suspected variants generated from TES, the target sites and their flanking sequences were amplified with PCR combined with sanger sequencing in all the individuals and in 100 normal controls.

## 2.4 | Bioinformatics analysis

The obtained variants were firstly analyzed in Human Gene Mutation Database (HGMD), NCBI SNP database

(dbSNP), and Cat-Map to confirm whether they were reported before. Pathogenicity of the missense variants was predicted by online algorithms including PROVEAN, SIFT, PolyPhen-2, and Mutation Taster to evaluate the functional impact of the amino acid substitution on the encoded protein. For splice site mutation, bioinformatic tools scSNV and SPIDEX software were employed to predict the pathogenicity. Multiple protein sequence alignments were performed using Clustal X to evaluate cross-species conservation. Kyte-Doolittle algorithm of ProtScale was adopted to analyze the hydrophobic properties and chemical parameters of protein. For secondary structure evaluation, online program GOR4 method was used to analyze alteration in the protein properties including alpha helix, beta bridge, extended strand as well as random coil. Three-dimensional (3D) models of both wild type and mutant proteins were generated using SWISS-MODEL server program. Illustrations were prepared using PyMOL.

## 3 | RESULTS

### 3.1 | Family enrollment and clinical data

A total of 53 participants (23 affected and 30 unaffected) from six unrelated Chinese families were recruited. All families exhibited isolated bilateral early onset cataracts with autosomal dominant mode of inheritance. The pedigree charts and phenotypes are provided in Figure 1.

Pedigree 1 is a four-generation family from which 15 individuals were enrolled (seven affected and eight unaffected). Ophthalmic examinations revealed bilateral nuclear cataract. According to the medical history, age of onset of cataract in this family was very early in childhood and all affected members needed surgery in the first decade of their life (Figure 1a).

Pedigree 2 is a four-generation family from which 15 individuals were enrolled (5 affected and 10 unaffected). The proband (IV:4) was a 17-year-old male presenting bilateral total cataract combined with spot-like cortex opacities. All other affected individuals displayed similar phenotype in their early age and were surgically removed later than their children and grandchildren (Figure 1b).

Pedigree 3 is a three-generation family from which 13 individuals were enrolled (5 affected and 8 unaffected). The cataract of the proband (II:2) was of posterior polar type with extensive pulverulent opacities scattered throughout the perinuclear cortex. High myopia and significant myopic fundus change were also noted in the proband. Whereas younger affected individuals (III:1, III:2) presented only with mild myopia and cataract phenotype was characterized as thin pulverulent opacities in perinuclear cortex. The other older affected individuals (II:3, II:5) both had cataract removal and were complicated by retinal detachment and poor visual function after retinal surgeries (Figure 1c).

Pedigree 4 has a relatively small family size, including 2 affected and 1 unaffected individual. The proband (II:1) was a 2-year-old boy whose parents observed opacified lens for the first time when he was 6 months old which showed opacities in the posterior pole of lens. His father (I:2) also started to have poor vision at a very young age and had a bilateral posterior pole cataract (Figure 1d).

Pedigree 5 also had a relatively small family size, including two affected and two unaffected individuals. The proband (II:1) exhibited bilateral snowflake-like opacities in lens cortex. However, her mother (I:2) displayed milder but similar cataract phenotype as her daughter (Figure 1e).

Pedigree 6 consists of 3 individuals (two affected and one unaffected). The proband (I:2) was diagnosed as bilateral zonular cataract and his 2-year-old son (II:1) displayed similar phenotype. We failed to obtain photograph of II:1 because he is in early childhood. Both patients in this family were unaware of their cataracts until the examinations (Figure 1f).

### 3.2 | Mutation analysis

Targeted exome sequence enriched 551 exons and covered 95.4% targeted regions, with an average median depth of 500× across all samples and accuracy of a variant call more than 99%. Through TES, we detected a total of six mutations in four different β-crystallin genes, they were missense mutations c.209A>C (p.Q70P) of *CRYBB1* in family 1, c.67G>C (p.E23Q), c.146C>T (p.A49V), c.562C>T (p.R188C) of *CRYBB2* in family 2, 3, 4, respectively, c.41T>A (p.M14K) of *CRYBA4* in family 5, and one splice mutation c.75+1 G>A of *CRYBB3* gene in family 6 (Figures 2a–e and 4a). The results of sanger sequencing indicated that each of these mutations showed complete co-segregation with the disease and were not detected in 100 normal controls.

