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ORIGINAL INVESTIGATION

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A deletion mutation in the βA1/A3 crystallin gene (*CRYBA1/A3*) is associated with autosomal dominant congenital nuclear cataract in a Chinese family

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Abstract Congenital cataracts are an important cause of blindness worldwide. In a family of Chinese descent, a dominant congenital nuclear cataract locus was mapped to chromosome 17q11.1-12. The maximum LOD score, 2.49, at recombination fraction 0, was obtained for marker D17S1294. The results of both linkage and haplotype analyses defined a disease-gene to an 11.78-cM region harboring the gene coding for βA1/A3 crystallin (*CRYBA1/A3*). Mutation analysis of the *CRYBA1/A3* gene identified a 3-bp deletion in exon 4, which cosegregated with the disease risk in this family and was not observed in 100 normal chromosomes. This mutation resulted in the deletion of a highly conserved glycine at codon 91 (ΔG91) and could be associated with an incorrect folding of βA1/A3 crystallin. It highlights the physiological importance of crystallin and supports the role of *CRYBA1/A3* in human cataracts formation.

Introduction

Congenital cataracts are phenotypically and genetically heterogeneous, with an estimated prevalence of 1–6 per 10,000 live births (Lambert and Drack 1996). Approximately one-third of all cases are familial, and autosomal dominant cataract (ADC) appears to be the most common transmission mode (Ionides et al. 1999). Despite congenital cataracts being a leading cause of blindness worldwide, the mechanisms of lens opacification remain poorly understood (Arnold 1998).

So far, causative mutations in ADC have been identified in 12 distinct genes, including seven genes coding for crystallins: *CRYAA* on chromosome 21q (Litt et al. 1998), *CRYAB* on 11q (Berry et al. 2001), *CRYBA1/A3* on 17q (Kannabiran et al. 1998; Bateman et al. 2000), *CRYBB1* on 22q (Mackay et al. 2002), *CRYBB2* on 22q (Litt et al. 1997; Gill et al. 2000; Vanita et al. 2001), *CRYGC* on 2q (Heon et al. 1999; Ren et al. 2000), and *CRYGD* on 2q (Stephan et al. 1999; Nandrot et al. 2003); as well as two coding for gap junctional channel proteins, *GJA3* on 13q (Mackay et al. 1999; Rees et al. 2000), and *GJA8* on 1q (Shiels et al. 1998; Berry et al. 1999; Polyakov et al. 2001), one for heat-shock transcription factor 4, *HSF4* on 16q (Bu et al. 2002), one for major intrinsic protein, *MIP* on 12q (Berry et al. 2000), and one for beaded-filament structural protein-2, *BFSP2* on 3q (Conley et al. 2000; Jakobs et al. 2000). Only two genes are associated with human autosomal recessive cataracts: one coding for crystallin gene, *CRYAA* on 21q (Pras et al. 2000), the other for intrinsic membrane protein 19, *MP19* on 19q (Pras et al. 2002). Of interest, the *CRYAA* gene causes both autosomal dominant and autosomal recessive congenital cataracts (Litt et al. 1998; Pras et al. 2000).

Nuclear cataract, which is one of most familial types of severe congenital cataracts, was first reported by Brown (1924). The opacity was located at the center of the lens, which can have a remarkable effect on visual acuity. So far, dominantly inherited nuclear cataracts have been linked to 2p12 (MIM 607304) (Khaliq et al. 2002) and *CRYAA* on 21q (MIM 123580) (Litt et al. 1998).

In this report, we linked the dominantly inherited nuclear cataracts affecting a Chinese family to 17q11.1-12, and identified a deletion mutation in the *CRYBA1/A3* gene that is responsible for the cataracts in this pedigree. This is the first report of nuclear cataracts caused by this gene.

