Congenital cataracts due to a novel 2-bp deletion in *CRYBA1/A3*

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Abstract. Congenital cataracts, which are a clinically and genetically heterogeneous group of eye disorders, lead to visual impairment and are a significant cause of blindness in childhood. A major proportion of the causative mutations for congenital cataracts are found in crystallin genes. In the present study, a novel deletion mutation (c.590-591delAG) in exon 6 of *CRYBA1/A3* was identified in a large family with autosomal dominant congenital cataracts. An increase in local hydrophobicity was predicted around the mutation site; however, further studies are required to determine the exact effect of the mutation on βA1/A3-crystallin structure and function. To the best of our knowledge, this is the first report of an association between a frameshift mutation in exon 6 of *CRYBA1/A3* and congenital cataracts.

Introduction

Cataracts are opacities of the crystalline lens that can be categorized into early onset (congenital or infantile) and age-related conditions. Congenital cataracts exhibit a prevalence of ~1-6 cases per 10,000 live births and are a significant cause of blindness in childhood (1). Among the cases of congenital cataracts, approximately one-third are hereditary, with most occurring in a nonsyndromic autosomal dominant fashion (2). Congenital cataracts exhibit high clinical and genetic heterogeneity. A total of 40 genetic loci have been associated with congenital cataracts, with ≥26 susceptibility genes cloned and sequenced (3). Among the disease-causing mutations identified, ~50% are found in crystallins, 25% in connexins and the remainder within genes such as heat shock transcription factor-4, aquaporin-0 and beaded filament structural protein-2 (3). Crystallins are abundant, soluble proteins located in the eye lens. The major human crystallins comprise 90% of protein in the mature lens and contain two different superfamilies: the small heat-shock proteins (α-crystallins) and the βγ-crystallins.

In this study a functional candidate approach was used to investigate the known crystallin genes, including *CRYAA*, *CRYAB*, *CRYBA1/A3*, *CRYBB1*, *CRYBB2*, *CRYGC*, *CRYGD* and *CRYGS*, in which a major proportion of the mutations identified in a large family with congenital cataracts were found.

Subjects and methods

Subjects. A four-generation Chinese family with autosomal dominant congenital cataracts was identified from the Shijiazhuang Obstetrics and Gynecology Hospital (Shijiazhuang, China) (Fig. 1). The study was approved by the Ethics Committee of the Shijiazhuang Obstetrics and Gynecology Hospital, and the study protocol followed the principles of the Declaration of Helsinki. Informed consent was obtained from all family participants. One hundred unrelated subjects without eye disease were recruited from the Shijiazhuang Obstetrics and Gynecology Hospital as unaffected controls. A history of cataract extraction or ophthalmologic examination was used to determine affected family members. In total, eight family members (III:1, III:3, III:6, III:7, III:8, IV:1, IV:2 and IV:3) participated in the study. All participating family members and controls underwent ophthalmic examination, including visual acuity, slit-lamp and fundus examination. Phenotypes were documented by slit-lamp photography and 5 ml venous blood was collected in BD Vacutainer™ tubes (BD Biosciences, San Jose, CA, USA) containing EDTA for further analysis. Genomic DNA was extracted by QIAamp DNA Blood Mini kits (Qiagen Sciences, Inc., Germantown, MD, USA).

Mutation detection. All coding exons and intron-exon junctions of candidate genes known to be associated with congenital cataracts were amplified by polymerase chain reaction (PCR) using the primers listed in Table I. Each reaction mix (25 μl) contained 20 ng genomic DNA, 1X PCR buffer, 1.5 mM $MgCl_2$, 0.2 mM deoxynucleotide triphosphates, 0.5 μM each of forward and reverse primer and 2.5 units Taq DNA polymerase (Tiangen Biotech, Beijing, China). The PCR conditions for DNA amplification were: 95˚C for 5 min, followed by

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35 cycles at 95˚C for 30 sec, 57-63˚C for 30 sec (annealing temperature dependent on the primer), 72˚C for 30 sec, with a final extension at 72˚C for 10 min. The PCR products were sequenced using an ABI 3730 Automated Sequencer (Applied Biosystems, Foster City, CA, USA). Sequencing results were analyzed using Chromas 2.33 (http://www.technelysium.com.au/chromas.html) and compared with the National Center for Biotechnology Information (NCBI) reference sequences (http://www.ncbi.nlm.nih.gov/).

