

A novel γ D-crystallin mutation causes mild changes in protein properties but leads to congenital coralliform cataract

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Purpose: To identify the genetic lesions for congenital coralliform cataract.

Methods: Two Chinese families with autosomal dominant coralliform cataract, 12 affected and 14 unaffected individuals, were recruited. Fifteen known genes associated with autosomal dominant congenital cataract were screened by two-point linkage analysis with gene based single nucleotide polymorphisms and microsatellite markers. Sequence variations were identified. Recombinant FLAG-tagged wild type or mutant γ D-crystallin was expressed in human lens epithelial cells and COS-7 cells. Protein solubility and intracellular distribution were analyzed by western blotting and immunofluorescence, respectively.

Results: A novel heterozygous change, c.43C>A (R15S) of γ D-crystallin (*CRYGD*) co-segregated with coralliform cataract in one family and a known substitution, c.70C>A (P24T), in the other family. Unaffected family members and 103 unrelated control subjects did not carry these mutations. Similar to the wild type protein, R15S γ D-crystallin was detergent soluble and was located in the cytoplasm. ProtScale and ScanProsite analyses revealed raised local hydrophobicity and the creation of a hypothetical casein kinase II phosphorylation site.

Conclusions: A novel R15S mutation caused congenital coralliform cataract in a Chinese family. R15S possessed similar properties to the wild type γ D-crystallin, but its predicted increase of hydrophobicity and putative phosphorylation site could lead to protein aggregation, subsequently causing opacification in lens.

Congenital cataract refers to lens opacification presented at birth or developed shortly thereafter. Its prevalence is up to 7.2 per 10,000 live births and renders about 10% of childhood blindness worldwide [1-7]. If left untreated, permanent visual loss usually occurs. Various etiological factors have been identified including infection, metabolic disorders, and genetic defects. About 18% of affected children have known family history of cataract [8]. The most common mode of genetic lesion is a single gene determinant in Mendelian inheritance. Autosomal dominant congenital cataract (ADCC) is a major form. Autosomal recessive and X-linked inheritance also exists [9]. According to the outward appearance, size, and location of lens opacity, congenital cataract (CC) is classified into various subtypes: whole lens, nuclear, lamellar, cortical, polar, sutural, pulverulent, cerulean, coralliform, and other minor subtypes [10,11]. The development of each type of cataract can be caused by distinctive etiological factors, especially defects in lens crystallins [9].

More than 20 genes out of 34 genetic loci mapped for isolated congenital cataract have been identified with specific mutations [12]. More than half of CC families carry mutations in 10 crystallin genes (namely crystallin alpha A (*CRYAA*), crystallin alpha B (*CRYAB*), crystallin beta B1 (*CRYBB1*), crystallin beta B2 (*CRYBB2*), crystallin beta B3 (*CRYBB3*), crystallin beta A1 (*CRYBA1*), crystallin beta A4 (*CRYBA4*), crystallin gamma C (*CRYGC*), crystallin gamma D (*CRYGD*) and crystallin gamma S (*CRYGS*). About 25% of affected families have gene defects in membrane transport genes (major intrinsic protein of lens fiber (*MIP*), gap junction protein alpha 8 (*GJA8*), gap junction protein, alpha 3 (*GJA3*) and transmembrane protein 114 (*TMEM114*) [13] and lens intrinsic membrane protein 2 (*LIM2*). The remaining are caused by mutations in genes encoding cytoskeletal proteins (beaded filament structural protein 1 (*BFSP1*) [14] and beaded filament structural protein 2 (*BFSP2*)), transcription factors (paired-like homeodomain 3 (*PITX3*), v-maf musculoaponeurotic fibrosarcoma oncogene homolog (*MAF*), and heat shock transcription factor 4 (*HSF4*)), chromatin modifying protein (*CHMP4B*) [15], and glucosaminyl transferase 2 (*GCNT2*) [16]. Forkhead box E3 (*FOXE3*), eyes absent homolog 1 (*EYA1*), and paired box gene 6 (*PAX6*) have been reported to cause congenital cataract in some patients associated with other anterior segment anomalies [9]. The same mutation in different families or even within a family can result in drastically different morphologies and severity of lens opacification. On the other