### 3.3 | Bioinformatics analysis

Bioinformatics analysis by PROVEAN, SIFT, PolyPhen-2, and Mutation Taster of the missense mutations were listed (Figure 2f). In silico results predicted pathogenic of all the four variants *CRYBB1* p.Q70P, *CRYBB2* p.E23Q, *CRYBB2* p.R188C, and *CRYBA4* p.M14K, while the variant *CRYBB2*-p.A49V yielded results as tolerant (Figure 2f). Multiple sequence alignments indicated that Glutamine (Q) at position 70 of βB1-crystallin, Glutamic (E) at position 23, and Arginine (R) at position 188 of βB2-crystallin were highly conserved among different species. While Alanine (A) at position 49 of βB2-crystallin and Methionine (M) at position 14 of BA4-crystallin were relatively conserved

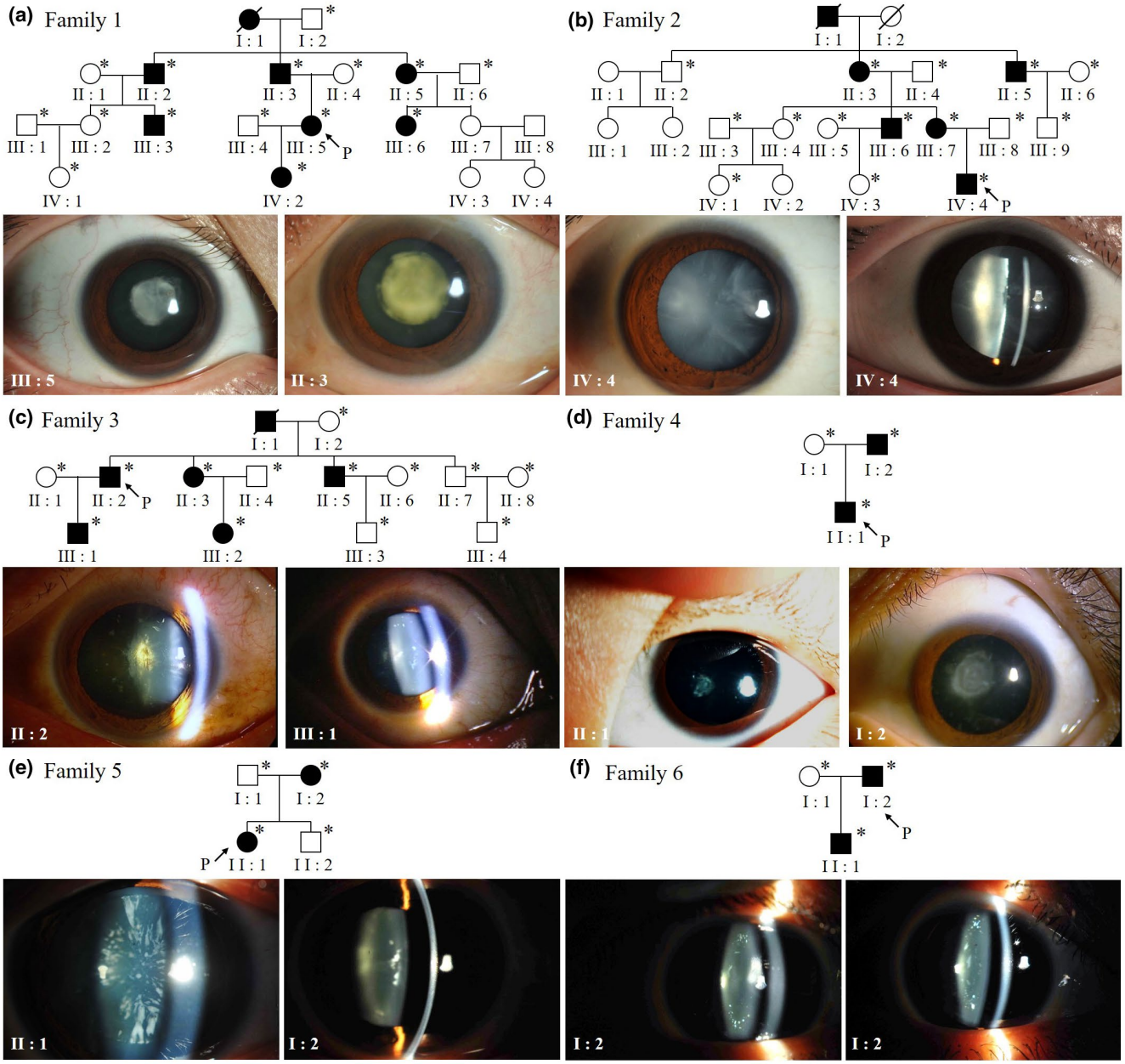
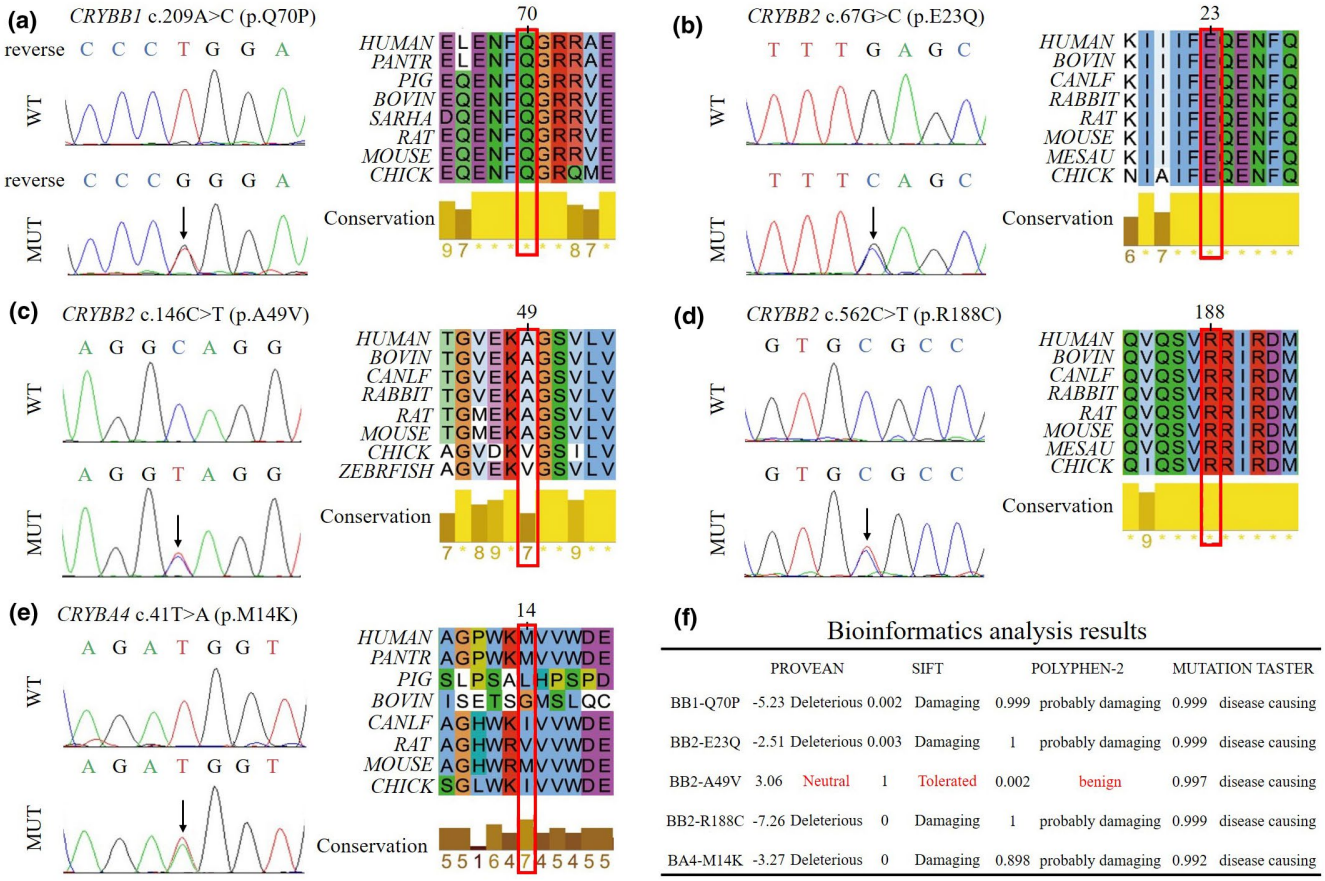