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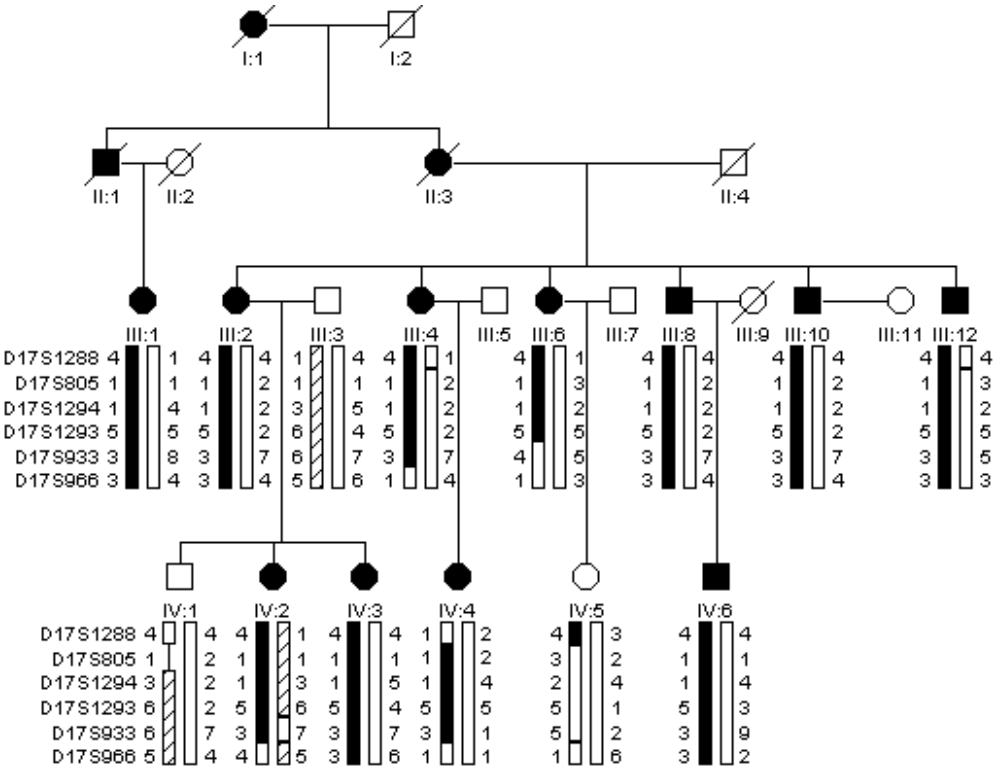

Fig. 1 Pedigree and haplotype of the autosomal dominant congenital cataract. *Open white* and *solid black symbols* denote unaffected and affected individuals, respectively. The affected haplotype is indicated by a *black vertical bar*. The sequence of markers is from centromere to telomere. Uninformative makers are indicated by a *vertical line* in the haplotype bar

Materials and methods

Clinical evaluation and DNA specimens

We studied a large family that is affected with congenital nuclear cataracts (Fig. 1). This family resides in a relatively isolated region of China. Fourteen members of the family participated the study; all participants gave informed consent, which was approved by the Institution Review Board of the Harbin Medical University, China. The dilated slit-lamp examinations were carried out. Among 14 participants, 11 individuals were identified to be affected with nuclear cataract, the remaining three were normal. The cataract was bilateral in all cases and consisted of a well-defined and dense opacity that was located at the embryonic nucleus of the lens and was 1.5–3 mm in diameter. The opacity was present at birth and developed during infancy but did not progress with age. There was no family history of other ocular or systemic abnormalities.

Blood samples were collected in EDTA and leukocyte genomic DNA was extracted.

