Identification of a CRYAB Mutation Associated with Autosomal Dominant Posterior Polar Cataract in a Chinese Family

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PURPOSE. A four-generation Chinese family with 13 members affected with autosomal dominant congenital posterior polar cataract was studied. The purpose of this study was to identify the disease-causing gene in the family and to validate that mutations in CRYAB, the αB-crystallin gene, cause the congenital cataract.

METHODS. Linkage analysis was performed with a panel of microsatellite markers flanking candidate genetic loci for cataracts, including 14 known autosomal dominant congenital cataract (ADCC) genes. For mutation analysis, the complete coding region and exon–intron boundaries of CRYAB were sequenced with DNA from the proband. Single-strand conformation polymorphism (SSCP) analysis for exon 1 of CRYAB was performed in all family members and 200 normal control subjects.

RESULTS. The disease gene in the Chinese family was mapped to chromosome 11 in region q22.3 with a maximum lod score of 4.52. Direct DNA sequence of CRYAB revealed a heterozygous C→T transition at nucleotide 58, resulting in a novel 58 C→T (Pro20Ser) mutation. The Pro20Ser mutation cosegregated with all affected individuals and was not present in unaffected members in the family or in 200 normal control subjects. The mutation occurs at the evolutionarily conserved residue Pro20 in the N-terminal region of αB-crystallin.

CONCLUSIONS. To date, only one CRYAB mutation has been associated with congenital isolated cataract. This study identified a second novel mutation in CRYAB in a large Chinese cataract family. Together, these results provide strong evidence that CRYAB is a pathogenic gene for congenital cataract.

(Congenital) cataract is a highly heterogeneous disorder at both the genetic and clinical–phenotypic levels. Clinically, cataracts can be classified into many different types—for example, posterior polar, anterior polar, Lamellar type, and others. Genetically, multiple genes are involved. Recently, remarkable advances have been made in identifying the genes for cataracts.1–3,14–16,24,32 Fourteen genes have been identified for autosomal dominant cataracts, including (1) the genes encoding the crystallins CRYAA,4 CRYAB,5 CRYBB1,6 CRYBB2,5 CRYBA1,8 CRYGD,9 and CRYGD10; (2) the transcription factor genes MAF, PITX3, and HSF411–13; (3) the genes for membrane transport proteins MIP, CX46, and CX5014–16; and (4) the cytoskeletal protein BPES2.17 Mutations in four genes (LIM2, CRYAA, HSF4, GCNT2, and CRYBB3) have been implicated in autosomal recessive cataract.18–22 Two additional genetic loci have been defined for cataracts on the short arm of chromosome 3 and on chromosome 9, region q1342.23–24 but the specific genes at these loci remain to be identified.

The water-soluble lens crystallins constitute approximately 90% of the total lens proteins. They are divided into α-, β-, and γ-crystallins according to the order of their elution on gel exclusion chromatography. Because they are very stable proteins and have an essential role in maintaining lens transparency, the crystallins are thought to be good candidate genes for inherited cataracts. α-Crystallin is the most abundant soluble protein in the lens and is composed of 30 to 40 copies of αA and αB subunits with a 3:1 ratio (encoded by CRYAA and CRYAB genes, respectively). CRYAA is expressed at a high level in the lens and is found in only trace amounts in a few nonlenticular tissues.25–27 Mutations in CRYAA have been identified in patients with autosomal dominant congenital cataract (ADCC) and autosomal recessive congenital cataract (ARCC) in different families.4,19 CRYAB is widely expressed in several nonocular tissues, including cardiac and skeletal muscle. In 1998, Vicart et al.28 identified one CRYAB mutation, Arg120Gly, in a French family with desmin-related myopathy. Discrete lens opacities were noted in some patients in the family. Two new CRYAB mutations were identified in two patients with desmin-related myofibrillar myopathy (without cataract) by Seelen and Engel29 in 2003: a 2-bp deletion (464delCT) and a nonsense mutation Gln151Stop. In 2001, Berry et al.30 identified the first CRYAB mutation associated with isolated posterior polar cataract, deletion 450delA that resulted in a frameshift in codon 150 in an English family. The deletion remains the only mutation of CRYAB that has been associated with nonsyndromic ADCC to date. It is not clear why some mutations in CRYAB cause muscle system disorders and/or cardiovascular defects, whereas the other mutation causes nonsyndromic ADCC. Some investigators suggested that...
the congenital cataract disease gene in the 11q22-22.3 region may be a different gene from CRYAB.\textsuperscript{30}

In the present study, we investigated a large Chinese family with autosomal dominant congenital posterior polar cataract and identified a novel Pro20Ser mutation of CRYAB that cosegregates with the disease in the family. Our results strongly suggest that mutations in CRYAB cause nonsyndromic cataract.