**FIGURE 1** Pedigrees and Cataract phenotypes. (a) Pedigree chart of Family 1 and phenotype of nuclear cataract in the affected member (III:5 and II:3). (b) Pedigree chart of Family 2 and phenotype of total cataract in the proband (IV:4). (c) Pedigree chart of Family 3 and phenotype of posterior polar cataract in the affected member (II:2 and III:1). (d) Pedigree chart of Family 4 and phenotype of posterior pole cataract in the affected member (II:1 and I:2). (e) Pedigree chart of Family 5 and phenotype of snowflake-like cataract in the affected member (II:1 and I:2). (f) Pedigree chart of Family 6 and phenotype of zonular cataract in the affected member (II:1). Squares and circles indicate males and females, respectively. Open and filled symbols indicate unaffected and affected individuals, respectively. The proband is marked with an arrow and the diagonal line indicates a deceased individual. Asterisks indicate individuals attend this study

(highlighted in red box, Figure 2a–e). By ProtScale analysis, local hydrophobicity at and near the altered R188 region significantly increased, around the altered Q70 and A49 region relatively increased, and around the altered M14 region dramatically decreased. Hydrophobicity at the mutant site and neighboring regions of E23 did not show any change compared to normal protein (highlighted in red box, Figure 3a–e). Consistently, molecular modeling by GOR4 also showed that *CRYBB2* p.R188C mutation caused significant change in secondary structure which led to replacement of extended strand with random coil. The *CRYBB1* p.Q70P and *CRYBA4* p.M14K mutation also altered the secondary structure while *CRYBB2* p.E23Q did not show any change (highlighted in red box, Figure 3a–e). 3D models indicated different structures of the altered amino acid between wild-type and mutant proteins (Figure 3a–e). The *CRYBB3* c.75+1 G>A mutation was




| | PROVEAN | | SIFT | | POLYPHEN-2 | | MUTATION TASTER | |
|---|---|---|---|---|---|---|---|---|
| BB1-Q70P | -5.23 | Deleterious | 0.002 | Damaging | 0.999 | probably damaging | 0.999 | disease causing |
| BB2-E23Q | -2.51 | Deleterious | 0.003 | Damaging | 1 | probably damaging | 0.999 | disease causing |
| BB2-A49V | 3.06 | Neutral | 1 | Tolerated | 0.002 | benign | 0.997 | disease causing |
| BB2-R188C | -7.26 | Deleterious | 0 | Damaging | 1 | probably damaging | 0.999 | disease causing |
| BA4-M14K | -3.27 | Deleterious | 0 | Damaging | 0.898 | probably damaging | 0.992 | disease causing |

**FIGURE 2** Sequence chromatograms and in silico predictions. (a–e) Sequence chromatograms and multiple sequence alignments for c.209A>C (p.Q70P) of *CRYBB1* in family 1, c.67G>C (p.E23Q) of *CRYBB2* in family 2, c.146C>T (p.A49V) of *CRYBB2* in family 3, c.562C>T (p.R188C) of *CRYBB2* in family 4, c.41 T>A (p.M14K) of *CRYBA4* in family 5. (f) Bioinformatics analysis by PROVEAN, SIFT, PolyPhen-2, and Mutation Taster of the missense mutations

also predicted highly damaging by Mutation Taster (score of 0.999), scSNV (ADA_SCORE of 1.000, and RF_SCORE of 0.954), and SPIDEX (dpsi_zscore of −3.421). According to BDGP and NetGene2 tools, the mutation was predicted to have a high risk of leading to a broken site, subsequently, a new splice site was generated and a premature stop codon was present after 88 codons resulting in a short peptide of 113 amino acids (Figure 4b). Computer-assisted prediction by ProtScale and GOR4 showed that the mutation remarkably altered hydrophobicity, secondary, and tertiary structure features of βB3-crystallin (Figure 4c,d).

## 4 | DISCUSSION

Transparency of the lens is achieved by precise architecture of the lens proteins in terms of their concentration, stability, and supramolecular organization (Hejtmancik et al., 2015; Jaenicke, 1994). Any subtle alterations in protein structure or molecular properties could cause degradation or aggregation, ultimately leading to lens opacity (Moreau & King, 2012). Crystallin is the most abundant protein in the human lens and crystallin genes are the largest known gene family linked with congenital cataracts, among which pathogenic mutations have been mostly reported in β-crystallin gene-cluster (Li et al., 2020; Shiels & Hejtmancik, 2017). The current study reported six mutations affecting different members of β-crystallin gene family which were causative for hereditary cataract patients. We believe our finding of these disease-causing mutations were of importance for study of congenital cataracts.

*CRYBB1* p.Q70P mutation identified in Family 1 was associated with congenital nuclear cataract. It was a recurrent mutation which was reported before (Ji et al., 2020). βB1-crystallin comprises 9% of total crystallin in human lens and is a major subunit of the β-crystallin (Lampi et al., 1997). A total of 23 mutations in *CRYBB1* have been identified in sporadic or familial cataract cases and almost half produced nuclear cataract, which was consistent with its high expression level in lens nucleus (Huang & Xie, 2010) (Figure 5a). 3D models revealed substitution of a highly conserved hydrophilic polar Glutamine by a hydrophobic unpolar Proline in Greek key I of βB1-crystallin, consequently, the hydrophobicity index of the corresponding region increased and