Genotyping and linkage analysis

The initial strategy consisted of screening 12 known candidate genes related to ADC formation and one locus related to congenital nuclear cataract (Khaliq et al. 2002). The primer sequences were taken from the Genome Database (http://www.gdb.org). Microsatellites used in this study included: D1S252, D1S305, D1S2721, D2S157, D2S325, D2S2333, D3S1290, D3S1744, D11S1986, D11S898, D12S90, D12S1676, D13S175, D13S1236, D16S421, D16S3043, D17S1294, D21S212, CRYBB2, D22S1174, TOP1P2, D22S315. Fifty nanograms of template DNA was used in a 25-µl reaction volume, with 2.5 mM of dNTPs, 10 pmol of fluorescently labeled forward primers and unlabeled reverse primers, 1.0 U Taq DNA polymerase, and 10×PCR buffer [100 mM Tris-HCl, 80 mM $(NH_4)_2SO_4$, 100 mM KCl, and 25 mM $MgCl_2$, (pH 9.0); Sangon, Shanghai, China]. After an initial denaturation of 5 min at 95 °C, 31 cycles were performed at 94 °C for 30 s, 53–58 °C for 30 s, and 72 °C for 1 min, followed by an extension at 72 °C for 10 min and a final hold at 4 °C. PCR products were mixed with loading buffer equally (v/v), as well as with 0.5 µl of internal lane standard (ROX-400; Perkin-Elmer Applied Biosystems). PCR products were denatured at 98 °C for 2 min, and were electrophoresed on 4% denaturing polyacrylamide gels on an automatic DNA sequencer (ABI-PRISM 377; Perkin-Elmer Applied Biosystems). Data were collected by GENESCAN 2.1 software (Perkin-Elmer Applied Biosystems) and individual genotypes were analyzed with GENOTYPER version 2.0 (Perkin-Elmer Applied Biosystems).

Two-point linkage analysis was performed with LINKAGE program package version 5.1 (freely available at http://linkage.rockfeller.edu). Autosomal dominant inheritance, with a full penetrance and a disease-gene frequency of 0.0001 was considered. The marker allele frequencies were assumed to be uniformly distributed.

Mutation analysis

A strong candidate gene, the βA1/A3 crystallin gene (*CRYBA1/A3)* (GenBank accession number NM_005208), is comprised of six exons. To screen the coding regions of *CRYBA1/A3*, gene-specific PCR primers were designed flanking each exon and intron-exon junction (Table 1). All primers were derived from relevant articles, except exon 2 (Kannabiran et al. 1998; Bateman et al. 2000).

The purified PCR products (using an Agarose Gel DNA Purification kit; TAKARA, Dalian, China) were sequenced from both directions using an automated fluorescence sequencer (ABI PRISM 377; Perkin-Elmer Applied Biosystems). One affected (III:2) and an unaffected family member (IV:5) were compared. After identifing a mutation in exon 4, all family members and 50 unrelated normal individuals were screened.

Results

Autosomal dominant inheritance of the cataract was supported by the presence of affected individuals in each of the four generations, and male-to-male transmission (Fig. 1).

Table 1 PCR primers, annealing temperature and size of *CRYBA1/A3* exons

Exon	Sequence	Annealing temperature	Fragment size
Exon 1 F	5′-GGTCTTAGGAAGATCCCAAG-3′	58 °C	394 bp
Exon 1 R	5′-AAGGAGAGGAAGGGCAAGGG-3′		
Exon 2 F	5′-CCTTTCAAGGTATTCCCTCA-3′	54 °C	276 bp
Exon 2 R	5′-CACTGGAGCTTGTGTGGA-3′		
Exon 3 F	5′-CAATCCTCCCTCCACCTC-3′	53 °C	520 bp
Exon 3 R	5′-TCCTTCCTTCAGCTTTGG-3′		
Exon 4 F	5′-GAACACCATGAACAAACACTAC-3′	57 °C	205 bp
Exon 4 R	5′-ACGGAAGTGGAAATTTCAGAG-3′		
Exon 5 F	5′-TGCTTCCTTGTATAATCC-3′	53 °C	306 bp
Exon 5 R	5′-ACTATTGATGCAACCTCAGG-3′		
Exon 6 F	5′-CATCTCATACCATTGTGTTGAG-3′	56 °C	315 bp
Exon 6 R	5′-ACTTTCTAGAGTGCTTAGCAAG-3′		

After excluding candidate genes which lack segregation between the markers and the disease, a maximum LOD score of 2.49 without recombination (θ=0), was obtained with D17S1294 on chromosome 17q11.1-12. Five additional markers flanking D17S1294 were analysed in this pedigree (Table 2). The order and genetic distances of the markers were derived from the Marshfield database (http://research. marshfieldclinic.org).