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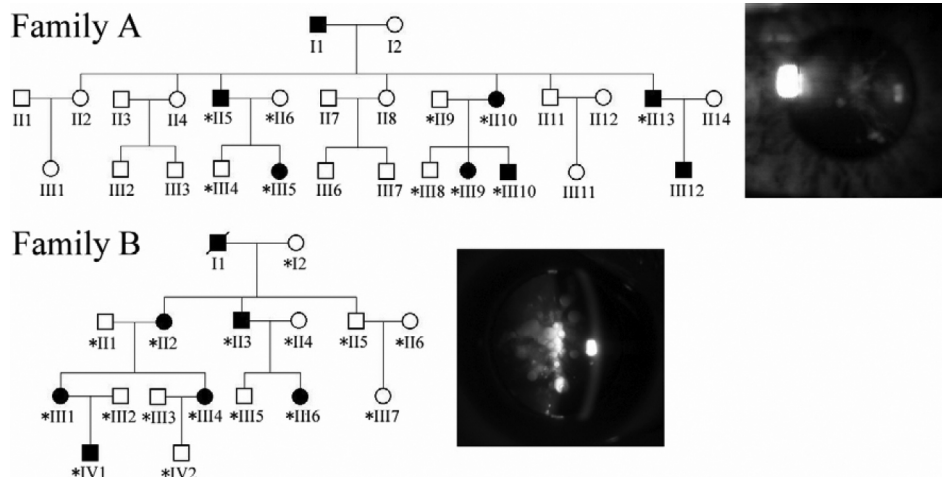


Figure 1. Pedigree of coralliform cataract families. The asterisk indicates family members who attended this study. The lens photograph from II-13 in Family A showed a line-shape opacity with a larger white dot end and tubular opacity radiating from the center of the lens. The lens photograph from II-2 in Family B showed a tubular and irregular opacity extending from center of the lens. Large white dots accumulated at the end of tubular protrusions.

hand, similar or identical cataract presentation may arise from mutations of different genes. These observations suggest that additional genes or modifying factors such as environmental regulators could play important roles in cataract onset, progression, and maturation.

CRYGD is a structural protein essential for lens transparency. Mutations of *CRYGD* are common genetic lesions causing different types of congenital cataracts. Among the reported families with congenital cataract caused by mutations of crystallin, one-third of them were associated with *CRYGD*. Until now, a total of 11 cataract-causing mutations (UniProt) have been reported including R15C, P24S, P24T, R37S, R59H, G61C, E107A, Y134X, W156X, G165fsX8, and R168W [17-21]. In this study, we added a novel R15S mutation to this list, affirming a causative role of *CRYGD* in coralliform type of congenital cataract.

METHODS

Patients and controls: This study adhered to the tenets of the Declaration of Helsinki and was approved by the ethics committees for medical research at The Chinese University of Hong Kong (Hong Kong, China) and Zhejiang University (Hangzhou, China). Two Chinese families with autosomal dominant congenital cataract were recruited at the University Eye Center (The Chinese University of Hong Kong; Family A; Figure 1) and the Department of Ophthalmology at the First Affiliated Hospital (College of Medicine at Zhejiang University; Family B; Figure 1). A total of 26 family members including 12 affected and 14 unaffected individuals attended this study, and informed consents were obtained from all participants. Unrelated Chinese control subjects ($n=103$) attending the hospital clinics for ophthalmic examinations were also recruited. They did not have any eye diseases except senile cataract and mild floaters. All subjects underwent complete ophthalmoscopic examinations. Family history and ophthalmic examination were documented by senior ophthalmologists. Peripheral venous blood was collected for

genomic DNA extraction using QIAamp DNA kit (Qiagen, Valencia, CA).

Candidate gene screening: Fifteen candidate genes that account for most cases of ADCC were taken for linkage analysis (Table 1). A gene exclusion strategy was conducted by screening with single nucleotide polymorphisms (SNPs) and microsatellite markers. We obtained gene-based SNP markers in Han Chinese from HapMap with ABI SNP browser v.3.5 (Applied Biosystems, Foster, CA). TaqMan SNP genotyping assay and allelic discrimination was conducted on an ABI PRISM 7000 sequence detection system (Applied Biosystems). For genes without informative SNP markers or those that could not be excluded by SNP linkage analysis, microsatellite markers flanking to the target genes were chosen from the Marshfield genetic map. GeneScan was conducted on ABIPRISM[®] 377 DNA sequencer (Applied Biosystems). The pedigree and genotyping data were managed by GenoPedigree 1.0 and GeneBase 2.0.1. (Applied Biosystems). Two point LOD scores were calculated by the MLINK subprogram of FASTLINKAGE v.4.1P. A gene frequency of 0.0001 and penetrance of 100% were assumed for ADCC.