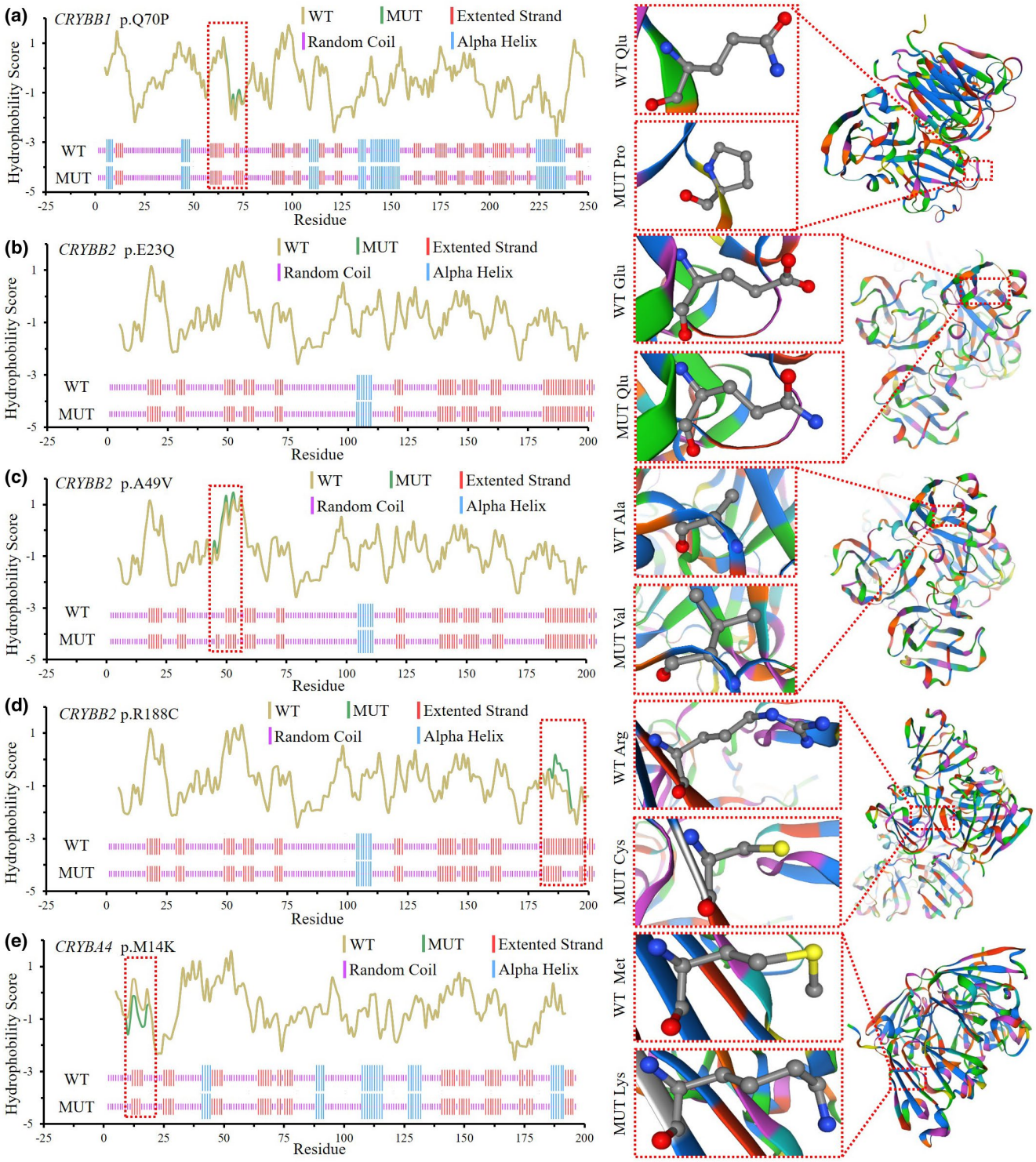



**FIGURE 3** Hydrophobicity, secondary, and tertiary structure prediction. (a) Local hydrophobicity at and near the altered *CRYBB1* Q70 region relatively increased, the secondary structure and the amino acid structure altered. (b) Local hydrophobicity at and near the *CRYBB2* p.E23Q region and the secondary structure did not show any change, the amino acid structure altered. (c) Local hydrophobicity at and near the altered *CRYBB2* A49 region relatively increased, the secondary structure and the amino acid structure altered. (d) Local hydrophobicity at and near the altered *CRYBB2* R188 region significantly increased, the secondary structure and the amino acid structure significantly altered. (e) Local hydrophobicity at and near the altered *CRYBA*4 M14 region dramatically decreased, the secondary structure and the amino acid structure altered

secondary structure of the protein changed, as illustrated in silico results. Two previously described mutations S228P and X253R, also located in Greek key motif of βB1 crystallin, had been shown to promote βB1-crystallin aggregation and degradation, ultimately leading to lens opacification (Leng et al., 2016; Qi et al., 2016). We speculated the *CRYBB1*