**Materials and Methods**

**Subjects**

The Chinese family with multiple individuals affected with cataract was enrolled in this study in Shandong province of the Peoples Republic of China. Clinical diagnosis was performed for the participants with a full ocular and systemic medical assessment, which included muscular tension and muscular reflex of proximal and distal muscles of the lower and upper limbs, electromyography, the serum creatine kinase level, electrocardiography (ECG), and echocardiography. These examinations excluded the possibility that the patients in the family had any cardiovascular or muscular defects.

Informed consent was obtained from the participants in accordance with the study protocols approved by the Ethics Committee of Huazhong University of Science and Technology, and the study adhered to the guidelines of the Declaration of Helsinki.

**Genotyping**

Genomic DNA was prepared from whole-blood (DNA Isolation Kit for Mammalian Blood; Roche Diagnostics Co., Indianapolis, IN). A panel of candidate genetic loci for cataracts, including 14 known ADCC genes, was selected for preliminary linkage and haplotype analysis. The markers were selected from the LMS ver. 2.5-MD10 marker set (Applied Biosystems, Inc. [ABI], Foster City, CA), and they flank the 14 known ADCC genes. Additional microsatellite markers were identified from the Marshfield Genetic Database for fine-mapping of the disease gene in the family (http://www.marshfieldclinic.org/provided in the public domain by the Marshfield Clinic, Marshfield, WI).

Markers were genotyped (model 3100 Genetic Analyzer; ABI) at the Huazhong University of Science and Technology Human Genome Research Center. Genotypes were analyzed on computer (GeneMapper 2.5 software; ABI). Two-point logarithm of the odds (lod) scores were calculated on computer (Linkage Package 5.2 program/ftp://linkage.rockefeller.edu/software/linkage/; provided in the public domain by Rockefeller University, New York, NY), assuming an autosomal dominant inheritance pattern, a gene frequency of 0.001, a full penetrance rate, and allele frequencies specific to a Chinese population. The allele frequencies of each marker were computed using genotyping data from 50 independent Chinese individuals.

**Mutation Analysis**

The disease gene in the Chinese cataract family was mapped to 11q22-22.3, where the CRYAB gene is located. The whole coding region and exon-intron boundaries of CRYAB were PCR amplified. The PCR products were extracted (QIAquick Gel Extraction Kit; Qiagen Inc., Valencia, CA) and sequenced with both forward and reverse primers. DNA sequence analysis was performed by dye termination sequencing (BigDye Terminator Cycle Sequencing v3.1 kit and the Prism 3100 Genetic Analyzer; ABI).

PCR primer pairs were designed from intronic sequences flanking the three CRYAB exons (NT_033899): exon 1, 1 forward (F) 5'-ATATATAAGGGGCTGGCTGTA-3' and 1 reverse (R) 5'-CAGGGTAG-GAAAGGAAAATGTG-3'; exon 2, 2F, 5'-AGGATGAATTACCGAACA-3' and 2R, 5'-ACCCCTGATCCCGACTGTTAT-3'; and exon 3, 3F 5'-TGATTCTGGGCGGTTGTAATGTT-3' and 3R (5'-AGCTTGATAATT-TGGGCGCTGC-3').

Single-strand conformational polymorphism (SSCP) analysis was performed by a standard method described previously.\textsuperscript{31-33}

**FIGURE 1.** Pedigree structure of a large Chinese family with autosomal dominate congenital cataract. Affected males and females are indicated by filled squares and circles, respectively, and normal individuals are shown as open symbols. The deceased individual is indicated by a slash through the symbol. Arrow: the proband (III:7). Genotyping data and results from haplotype analysis for five markers on chromosome 11 (D11S4175, D11S898, D11S1778, D11S1986, and D11S908) are shown below each symbol. Haplotype analysis defined the causative gene between D11S4175 and D11S908. The CRYAB gene is located between D11S1986 and D11S908.
RESULTS

We characterized a four-generation Chinese family with congenital cataract (Fig. 1). Twenty-one family members (13 affected and 8 unaffected) participated in the study. The cataract in the family was inherited in an autosomal dominant mode, and appeared to be bilateral, congenital posterior polar type (Fig. 2). All affected individuals started to have poor vision at a very young age, which further progressed to a single well-defined plaque confined to the posterior pole of the lens. The plaque was 0.6 to 3 mm in diameter by the age of 6 to 8 years. No other systemic findings in the cardiovascular and muscular systems or other ocular defects were identified in all the affected members, including II-1 (Fig. 1), who was 65 years of age. All affected patients required cataract surgeries and intraocular lens implantation before the age of 10 years. A reasonably good visual function was obtained after surgery, which suggests that visual deprivation was minimal in the critical period of visual development.