Mutation analysis: All coding exons and splice sites of *CRYAA*, *CRYAB*, *CRYGC*, *CRYGD*, and *MAF* were sequenced using BigDye terminator v3.1 cycle sequencing kit (Applied Biosystems) and specific primers (Table 2) and detected by ABIPRISM[™] 377 DNA sequencer. The data were compared with sequences from NCBI GenBank (*CRYAA*: NM_000394, *CRYAB*: NG_009824; *CRYGC*: NM_020989; *CRYGD*: NM_006891.3; *MAF*: NM_001031804).

Computational analysis: Effects of amino acid changes on the *CRYGD* protein structure, the isoelectric point (pI), and molecular weight (MW) were examined by ExPASy proteomics. Local hydrophobicity was predicted by ProtScale. The protein sequence was scanned by ScanProsite to predict the effect of the mutation on specific motifs.

TABLE 1. SNPs AND MICROSATELLITE MARKERS SELECTED IN 15 KNOWN ADCC CANDIDATE GENES FOR THE LINKAGE ANALYSIS.

Gene	Gene ID	Chromosome location	SNPs	STRs
<i>CRYAA</i>	1409	21q22.3	rs870137	D21S1890 D21S266 D21S1255
<i>CRYAB</i>	1410	11q22–22.3	rs2070894 rs1940392 rs14133 rs10502149	D11S1986 D11S4078 D11S1793 D11S4151
<i>CRYBB1</i>	1414	22q11	rs4822752 rs5752351 rs2071859 rs4822749 rs5761618	
<i>CRYBB2</i>	1415	22q11.2	rs739315 rs5752084 rs969623	D22S315 D22S1174
<i>CRYBA1</i>	1411	17q11.1–12	rs8080081	
<i>CRYGC</i>	1420	2q33–35	rs2242071	D2S2208 D2S2361
<i>CRYGD</i>	1421	2q33–35	rs2305429 rs3774803	D3S1262 D3S3570
<i>GRYGS</i>	1427	3q25-qter	rs11917060 rs1447670 rs4686428	
<i>MIP</i>	4284	12q12	rs7953824 rs2269348 rs3809125 rs10876890	D12S1632 D12S1691
<i>GJA8</i>	2703	1q21–25	rs7541950 rs2132397	D1S498 D1S442
<i>GJA3</i>	2700	13q11–13	rs4769953 rs1886176	
<i>BFSP2</i>	8419	3q21–22	rs1153876 rs666067 rs4854585 rs6762405 rs931099	D3S1290 D3S3713 D3S3657 D3S1292
<i>PITX3</i>	5309	10q24–25	rs3758553	D10S1267
<i>MAF</i>	4094	16q23.2	rs2288066	D16S3040 D16S504 D16S503
<i>HSF4</i>	3299	16q22	Hcv25749941 Hcv25613880 rs11642409 rs9033	

Gene name, gene ID in NCBI [GeneBank](#), and the chromosomal location for 15 candidate genes were shown in this table. All single nucleotide polymorphism (SNP) markers and microsatellite markers used in this study were listed, respectively.

CRYGD expression, mutagenesis, and transfection: Human full-length wild type *CRYGD* was cloned to p3XFLAG-myc-CMVTM-25 (Sigma, St Louis, MO) for epitope tagging to generate pFLAG/myc-*CRYGD*^{WT} [19]. Missense mutations were introduced by a site-directed mutagenesis kit (Stratagene, La Jolla, CA) with specific oligonucleotides (Table 3). Correctness of the construct sequence was

confirmed by direct sequencing. Preparation of pFLAG-*CRYGD*^{G165fs} was described as before [19]. Human lens B3 epithelial cells and COS-7 cells (ATCC, Manassas, VA) were maintained in Eagle's minimum essential medium (Invitrogen, Carlsbad, CA) supplemented with 10% fetal bovine serum and antibiotics [19]. Cells were seeded with 5×10^5 cells in a 60 mm (diameter) culture dish (Nunc,

TABLE 2. SPECIFIC PRIMERS FOR DIRECT SEQUENCING.