The results of both linkage and haplotype analyses (Fig. 1) defined a disease-gene to an 11.78-cM region bounded by D17S1288 and D17S933.

Direct sequencing of amplified exons, a 3-bp (GGA) deletion was detected at nucleotide position 278–280 (GenBank accession number NM_005208) of exon 4 (Fig. 2B).

This deletion is predicted to cause an in-frame deletion of a glycine residue at position 91 (ΔG91) from the mature gene product (Entrez-protein accession number P_05813) and produced an aberrant protein consisting of 214 residues. No other exonic sequence variants were observed in one affected (III:2) and an unaffected family member (IV:5). The six base changes reported by Lampi et al. (1997) were confirmed. In addition, this mutation was detected in all affected members in this family, but neither in normal relatives nor in 50 unrelated individuals, excluding the possibility that it is a rare polymorphism.

Discussion

In mammals, three major groups of lens proteins can be distinguished: the α-, β-, and γ-crystallins. The β- and γ-crystallins are also considered to be superfamilies. Crystallography has shown that both the β- and γ-crystallins consist of two similar domains (the N-terminal domain and C-terminal domain) separated by a short connecting peptide. Each domain folds into two similar "Greek key" motifs, with distinctive β-sheet folding. Each Greek key motif consists of four consecutive anti-parallel β-strands, known as a, b, c and d strands (Fig. 3) (Blundell et al. 1981; Slingsby and Clout. 1999). The four motifs form four β-sheets: two (the β1- and β3-sheets) lie on the outside of the molecule, and two (the β2- and β4-sheets) are in partial contact (domain association) (Fu and Liang 2003).

The β-crystallin family consists of seven proteins, βB1-, βB2-, βB3-, and βA4-crystallin on chromosome 22q11.2-13.1 (Hulsebos et al. 1991, van Rens et al. 1992), βA1/A3-crystallin on 17q11.1-12 (Sparkes et al. 1986) and βA2-crystallin on 2q33-35 (Hulsebos et al. 1995). Different β-crystallin proteins can interact with each other to form oligomers of different sizes range from dimers to octamers (Werten et al. 1996) and can also interact with other lens proteins. The protein-protein interactions are predicted to be key in maintaining the transparency of lens (Bax et al. 1990; Russell and Chambers 1990). It is pre-

Table 2 Two-point lod linkage data between cataract locus and chromosome 17 markers (*IMD* intermarker distances)

Marker	IMD[b] (cM)	Lod score at a recombination fraction of θ=								
		0	0.01	0.05	0.1	0.2	0.3	0.4	Z_{max}	θ_{max}
D17S1288	1.07	–∞	–2.04	–0.77	–0.32	–0.03	0.03	0.02	0.03	0.3
D17S805	3.74	2.03	1.98	1.79	1.56	1.11	0.70	0.32	2.03	0
D17S1294	5.74	2.49	2.44	2.22	1.94	1.40	0.88	0.39	2.49	0
D17S1293	1.23	2.22	2.16	1.95	1.68	1.15	0.65	0.24	2.22	0
D17S933	0.54	–∞	1.58	2.04	2.03	1.67	1.12	0.49	2.04	0.05
D17S966		–∞	–4.77	–2.15	–1.17	–0.43	–0.17	–0.06	–0.06	0.4