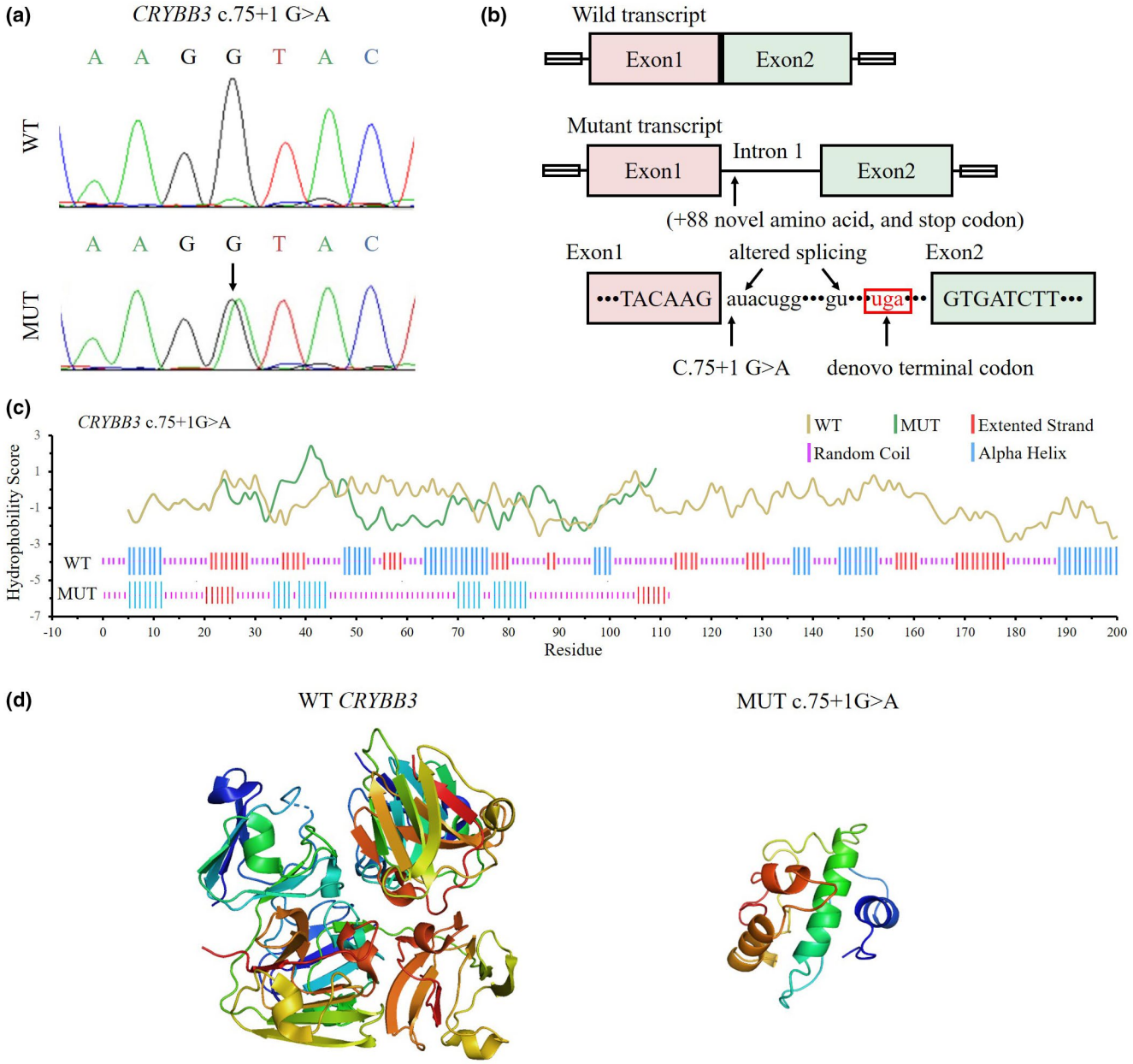



**FIGURE 4** Sequence chromatograms, hydrophobicity, secondary, and tertiary structure prediction. (a and b) Sequence chromatograms of *CRYBB3* c.75+1 G>A and NetGene2 prediction of a new splice site and a premature stop codon. (c) Hydrophobicity and the secondary structure of wild type and mutant protein. (d) 3-D structure of the mutant CRYBB3 protein which presented a short peptide

p.Q70P mutation may also produce cataract in human lens through crystallin aggregation formation and degradation. In all, our data above together suggested that *CRYBB1* p.Q70P mutation is sufficient to be the disease-causing mutation for congenital cataract in the family.