Gene	Amplicon	Primer sequences
<i>CRYAA</i>	1	5'-CTCCAGGTCCCCGTGGTA
		5'-AGGAGAGGCCAGCACCAC
	2	5'-CTGTCTCTGCCAACCCAG
		5'-CTGTCCCACCTCTCAGTGCC
	3	5'-AATGATCCTGCGATTTTGGAG
		5'-GGAAGCAAAGGAAGACAGACACC
<i>CRYAB</i>	1	5'-TG TAGCTGCAGCTGAAGGAG
		5'-TTCCAGTAAGGACTCTCCCG
	2	5'-GAAGGATGAATTACCCGGACAG
		5'-AGACATTGATTTGTAACCCCTGATC
	3	5'-GAGTTCTGGGCAGGTGATAAT
		5'-CTGGTGGGGAACTTTCTTG
<i>CRYGC</i>	1	5'-TGCATAAAATCCCCTTACCGC
		5'-ACTCTGGCGGCATGATGG
	2	5'-AGACTCATTTGCTTTTTTCCATCC
		5'-GAATGACAGAAGTCAGCAATTGC
<i>CRYGD</i>	1	5'-CAGCAGCCCTCCTGCTAT
		5'-GCTTATGTGGGGAGCAAACCT
	2	5'-CTTTTCTTCTCTTTTTATTTCTGGGTCC
		5'-GAAAGACACAAGCAAATCAGTGCC
<i>MAF</i>	1	5'-CTCCTGCAGCCCATCTGG
		5'-CTGGTGGCTGTTGCTGATG
	2	5'-CATCAGCAACAGCCACCAG
		5'-GAGAAGCGGTTCGTCGAAGT
	3	5'-ACTTCGACGACCGCTTCTC
		5'-TGGCGAGCATGGCTCTAG
	4	5'-CCTTTACGCTGCGTTTGATC
		5'-AACCCCCAGACAAGAGGC

Five genes which were sequenced were listed. Forward and reverse primer sequences were provided for each amplicon of each gene.

TABLE 3. SENSE OLIGONUCLEOTIDES FOR SITE-DIRECTED MUTAGENESIS IN *CRYGD*.

Mutations	Oligonucleotides with specific base change (<u>underlined</u>)
R15C	5'-GACCGGGGCTTCCAGGGCT <u>G</u> GCCACTATGAATGCAGC
R15S	5'-GACCGGGGCTTCCAGGGC <u>A</u> GCCACTATGAATGCAGC
P24T	5'-GAATGCAGCAGCGACCAC <u>A</u> CCAACCTGCAGCCCTAC
G61C	5'-TACTTCCTGCGCCGCT <u>G</u> GCGACTATGCCGAC

Constructs of four mutations in *CRYGD* were made. The sense oligonucleotides used in site-directed mutagenesis experiment were listed, respectively.

Rochester, NY) overnight before transfection. Expression construct containing wild type or mutant *CRYGD* was mixed with FuGene HD reagent (Roche, Basel, Switzerland) at a ratio of 3 μ l of FuGene per 1 μ g of vector DNA in Opti-MEM® I (Invitrogen) supplemented with GlutaMAX™-I (Invitrogen)

and incubated for 30 min. The mixture was then added to cells for up to 48 h.

Detergent solubility of wild type and mutant CRYGD: Cells transiently expressing wild type or mutant *CRYGD* were washed twice with ice-cold PBS and lysed in 2.5×10^6 cells/ml lysis buffer, which contained 0.5% Triton X-100 (Tx; Sigma),