Bioinformatics analysis. The amino acid sequences of CRYBA1/A3 protein from four species (human, mouse, rat and cow) were obtained from the NCBI GenBank (https://www.ncbi.nih.gov/genbank/). Conservation analysis was performed using CLC Main Workbench Software (CLC bio, Aarhus, Denmark). Hydrophobicity changes were predicted using ProtScale (http://web.expasy.org/protscale/).

Results

Clinical evaluation. Using medical records and ophthalmic examinations, five family members (III:1, III:7, IV:1, IV:2 and IV:3) were diagnosed with bilateral congenital cataracts. Slit-lamp examination of the left eye of the proband (IV:3) showed nuclear lens opacities (Fig. 2). No other ocular or systemic abnormalities were identified.

Mutation analysis. By direct sequencing of the coding regions of candidate genes, a novel heterozygous 2-bp deletion mutation (c.590-591delAG) in exon 6 of *CRYBA1/A3* was identified (Fig. 3). This deletion led to a frameshift starting at amino acid residue 197, with a substitution of glutamic acid to valine, followed by an altered amino acid sequence (wild type, -EWGSHAQTSQIQSIRRIQQ; mutant, -VGLSCPDFADPIDSPNPTV). This altered sequence was identified in all affected family members, but was not detected in any unaffected family members or the 100 unrelated control subjects.

Bioinformatic analysis. Conservation analysis revealed that the sequence from amino acid residue 197 to the COOH-terminal end is highly conserved among species (Fig. 4). Furthermore, an increase in local hydrophobicity around the mutation site was predicted by ProtScale (Fig. 5).

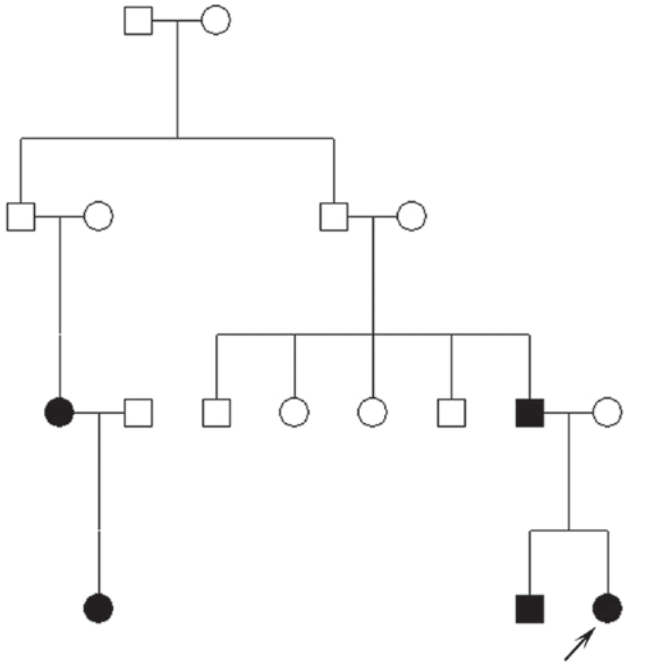

Figure 1. Chinese congenital cataract pedigree. The arrow indicates the proband. Black shading represents family members with bilateral congenital cataracts.

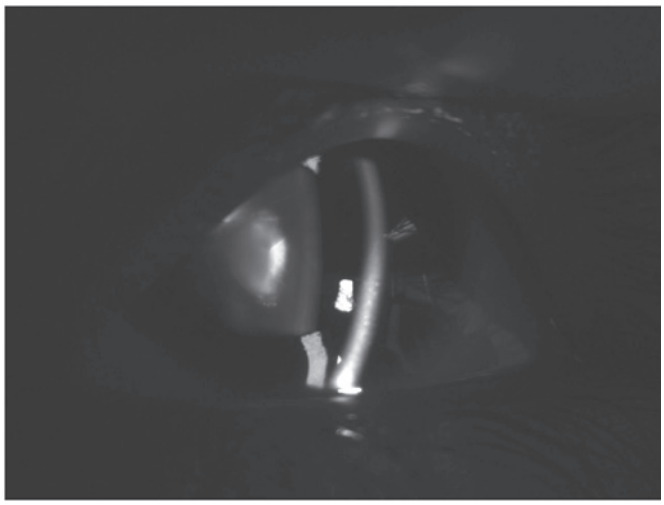

Figure 2. Slit-lamp photograph of the left eye of the proband.