βB2-crystallin is the most abundant β-crystallin and mutations in *CRYBB2* account for approximately half cataract families (Lampi et al., 1997) (Figure 5b). Most *CRYBB2* mutations are found in Greek key III and IV and acted as Greek-key motif breakers which impact on protein folding, solubility, dynamic oligomeric equilibrium, or protein-protein interaction, consequently leading to cataract formation (Chen et al., 2013; Zhao et al., 2017). In the present study, three missense mutations of *CRYBB2* p.E23Q, p.A49V, and p.R188C were detected. The R188 residue located in the most reported Greek key IV motif was a recurrent mutation site in which a previously reported mutation p.R188H was identified in a family with anterior axial embryonal nuclear cataract(Weisschuh et al., 2012). While p.R188C mutation was identified in a posterior pole cataract family in the present study. The difference in phenotypes clearly demonstrated genetic and clinical discrepancy of congenital cataracts. Our previous study of p.R188H revealed that the mutation promoted βB2-crystallin aggregation through perturbing the dynamic

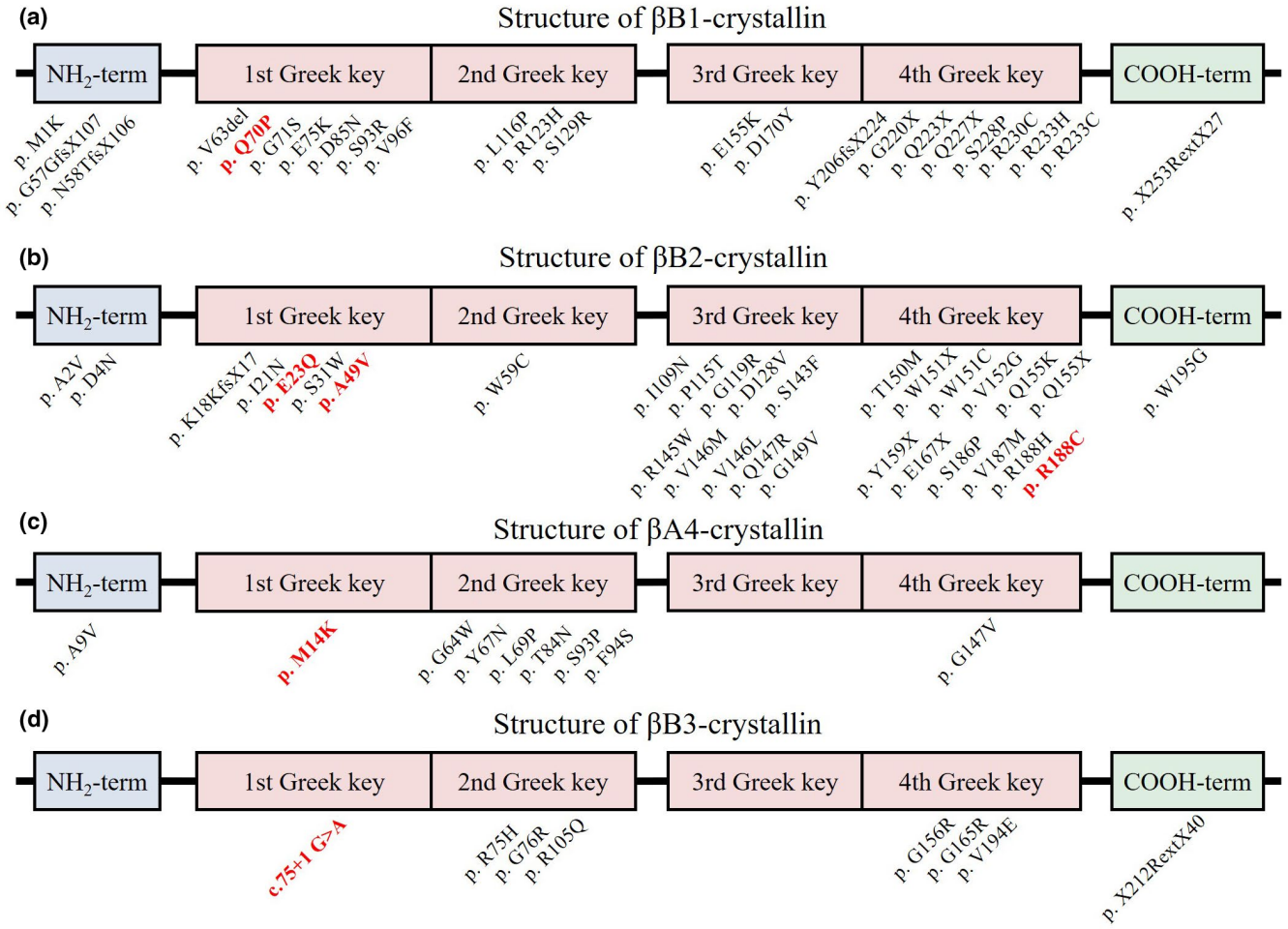



**FIGURE 5** Schematic of the human βB-crystallin protein and previously reported variants. (a–d) Human βB1-crystallin, βB2-crystallin, βA4-crystallin, and βB3-crystallin protein is folded in four Greek key motifs. The mutations identified in this study were highlighted in red