Linkage analysis excluded previously identified genes for ADCC, except for CRYAB. Further haplotype analysis confirmed the linkage of the disease gene to CRYAB on 11q22-22.3 (Fig. 1). Linkage analysis yielded a maximum two-point lod score of 4.52 for marker D11S1778 (recombination fraction $\theta = 0$; Table 1). Co-segregation of marker D11S1778 close to CRYAB with all affected individuals in the family and a lod score of 4.52 for the marker suggest that the disease gene in the family may be the CRYAB gene.

To identify the causative mutation in the ADCC family, we sequenced the whole coding region and exon–intron boundaries of the CRYAB gene. A single nucleotide change, C$\rightarrow$T at nucleotide 58 from the start codon ATG (NM_001885), was identified. The C$\rightarrow$T change results in substitution of the proline residue at codon 20 by a serine residue (Fig. 3A). The Pro20 residue is highly conserved during evolution and is located in the crystallin_N region of $\alpha$-crystallin (Fig. 3B). Direct DNA sequence analysis of the members of the family showed that the Pro20Ser mutation cosegregated with all affected individuals, and was not present in unaffected family members. SSCP analysis confirmed the results of DNA sequence analysis (Fig. 4). Further analysis with SSCP did not identify the mutation in 200 normal control subjects. These results suggest that the Pro20Ser mutation of CRYAB is not a rare polymorphism, but a causative mutation for the autosomal dominant congenital posterior polar cataract in the Chinese family.

DISCUSSION

In this study, we identified a novel mutation, Pro20Ser, in the CRYAB gene in a large four-generation Chinese family with nonsyndromic posterior polar cataract. The disease gene of this Chinese family is linked to 11q22-22.3, with a maximum lod score of 4.52, where two small heat shock proteins CRYAB and HSPB2 are harbored. The finding that HSPB2 expression is not detectable in the lenses makes it unlikely to be the candidate gene for ADCC. Instead, mutation analysis of CRYAB identified a novel mutation Pro20Ser that cosegregated with all affected individuals, but was not present in unaffected members of the family or the 200 normal control subjects. The mutation occurs at an evolutionarily conserved residue Pro20 in the $\alpha$-crystallin. This is the second novel mutation in CRYAB that has been linked to isolated cataract, and our results strongly indicate that mutations in CRYAB cause cataracts. It is interesting to note that the Pro20Ser mutation is located at the N terminus of CRYAB, whereas the previously identified 450delA mutation associated with cataract and other CRYAB mutations associated with myopathy are all located at the C terminus.

Vicart et al. identified a missense mutation Arg120Gly in a French family, and Selcen and Engel identified two other CRYAB mutations in two sporadic patients with myofibrillar myopathy. The latter two mutations are one 2-bp deletion (464delCT) that results in a truncated protein of 162 amino acids from the normal 175-amino-acid $\alpha$-crystallin and a 451C$\rightarrow$T transition resulting in a stop codon mutation Gln151Stop that also truncates $\alpha$-crystallin. Both mutations are in the C-terminal domain of $\alpha$-crystallin, which is necessary for the chaperone function of the protein. Expression studies suggest that both mutations have a dominant-negative effect. Berry et al. studied a four-generation family of English descent and identified a CRYAB mutation associated with cataract, a 1-bp deletion 450delA that results in a frameshift at codon 150. Together with the report by Berry et al., the present study demonstrates that mutations in the CRYAB gene indeed cause cataract. It is intriguing that mutations in the same CRYAB gene cause two seemingly distinct diseases, but the mechanism for this is unknown. It is interesting to note that myopathy patients with the mutation Arg120Gly also dis-