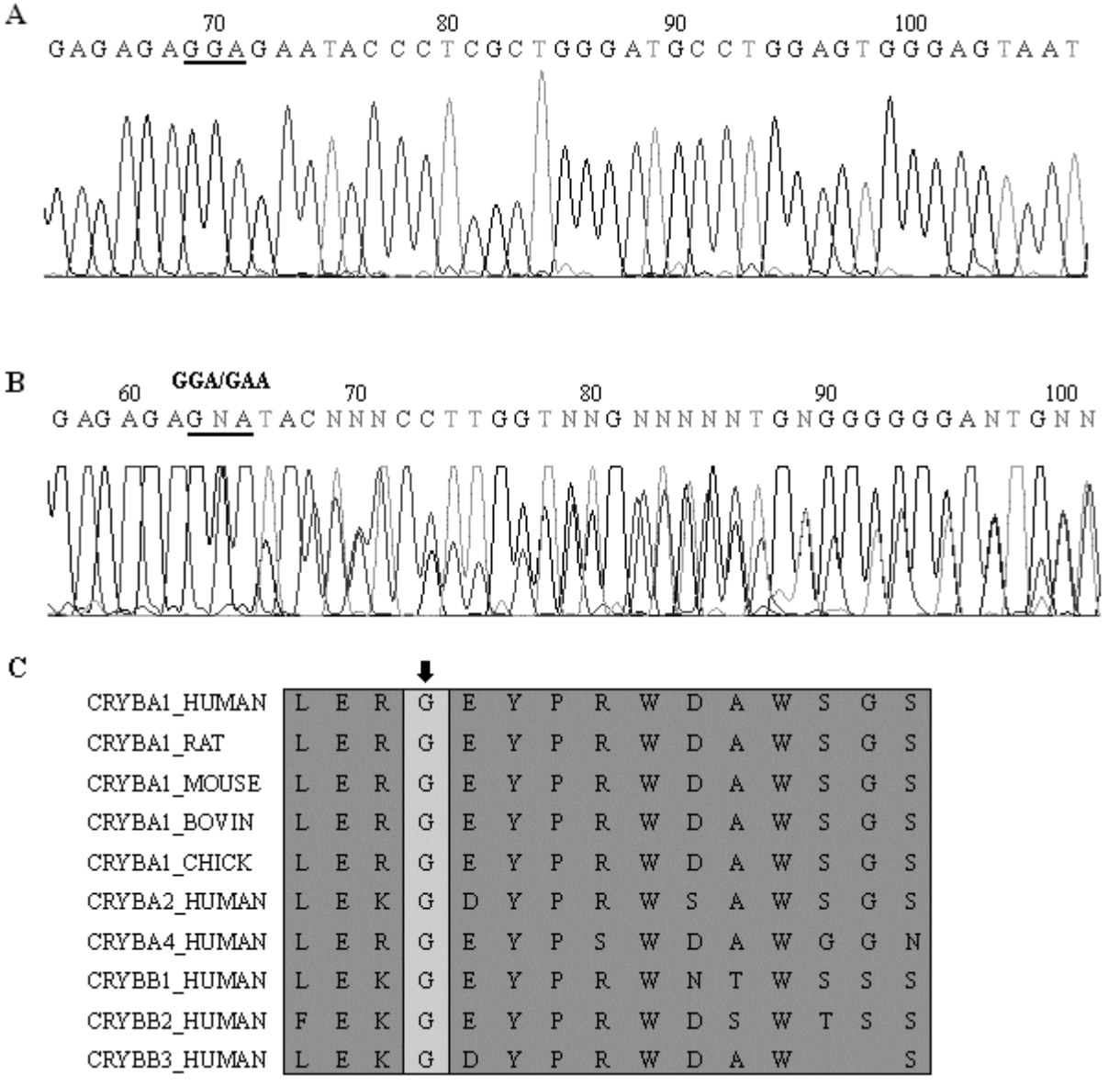

Fig. 2A–C Sequence analysis of *CRYBA1/A3* at exon 4. **A** Sequence chromatograms of the wild-type allele, showing the translation of glycine (GGA) at codon 91 (*underlined*). **B** Sequence chromatograms of mutant allele showing a 3-bp deletion (*underlined*). **C** A multiple alignment of protein sequence of CRYBA1/A3 with the corresponding segments in different species, and five other β-crystallins in humans. The sequences were selected using BLASTP (http://www.ncbi.nlm.nih.gov/BLAST). The *arrow* indicates the ΔG91 mutated position

dicted that the transparency of the lens depends on the tertiary structure of the crystallins. A disruption of stabilization or oligomerization by any means would result in lens opacification (Delaye and Tardieu 1983).