oligomeric equilibrium, which highlights the importance of the last strand of β-crystallin in congenital cataract formation (Xi et al., 2014). We speculated the pathogenic mechanism of p.R188C mutation might be similar. The p.E23Q mutation led to the replacement of a highly conserved amino acid in Greek key I and in silico results suggested that the variation was most likely pathogenic. However, as both Glutamic (E) and Glutamine (Q) are hydrophilic, no significant change was observed in protein hydrophobicity or secondary structure. We could preliminarily consider that this alteration might influence the protein properties other than protein hydrophobicity or solubility, to cause cataract formation. The p.A49V mutation was completely co-segregated with congenital cataract in Family 3. However, bioinformatics analysis indicated that this mutation may not be pathogenic, which raises doubt about its pathogenicity. We could not exclude this to be a rare polymorphism. However, no functional study on mutations in Greek key I have been reported. Further experimental investigations are highlighted to elucidate the question.

To date, 8 pathogenic mutations have been reported in *CRYBA4* gene which most occurred in Greek Key II motif (Figure 5c). In the present study, a p.M14K mutation was identified underlying a congenital snow-like cataract family, which was the first reported mutation located in Greek Key I. By multiple sequence alignments, the M14 residue was partially conserved but always replaced by hydrophobic residues. Protein structure modeling showed that the substitution from a hydrophobic nonpolar Methionine (M) to hydrophilic polar Lysine (K) was predicted to reduce the hydrophobicity in the corresponding region and alter the secondary structure of the CRYBA4 protein, as a result, the mutant structure would be unstable and protein folding would consequently be impaired, most probably leading to protein aggregation. One previously reported mutation p.G64W in Greek Key II motif of CRYBA4 has been studied which led to protein misfolding, decreased stability, and blocked interaction with CRYBB1, consequently resulting in disturbance of lens transparency (Li et al., 2019). We also speculated that each of the above effects is expected to involve in the pathogenesis of p.M14K mutation.

βB3-crystallin is the least well studied (Riazuddin et al., 2005). The identified mutation c.75+1 G>A was the first report of splice site mutation of *CRYBB3* (Figure 5d). The G at

position c.75+1 splice site is a highly conserved donor site. The mutation was expected to cause wrong junction of the exons in CRYBB3 which has direct influence on splicing of mature mRNA. As predicted, the mutation recruited a new cryptic splice site downstream the mutation site (position 208, +1741nt, 0.48), consequently, a UGA premature stop codon of the polypeptide was present after 88 codons, which mimics a nonsense mutation at the protein level resulting in a short peptide of 113 amino acids. The truncation of protein may further affect function of the CRYBB3 protein. In this condition, we could preliminarily consider that the surveillance of mRNAs for transcripts cannot be completely translated leading to their rapid degradation by nonsense-mediated decay (NMD), which would cause improper folding and structural instability of βB3-crystallin (Hug et al., 2016; Kurosaki & Maquat, 2016).

Detection of gene mutations would help to understand the function of crystalline and pathophysiology of cataracts. However, the large number of crystalline genes known to cause congenital cataracts complicate molecular diagnosis using traditional sequencing. In the present study, we diagnose congenital cataract genetically using enrichment of targeted genes and proved that it is a rapid, high-throughput, and cost-efficient method.

In conclusion, our findings expanded the mutation spectrum of β-crystallin gene mutations in congenital cataracts as well as their associated phenotypes. The results provided further evidence for importance of β-crystallin to congenital cataractogenesis. Identifications of causal variants associated with cataract aid in a better understanding of the biology of ocular lens and pathogenesis of cataract. Further research into the functional effects of these mutations, is warranted to clarify pathogenicity.

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## CONFLICT OF INTEREST

The authors declare no conflict of interest.

## AUTHORS CONTRIBUTIONS

K. Y and Y. Y conceived and designed the study. Y. Y and J. L helped with patient recruitment and clinical information. Y. Q and Y. Y collected the blood samples and performed the ophthalmic examinations. Y. Y, Y. Q, and Y. Y performed the molecular experiments and analyzed the data. Y. Y wrote the paper. All authors read and approved the final manuscript.

## DATA AVAILABILITY STATEMENT

The data that support the findings during the current study may be available from the corresponding author upon reasonable request.

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## SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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