A new congenital nuclear cataract caused by a missense mutation in the γD-crystallin gene (CRYGD) in a Chinese family

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Purpose: To identify genetic defects associated with nuclear golden crystal autosomal dominant congenital cataract (ADCC) in a Chinese pedigree in the north of China.

Methods: Clinical data were collected and the phenotype of the affected members in this family was recorded by slit lamp photography. Genomic DNA was isolated from peripheral blood. Linkage analyses excluded all known loci except that in 2q33-q35. Mutation analysis of CRYG S was carried by direct sequencing of the PCR products.

Results: Sequencing of the coding regions of CRYGA, CRYGB, CRYGC, and CRYGD showed the presence of a heterozygous C>A transversion at nt109 of the coding sequence (R36S) in exon 2 of CRYGD, which co-segregated with the affected members.

Conclusions: The R36S mutation in CRYGD identified in this Chinese family caused a nuclear golden crystal cataract phenotype not described before. This finding is an additional indication that there may be phenotypic heterogeneity of cataract, especially in different races.

Congenital cataracts are a significant cause of visual impairment in childhood. They have a high incidence and are a significant cause of vision loss worldwide causing approximately one tenth of childhood blindness [1]. Roughly 50% of congenital cataracts are hereditary and family studies have revealed that approximately 30% of children with bilateral isolated congenital cataract had a genetic basis [2].

Congenital cataract is phenotypically and genetically heterogeneous. To date, congenital cataracts, isolated or syndromic forms, have been mapped to 27 genetic loci, and the disease-associated mutations have been identified in 18 genes, including those coding for αA-crystallin [3,4], αB-crystallin [5], βA1-crystallin [6,7], βB1-crystallin [8], βB2-crystallin [9], γC-crystallin [10], γD-crystallin [11-13], beaded filament structural protein 2 [14], heat shock transcription factor 4 [15], gap junction protein alpha-3 [16], gap junction protein alpha-8 [17], paired-like homeodomain transcription factor-3 [18,19], ferritin [20], galactokinase 1 [21], glucosaminyl(N-acetyl)transferase 2 [22], major intrinsic protein of lens fiber (MIP) [23], lens intrinsic membrane protein 2 (LIM2) [24], and paired box gene 6 [25]. Among them, 12 distinct genes have been identified to cause nonsyndromic autosomal dominant cataracts, including seven genes coding for crystallin (CRYAA, CRYAB, CRYBA1/A3, CRYBB1, CRYBB2, CRYGC, CRYGD) and two genes coding for gap junctional channel protein (GJA3, GJA8), one gene coding for heat-shock transcription factor 4 gene (HSF4), one gene coding for major intrinsic protein (MIP), and one gene coding for beaded filament structural protein 2 (BFSP2). Also, a mutation in CRYGS with autosomal dominant cataract in humans was recently reported [26].

In our study, we performed linkage analysis on a four generation Chinese family with nuclear golden crystal cataracts by using STRs markers on 12 ADCC loci and haplotype analysis with makers on 2q33-q35. A missense mutation in exon 2 of the CRYGD gene was identified, which is responsible for the disease in this pedigree.

METHODS

Clinical evaluation: The family was ascertained by the Department of Ophthalmology, the Second Affiliated Hospital of Harbin Medicine University, Heilongjiang province, China. Informed consent in accordance with the Declaration of Helsinki and the Heilongjiang Institutional Review Board approval was obtained from all participants. The pedigree was a four generation family with 7 members affected (Figure 1). ADCC diagnosis was established by the presence of affected individuals in each generation and male to male transmission. Affected status was determined by the surgical records of cataract extraction for the patients or ophthalmologic examination, included slit lamp examination with dilated pupils, visual acuity testing, intraocular pressure measurement, fundus examination, and ultrasonic examination. Peripheral blood (5 ml) was collected from each of 5 available affected and 5 unaffected individuals in the family, and genomic DNA was extracted using standard protocol.