Discussion

Lens crystallin is fundamental for the establishment and maintenance of lens transparency (4). Previous studies in affected patients and transgenic animals indicate that mutations in crystallin genes cause cataracts (14-15). The human lens contains α-, β- and γ-crystallins, of which, β-crystallins comprise the greatest proportion (5). *CRYBA3/A1* is located at 17q11.2 and is a member of the β-crystallin family. The gene utilizes an alternate translation initiation site to encode two proteins (βA3- and βA1-crystallin) from a single mRNA, and consists of six exons. β-crystallin comprises seven protein domains: Four homologous Greek key motifs, a connecting peptide and NH_2- and COOH-terminal extensions. The first two exons of *CRYBA3/A1* encode the N-terminal arm and the Greek key motifs are encoded by exons 3-6 (6). βA3- and βA1-crystallin are identical, with the exception of 17 additional amino acid residues found on the NH_2-terminal arm of βA3-crystallin (7).

To date, several *CRYBA3/A1* gene mutations have been associated with autosomal dominant congenital cataracts (Table II). All known *CRYBA3/A1* gene mutations can be divided into two clusters, one located in the exon 3 splice site (8-9), and the other in an exon 4 in-frame deletion (10). In the present study, a novel 2-bp deletion mutation (590-591delAG) in exon 6 of *CRYBA1/A3* was identified. This mutation caused a frameshift and resulted in an alternative βA1/βA3-crystallin COOH-terminal. Typically, β- or γ-crystallin mutations cause major abnormalities in protein

Table I. Primers used for the polymerase chain reaction.

Name	Forward (5'-3')	Reverse (5'-3')
CRYAA-1	AGCAGCCTTCTTCATGAGC	CAAGACCAGAGTCCATCG
CRYAA-2	GGCAGGTGACCGAAGCATC	GAAGGCATGGTGCAGGTG
CRYAA-3	GCAGCTTCTCTGGCATGG	GGGAAGCAAAGGAAGACAGA
CRYAB-1	AACCCCTGACATCACCATTC	AAGGACTCTCCCGTCCTAGC
CRYAB-2	CCATCCCATTCCCTTACCTT	GCCTCCAAAGCTGATAGCAC
CRYAB-3	TCTCTCTGCCTCTTTCCTCA	CCTTGGAGCCCTCTAAATCA
CRYBA1-1	GGCAGAGGGAGAGCAGAGTG	CACTAGGCAGGAGAACTGGG
CRYBA1-2	AGTGAGCAGCAGAGCCAGAA	GGTCAGTCACTGCCTTATGG
CRYBA1-3	AAGCACAGAGTCAGACTGAAGT	CCCCTGTCTGAAGGGACCTG
CRYBA1-4	GTACAGCTCTACTGGGATTG	ACTGATGATAAATAGCATGAACG
CRYBA1-5	GAATGATAGCCATAGCACTAG	TACCGATACGTATGAAATCTGA
CRYBA1-6	CATCTCATACCATTGTGTTGAG	GCAAGGTCTCATGCTTGAGG
CRYBB1-1	CCCTGGCTGGGGTTGTTGA	TGCCTATCTGCCTGTCTGTTTCTC
CRYBB1-2	TAGCGGGGTAATGGAGGGTG	AGGATAAGAGTCTGGGGAGGTGG
CRYBB1-3	CCTGCACTGCTGGCTTTTATTTA	TCTCCAGAGCCCAGAACCATG
CRYBB1-4	CCAACTCCAAGGAAACAGGCATA	CCTCCCTACCCACCATCATCTC
CRYBB1-5	TAGACAGCAGTGGTCCCTGGAGA	AGCACTGGGAGACTGTGGAAGG
CRYBB1-6	CCTAGAAAAGGAAACCGAGGCC	AGCGAGGAAGTCACATCCCAGTA
CRYBB2-1	GTTTGGGGCCAGAGGGGAGTGGT	TGGGCTGGGGAGGGACTTTCAGTA
CRYBB2-2	CCTTCAGCATCCTTTGGGTTCTCT	GCAGTTCTAAAAGCTTCATCAGTC
CRYBB2-3	GTAGCCAGGATTCTGCCATAGGAA	GTGCCCTCTGGAGCATTTCATAGT
CRYBB2-4	GGCCCCCTCACCCATACTCA	CTTCCCTCCTGCCTCAACCTAATC
CRYBB2-5	CTTACCCTTGGGAAGTGGCAATGG	TCAAAGACCCACAGCAGACAAGTT
CRYGC-1	TGCATAAAATCCCCTTACCG	CCTCCCTGTAACCCACATTG
CRYGC-2	TGGTTGGACAAATTCTGGAAG	CCCACCCCATTCACTTCTTA
CRYGD-1	CAGCAGCCCTCCTGCTAT	GGGTCCTGACTTGAGGATGT
CRYGD-2	GCTTTTCTTCTCTTTTTATTTCTGG	AAGAAAGACACAAGCAAATCAGT
CRYGS-2	GAAACCATCAATAGCGTCTAAATG	TGAAAAGCGGGTAGGCTAAA
CRYGS-3	AATTAAGCCACCCAGCTCCT	GGGAGTACACAGTCCCCAGA
CRYGS-4	GACCTGCTGGTGATTTCCAT	CACTGTGGCGAGCACTGTAT

Table II. Summary of mutations in *CRYBA1/A3* responsible for congenital cataracts.