By in situ hybridization, Xu et al. (1988)had localized a gene that encodes crystallin, βA1 (MIM 123610) to chromosome 17q11.1-q12. The chromosomal localization, as well as the important role of the protein in maintaining lens transparency and development, and its association with

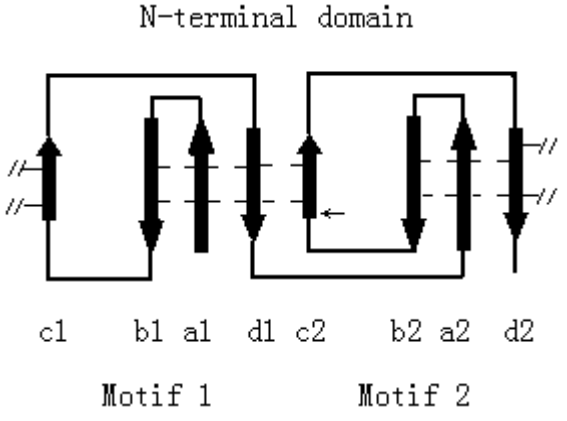

Fig. 3 Greek key motif topology. Strands are represented by *vertical arrows*. The lengths of β-strand are reflected by *arrow lengths*. The *horizontal arrow* indicates the site of the ΔG91 mutation in the N-terminal domain. The hydrogen bonds between strands are represented by *dashed lines*

cataract formation, suggested that *CRYBA1/A3* was an excellent candidate gene for this pedigree.

The βA1/A3-crystallin gene encodes both the βA3- and βA1-crystallins. The latter is 17 aa shorter than βA3-crystallin and they are generated by use of an alternative translation initiation site (Quax-Jeuken et al. 1984). The first two exons of the βA1-crystallin gene encode the N-terminal extension, and exons 3–6 encode the four Greek key motifs (Hogg et al. 1986).

Kannabiran et al. (1998) reported a splice mutation (G→A) in *CRYBA1/A3* in an Indian family affected with autosomal dominant zonular cataracts with sutural opacities (CCZS). Bateman et al. (2000) also reported a splice junction mutation (G→C) occurred in an identical locus.

Alignment of protein sequences of CRYBA1/A3 in five different species with five other β-crystallins of human species revealed that the glycine at this position is highly conserved (Fig. 2C). The deletion of this highly conserved glycine identified in our family is located at the c2 strand of the second Greek key motif, which is a key region for the tertiary structure of the βA1/A3-crystallin.The deletion could destroy the interstrand hydrogen bonds between c2 and d1 strands (Fig. 3), disrupting the proper folding of whole N-terminal domain, which, in turn, could destroy the intramolecular interactions between domains (Fu and Liang 2003), causing the disruption of the highly symmetrical structure of βA1/A3-crystallin. The change of tertiary structure ultimately may contribute to decrease the stability and/or solubility of this protein. If there is an aberrant aggregation of mutant protein, it may cause light scattering and finally lead to lens opacification.

The β-crystallins tend to abundantly expressed at an early developmental stage in elongating fiber cells, so they are found primarily in the lens nucleus (Aarts et al. 1989), which is consistent with the location of the opacity in the embryonic nucleus in this pedigree.

In conclusion, we have identified, in βA1/A3-crystallin gene (*CRYBA1/A3*), a novel deletion mutation (ΔG91) causing nuclear cataract. This mutation supports the previously proposed role of CRYBA1/A3 in the lens opacification process and may help delineate the functional domains of CRYBA1/A3. This is the first report of the autosomal dominant nuclear cataract caused by CRYBA1/A3, which should contribute to a better understanding of the relationships of genotype-phenotype.

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