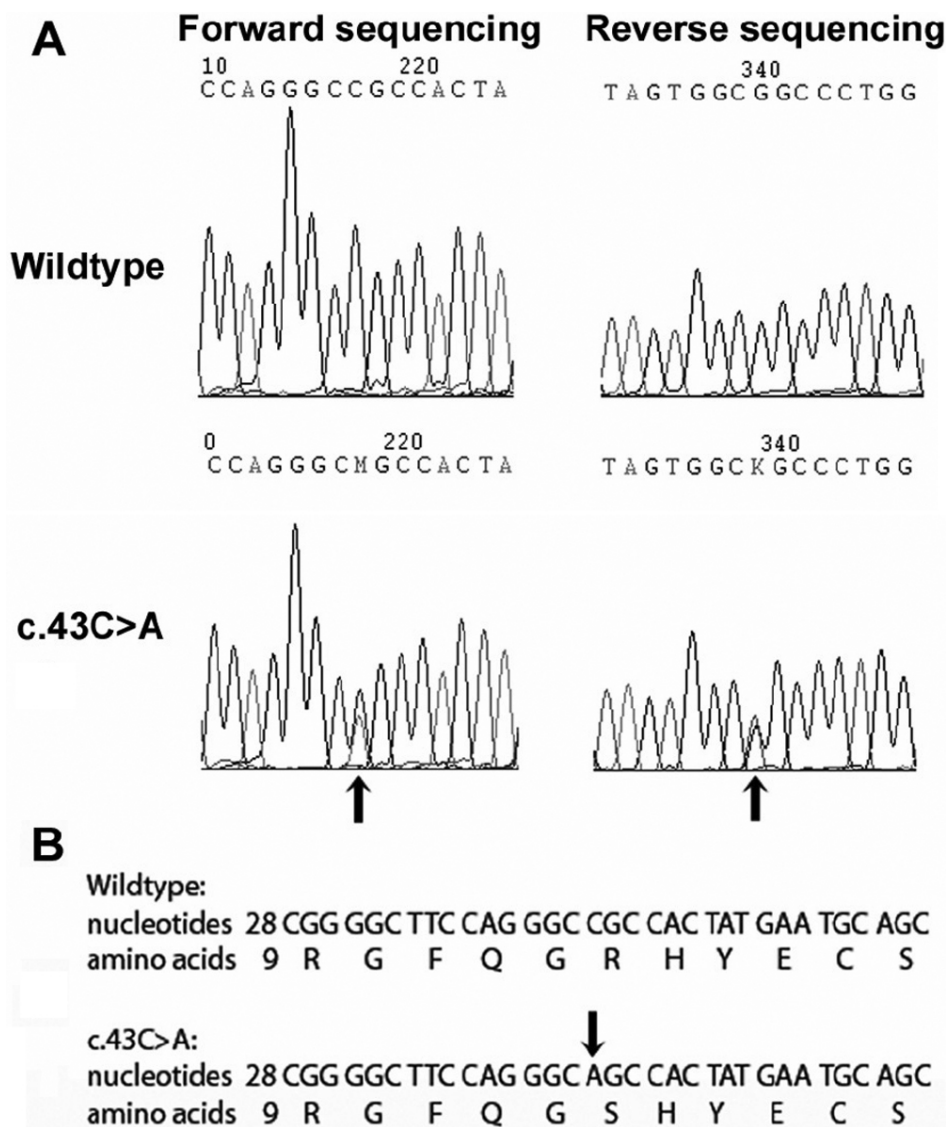


Figure 2. R15S mutation in Family A. **A:** The upper panel showed the forward (on the left) and the reverse (on the right) DNA segments of wildtype *CRYGD*. The lower panel displayed the forward and the reverse sequences of mutant *CRYGD*. The mutation of c.43C>A was indicated by arrow both in forward and reverse sequences. **B:** The DNA sequences in the upper lines and amino acid sequences in the lower lines were compared between wildtype and mutant *CRYGD*. The arrow denotes the nucleotide change.

for 2 min on ice [22]. After centrifugation, the Tx-soluble fraction was collected and denatured in SDS buffer containing 50 mM DTT. The pellet containing Tx-insoluble proteins was sonicated and denatured in SDS buffer containing 9 M urea. Both Tx-soluble and Tx-insoluble proteins equivalent to 7.5×10^4 cells were analyzed by western blotting using monoclonal antibodies against FLAG, Glyceraldehyde 3-phosphate dehydrogenase (GAPDH), or β -actin (Sigma).

Immunofluorescence staining: Cells grown on glass coverslips were fixed with freshly prepared neutral buffered 2% paraformaldehyde (Sigma) and permeabilized with 0.05% Tx [22] followed by incubations with mouse monoclonal antibodies against FLAG or mouse monoclonal antibodies against *CRYGD* (Abnova, Heidelberg, Germany). Rhodamine Red-X goat anti-mouse IgG (Invitrogen) was applied as the secondary antibody. The nuclei were contrast-stained with 4',6-diamidino-2-phenylindole (DAPI). The cells

were examined under fluorescence microscopy (DMRB, Leica, Germany), which was equipped with Spot RT color system (Diagnostic Instruments Inc., Sterling Heights, MI).

RESULTS

Clinical investigations: Two pedigrees exhibited coralliform type of cataract in an autosomal dominant mode of inheritance. All affected patients had bilateral lens opacification, characterized by the appearance of white lines and processes extending from the nucleus to peripheral cortex, resembling the shape of sea coral (Figure 1). This was classified as the coralliform type of cataract by senior ophthalmologists in two eye centers (D.S.P.F., D.L., and J.P.T.). The lens opacity was less severe in terms of size and density in Family A than in Family B and did not result in significant loss of visual acuity (VA). In family A, except for one patient, II10, who had low vision due to high myopia, VA

of other patients ranged from 0.67 to 0.8 without lens surgery. Patient III-10 was recorded to have normal lens transparency at his first eye examination at the age of 2.5 years, but he was diagnosed to have cataract at 10 years old. Other family members were diagnosed after the age of five years. In Family B, all patients showed cataract within the first year after birth. The lens opacity caused obvious vision loss ranging from 0.04 to 0.7. Four of the six patients received cataract surgeries before the age of 30.