Locus	Nucleotide	Amino acid
IVS3 (14)	IVS3+1 G.>C	Splice site mutation
IVS3 (5)	IVS3+1 G.>A	Splice site mutation
IVS3 (8)	IVS3+1 G.>T	Splice site mutation
IVS3 (9)	IVS3+2 T.>G	Splice site mutation
Exon 4 (10,13)	276-281delGGAGGA	p.90Glydel p.91Glydel
Exon 4	279-281delGGAGGA	p.91Glydel

IVS3, sequence of the 3rd intron.

structure, stability, solubility or the ability to oligomerize, and are predicted to precipitate from solution to cause lens opacity formation (11-12). The COOH-terminal of βA1/βA3-crystallin is highly conserved, and it has been reported that the C-terminal domain plays a role in structural stability (11). The ProtScale protein analysis in the present study showed a notable increase in local hydrophobicity around the deletion site in *CRYBA3/A1*. As demonstrated in other lens proteins, hydrophobicity is associated with crystallin activities, and an increased hydrophobic interaction can reduce protein solubility or lead to abnormal protein folding (12-13). In the present study, it was speculated that

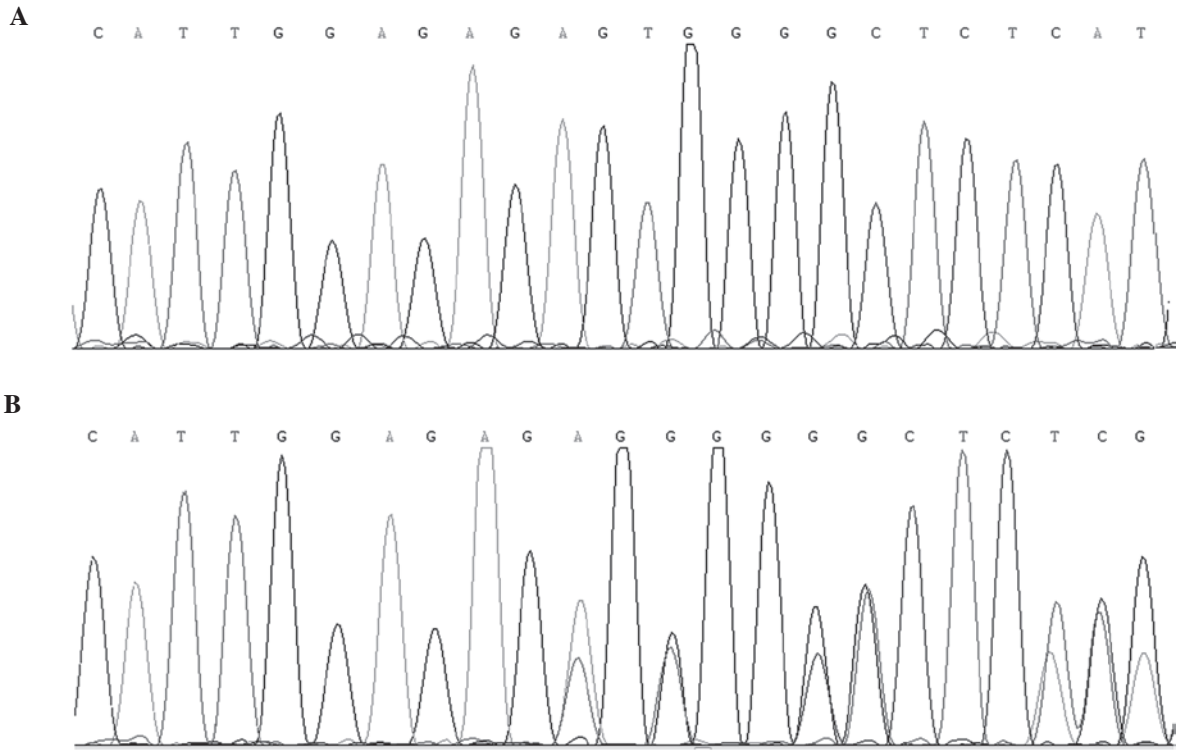

Figure 3. Partial sequence of *CRYBA1/A3* at exon 6. (A) Sequence of an unaffected individual. (B) Sequence of an affected individual. The deletion mutation c.590-591delAG was identified in all the affected participants, but was not found in unaffected family members or the 100 unrelated control subjects.