Table 1. Two-Point Lod Scores for Markers on 11q22-22.3

<table>
<thead>
<tr>
<th>Markers</th>
<th>cM</th>
<th>0.00</th>
<th>0.05</th>
<th>0.10</th>
<th>0.20</th>
<th>0.30</th>
<th>0.40</th>
<th>0.50</th>
<th>$Z_{\text{max}}$</th>
<th>$\theta$</th>
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<tbody>
<tr>
<td>D11S41775</td>
<td>91.47</td>
<td>$-\infty$</td>
<td>-1.86</td>
<td>-0.86</td>
<td>-0.10</td>
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<td>0.12</td>
<td>0.00</td>
<td>0.14</td>
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<td>0.00</td>
<td>0.00</td>
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<td>0.00</td>
<td>0.00</td>
<td>0.25</td>
</tr>
<tr>
<td>D11S1777</td>
<td>101.75</td>
<td>4.52</td>
<td>4.16</td>
<td>3.78</td>
<td>2.97</td>
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<tr>
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<td>3.91</td>
<td>3.60</td>
<td>3.27</td>
<td>2.56</td>
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<td>0.00</td>
<td>3.91</td>
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<td>-0.44</td>
<td>-0.19</td>
<td>-0.07</td>
<td>-0.02</td>
<td>0.00</td>
<td>0.00</td>
<td>0.50</td>
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$Z_{\text{max}}$, maximal lod score; $\theta$, recombination fraction.
played lens opacities, raising the possibility that different mutations in CRYAB can associate with myopathy, cataract, or both. Future genotype and phenotype correlation studies may clarify this question.

The molecular mechanism by which CRYAB mutations cause cataract is unknown. Brady et al. 35,36 generated mice with targeted disruptions of the genes that encode CRYAA35 and CRYAB36 and found that homozygous CRYAA knockout mice displayed microphthalmia, decreased lens size and weight, cataract formation before the age of 7 weeks, formation of CRYA35B-containing cytoplasmic inclusion bodies in lens fiber cells by 4 weeks of age, and a major shift of lenticular CRYA35B from the soluble to the insoluble phase. The lenses of the CRYAB knockout mice developed normally throughout life and were similar to lenses of wild-type mice. Compared with CRYAA knockout mice with severely decreased chaperone activity, the CRYAB-knockout mice have relatively normal chaperone activity, suggesting that αB-crystallin is not essential for proper lens development, whereas αA appears to be the more important protein in lens development and function. Considering the phenotype of the CRYAB-knockout mice, it is unlikely that the Pro20Ser mutation of CRYAB acts by a loss-of-function mechanism. As αB-crystallin interacts with αA-crystallin, mutant αB-crystallin with mutation Pro20Ser may act by a dominant negative mechanism that leads to dysfunction of αA-crystallin and the formation of cataract in the early life of mutation carriers. We cannot exclude the possibility that mutation Pro20Ser acts by a gain-of-function mechanism; however, if the 450delA mutation identified in the English family is a true mutation for cataract, this mechanism is less likely.

The mutation Pro20Ser reported in this study creates a new serine residue next to the phosphorylation site Ser19 in the CRYAB protein. There are three phosphorylation sites in CRYAB: Ser19, Ser45, and Ser59. Substitution of aspartate for serine residues, which mimics phosphorylated CRYAB, significantly reduces the chaperone efficacy of CRYAB.37 Further, Aquilina et al. 38 studied the aspartate mutations of serine residues known to be phosphorylated (Ser19Asp and Ser19Asp/Ser45Asp) and found that compared to wild-type CRYAB, the mutant proteins differ substantially in their ability...
to reduce protein aggregation. It is possible that the mutation Pro20Ser affects the chaperone function of CRYAB by changing the activity of phosphorylation site Ser19 of CRYAB in lens and causes cataract in the family.

Apoptosis is an essential cellular process for development of the lens, and induction of apoptosis in lens epithelial cells appears to be a common cellular mechanism that mediates stress-induced noncongenital cataractogenesis. The α-crystallin protein can prevent apoptosis induced by various agents including staurosporine, TNF, and UVA. Liu et al. demonstrated that αA-crystallin (encoded by CRYAA) and αB-crystallin (encoded by CRYAB) have differential functional mechanisms against UVA-induced apoptosis. It is possible that mutant αB-crystallin with mutation Pro20Ser no longer possesses the function to inhibit apoptosis of lens epithelial cells, which results in the development of cataract.

In summary, since the report of the only CRYAB mutation (deletion 450delA) associated with isolated posterior polar cataract in 2001, a controversy has been raised by Vero mann, who questioned whether CRYAB is the true cataract gene in the 11q22-22.3 region. Our results in this study provide strong evidence that mutations in CRYAB can cause cataracts.

Acknowledgments

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References