Linkage analysis and DNA sequencing: Through linkage analysis with selected SNPs and microsatellite markers (LOD

score equal to minus infinity), 10 ADCC-associated genes were excluded. Five other genes were subject to direct sequencing. These genes were *CRYAA* (D21S1255; LOD score 0.3), *CRYAB* (D11S4151; LOD score 0.9), *CRYGC*, *CRYGD* (D2S2361; LOD score 1.51), and *MAF* (D16S503; LOD score 0.9) in Family A and *CRYAB* (D11S1793; LOD score 2.41), *CRYGC*, and *CRYGD* (D2S2208; LOD score 2.41) in Family B. Two sequence variants in exon 2 of *CRYGD* (NCBI accession number [NM_006891.3](http://www.ncbi.nlm.nih.gov/nuccore/NM_006891.3)) were identified to segregate with cataract in these two families. In Family A, a novel missense transversion, c.43C>A, which led to a substitution of arginine with serine at the 15th amino acid position (R15S), was detected (Figure 2). In Family B, a reported missense change, c.70C>A, which substituted proline with threonine at the 24th amino acid position (P24T), was found. All patients showed heterozygous changes. Neither the normal family members nor the 103 unrelated healthy controls carried these changes.

Computational protein analysis of R15S CRYGD: By [ExPasy](#) proteomics, R15S CRYGD was predicted to have a reduced isoelectric point (pI) of 6.58 (compared to 7.0 for the wild type). The molecular weight (MW) was also slightly decreased (20.669 kDa for R15S CRYGD versus 20.738 kDa for wild type). By [ProtScale](#) analysis, the local hydrophobicity at and near the altered amino acid was increased (Figure 3). By [ScanProsite](#), a hypothetical casein kinase II phosphorylation site was created due to the R15S mutation (Table 4).

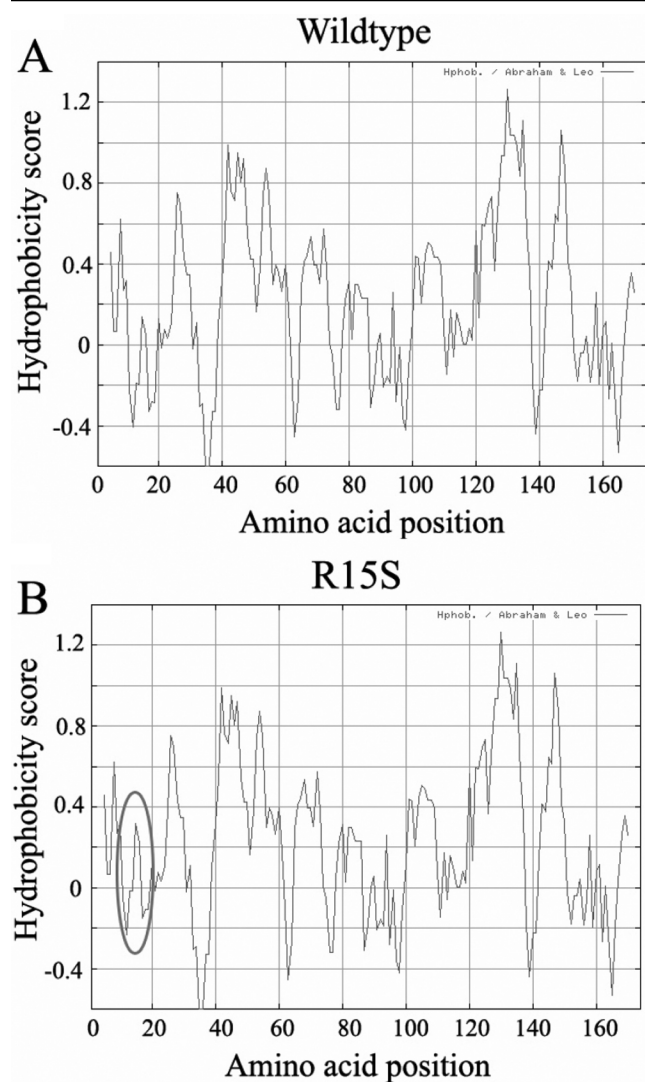


Figure 3. Hydrophobicity change of R15S CRYGD. The prediction by [ProtScale](#) analysis at [ExPasy](#) indicated an increase of local hydrophobicity around the site of R15S mutation (Circle in panel **B**). **A:** The curve showed the hydrophobicity score of each amino acid of wildtype CRYGD. **B:** The curve was the hydrophobicity of R15S CRYGD. At the position of the 15th amino acid and its neighboring locations, the hydrophobicity scores increased which was indicated by the circle.