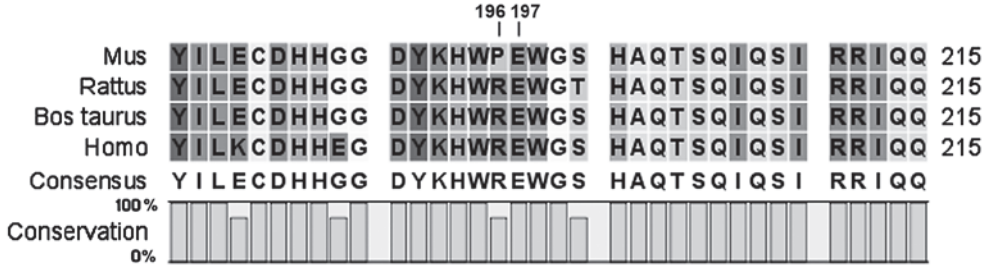

Figure 4. Multiple-sequence alignment of the amino acid sequence in CRYBA1/A3 protein from different species. The alignment data indicate that the 197th amino acid position is highly conserved (indicated by a dash) among the four species.

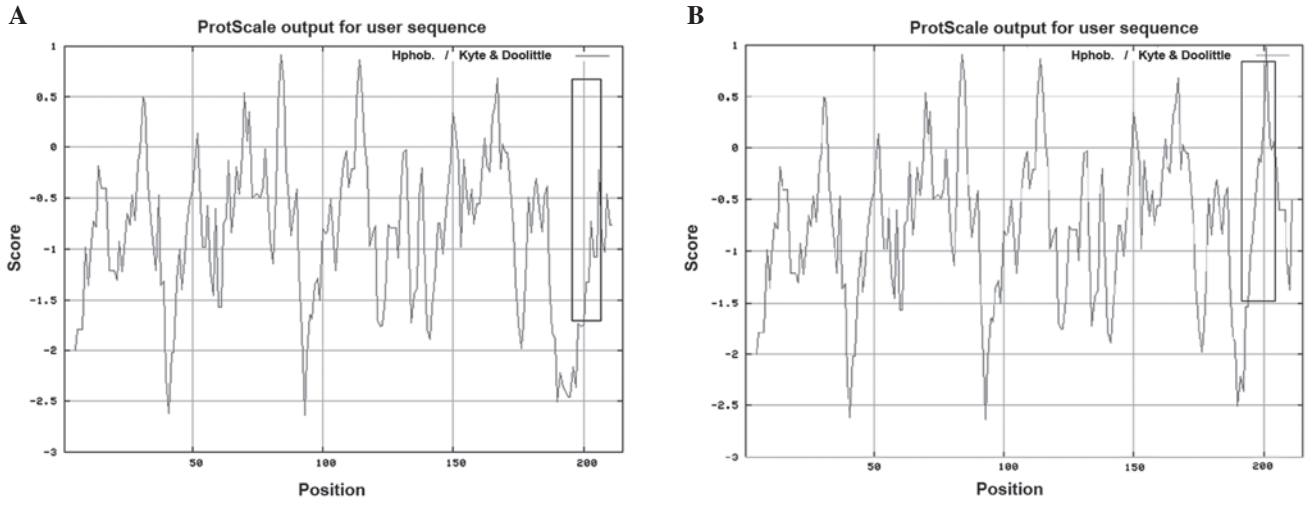

Figure 5. Altered hydrophobicity in CRYBA1/A3 protein. (A) The hydrophobicity of WT CRYBA1/A3 was predicted using the ProtScale program on the Expasy proteomics server. (B) Hydrophobicity of mutant-type CRYBA1/A3. Compared with the WT, the mutant type exhibits markedly enhanced hydrophobicity, which is indicated by the rectangle. WT, wild type.

the mutant COOH-terminal produces an abnormal protein structure with altered stability and/or solubility.

In conclusion, this is the first study, to the best of our knowledge, to associate a frameshift mutation in exon 6 of *CRYBA1/A3* with the development of congenital cataracts, and highlights the physiological importance of βA1/A3-crystallin. The possible effect of the mutation on βA1/A3-crystallin structure and function requires further investigation.

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