Cell specificity of R15S CRYGD solubility: Recombinant FLAG/myc-tagged wild type or cataract-causing mutant CRYGD (R15S, R15C, P24T, G61C, and G165fsX8) was expressed in human lens epithelial B3 cells and COS-7 cells. Tx-soluble and Tx-insoluble fractions were western blotted for FLAG to detect wild type and mutant CRYGD proteins. The result of expression in lens B3 cells showed that except for G165fsX8, all known mutants of CRYGD remained Tx-soluble, which is similar to the wild type protein (Figure 4). The majority of G165fsX8 was present as Tx-insoluble. However, when expressed in COS-7 cells, an appreciable amount of mutant CRYGD protein became Tx-insoluble (Figure 4) and wild type CRYGD remained Tx-soluble. Band densitometry followed by normalization with housekeeping proteins (GAPDH for Tx-soluble and β -actin for Tx-insoluble fractions) revealed that about 8% of R15C and 17% of R15S CRYGD were Tx-insoluble (compared to 0.2% of wild type CRYGD). Similar observations were obtained in triplicate experiments.

Cellular distribution: In COS-7 cells, recombinant FLAG/myc-tagged R15S CRYGD was located predominantly in the cytoplasm and slightly in the nuclei (data not shown). No obvious inclusion was observed. This was similar to that observed in cells expressing wild type CRYGD.

TABLE 4. COMPARISON OF WILD TYPE AND R15S CRYGD AMINO ACID SEQUENCES BY SCANPROSITE ANALYSIS.

Hypothetical sites	Wild type	R15S
Casein kinase II phosphorylation	5–8: TlyE	5–8: TlyE *15–18: ShyE
Protein kinase C phosphorylation	35–37: SaR 75–77: SvR 78–80: ScR 87–89: ShR 166–168: SIR	35–37: SaR 75–77: SvR 78–80: ScR 87–89: ShR 166–168: SIR
N-glycosylation	50–53: NYSG	50–53: NYSG
Cell attachment sequence	60–62: RGD	60–62: RGD
N-myristoylation	71–76: GLsdSV 158–163: GAtnAR	71–76: GLsdSV 158–163: GAtnAR
Tyrosine kinase phosphorylation	91–98: Rly, EredY	91–98: Rly, EredY

All predicted functional sites in both wildtype and R15S CRYGD were listed in this table. The number is amino acid position. The asterisk indicates the newly formed casein kinase II phosphorylation site in R15S CRYGD.

DISCUSSION

In this study, we identified a novel R15S mutation and a P24T change of CRYGD in two Chinese families showing ADCC of the coralliform type, which is a rare subtype of bilateral

static congenital cataracts characterized by the appearance of opacity resembling the shape of sea coral. It shows white or cerulean opacification arranged in fusiform or spindle shape, extending from the center of the lens to the periphery but never reaching the capsule [10,11]. The irregular pattern of opacity running across the anatomic boundary of the cortical region indicates an altered arrangement of lens fibers, which associates with light scattering and reduction of transparency [11]. Until now, autosomal dominant transmission was the only mode of inheritance reported for coralliform cataract. Two loci, 2p24-pter and 2q33–35, have been mapped, and the specific gene, *CRYGD* (UniProt), in the latter locus was identified to associate with this cataract subtype [23]. Among all *CRYGD* mutations, R15C, P24T, and G61C were reported to be responsible for the coralliform phenotype [21,24–27]. In this study, the novel R15S change in CRYGD was found to cause congenital coralliform cataract. The index patient, III-10, in in family A did not have a detectable lens opacity when he was 2.5 years old but was diagnosed with cataract at nine years of age. This was different from the effects of a previously reported R15C mutation of CRYGD, which caused the punctate type of congenital cataract at a much earlier age of disease onset [28]. Human CRYGD exists as a monomeric protein with a highly symmetric structure containing four Greek key motifs organized into two highly homologous β -sheet domains. The NH₂-terminal and COOH-terminal domains are covalently connected by a six-residue linker and interact non-covalently through the side chains of 10 amino acids across the domain interface. Due to these two conserved regions and a central hydrophobic domain interface, CRYGD exhibits high intrinsic stability [29,30]. For the R15C mutant, the additional reactive cysteine molecule at the protein surface could lead to the formation of disulfide cross-linkage, which in turn causes protein aggregation [31]. Nevertheless, substitution of the highly polar and charged arginine molecule

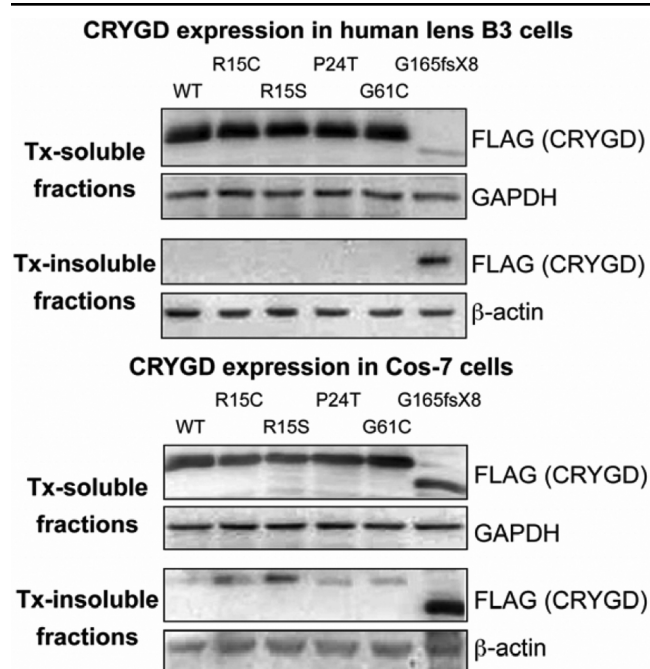


Figure 4. Detergent solubility assay of FLAG-tagged wild type and mutant CRYGD. Western blotting analysis showed cell type specific solubility changes of CRYGD mutants. When expressed in human lens B3 epithelial cells (upper set of blots), wild type (WT) and all known mutant CRYGD (including R15S), except G165fsX8, were completely soluble in 0.5% Triton X-100 extraction. G165fsX8 was mainly Tx-insoluble. When expressed in COS-7 cells (lower set of blots), R15C and R15S CRYGD became moderately Tx-insoluble, unlike the wild type and P24T and G61C mutants. G165fsX8 remained Tx-insoluble.

by a less polar serine molecule as in R15S may cause lesser effects on protein conformation than the formation of an additional disulfide bond due to cysteine. Therefore, the R15S mutant is associated with the less severe late onset of cataract phenotype in our studied family. Although we detected no alteration of biochemical properties due to R15S in our cell expression studies, increased hydrophobicity at the R15S mutation site as predicted by [ProtScale](#) might affect protein-protein interactions since R15 is located on a solvent accessible protein surface [28]. This would contribute to abnormal protein packing in the lens and would result in aggregate formation and thus lens opacification. Moreover, examination by [ScanProsite](#) predicted the creation of a putative casein kinase II phosphorylation site due to R15S. Whether this posttranslational modification is associated with protein interactions or oligomerization remains to be investigated. Additionally, an animal study had shown that the mutant γ -crystallin could cause cataract by the deposition of the misfolded protein as amyloid-like inclusions [32]. But this only explained those mutations which could affect protein structure severely, such as a frameshift mutation leading to protein truncation, ultimately causing mutant protein aggregate into inclusion. R15S was not supposed to have such a severe influence on protein structure. No obvious inclusion was observed in cells expressing R15S in this study.

The P24T mutant of CRYGD is associated with diverse opacity morphology [24,25,33-35]. The P24T and P24S mutants [18] are responsible for different crystallin cataracts such as the coralliform, aculeiform, fasciculiform, and crystal forms. In vitro expression of P24T CRYGD demonstrated reduced solubility when compared to the wild type protein [36,37]. The additional formation of a hydrogen bond might modify β -strand conformation, leading to protein insolubility rather than loss of stability. This might result in protein aggregation and then opacification in the lens.

In conclusion, a novel R15S mutation and a P24T mutation of CRYGD were identified in two Chinese families showing the coralliform type of congenital cataract. Different phenotypic features and onset time of disease in the two families accentuated the unique role of a single amino acid change in protein properties, which is crucial for disease pathogenesis. The enhanced hydrophobicity and a hypothetical phosphorylation site in the vicinity of R15S may affect the CRYGD protein interaction and the formation of the protein cluster.

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