Genotyping and linkage analysis: Twenty-one microsatellite markers close linked to 12 known ADCC loci were used to perform allele-sharing among patients in the fami-
ily, including D1S252, D1S305, D2S1782, D2S325, D3S1290, D3S1744, D11S898, D11S1986, D12S90, D12S1676, D13S1236, D13S175, D16S3034, D16S421, D17S1294, D17S1288, D21S212, D22S1174, D22S315, TOP1P2, CRYBB2 and three additional markers, D2S2318, D2S1384, and D2S1385, linked to the CRYGs. The oligonucleotide primer sequences were selected from The GDB Human Genome Database. The order and genetic distances of the markers were derived from the Marshfield database. Microsatellites were amplified by polymerase chain reaction (PCR) following standard methods. The genotypes were obtained by silver stain and manual inspection. The pedigree and haplotype was constructed by Cyrillic version 2.1 (MathStat Software; Victoria, Australia).

**Mutation analysis:** DNA samples from all available affected and unaffected family members of the family were screened for mutations in CRYGA, CRYGB, CRYGC, and CRYGD by direct cycle sequencing of the PCR products. The genomic sequence of CRYGs was obtained from the Ensemble genome data resources. Gene specific PCR primers were used to amplify the three exons and flanking introns sequences of CRYGA, CRYGB (sequences available upon request), CRYGC,

<table>
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<tr>
<th>Gene</th>
<th>Exon</th>
<th>Strand</th>
<th>Sequence (5’-3’)</th>
<th>Fragment size (bp)</th>
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<td>GTCAGCTGGAAGGAACATCC</td>
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<tr>
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<td></td>
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</tr>
<tr>
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<td>Forward</td>
<td>CATTTCCAGTGAATGCAAG</td>
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<tr>
<td></td>
<td></td>
<td>Reverse</td>
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</table>

Gene specific PCR primer sequences for all exons and flanking introns sequences of CRYGA, CRYGB, CRYGC, and CRYGD and the size of each PCR product.
and CRYGD (Table 1). PCR products were sequenced in both directions, and the data were collected and analyzed using ABI 3730XL sequencer (Perkin-Elmer, Applied Biosystems, Foster City, CA).

**RESULTS**

**Clinical findings:** All affected members showed nuclear cataract, presented at birth or developed during infancy, progressing slowly with age. One of them had had cataract extraction. In the other four patients without cataract extraction, the cataract was bilateral and consisted of a central pulverulent opacity affecting the embryonal, fetal, and infantile nucleus of the lens, characterized by golden crystal punctuate, with metal-like refractivity in the opaque nucleus (Figure 2, Figure 3). From the hospital records, the best corrected visual acuity of the affected eyes varied from 20/200 to 40/200 before cataract extraction. There was no family history of any other ocular or systemic abnormalities. Autosomal dominant inheritance of the phenotype was supported by the presence of affected individuals in each of the four generations and with a male to male transmission.

**Mutation detection:** Allele-sharing analysis among the affected members in the family excluded linkage with the 11 ADCC loci other than those on 2q33-35. Haplotype analysis on chromosome 2q33-35 showed that a block of 5 markers (black bar in Figure 1) was co-segregated with the disease in this family. Direct sequencing was performed to cover exons and flanking intron-exon boundary sequences. A heterozygous C>A transversion (Figure 4, Figure 5) was identified at nucleotide 109 in exon 2 of CRYGD in all affected members, but was not observed in any of the unaffected family members. Fifty unrelated control individuals were also sequenced and the possibility of a rare polymorphism was excluded.

**DISCUSSION**

In this report, after excluding all known loci corresponding to ADCC in a Chinese family except on 2q33-35, we have identified a locus on 2q33.3-q34 associated with the nuclear golden crystal cataract and found that a C>A transversion in exon 2 of CRYGD in all affected members of the family. This mutation co-segregated with the cataract in the family and no mutation was identified in the 100 independent control allele.

It is known that only CRYGC and CRYGD encode abundant lens γ-crystallins in humans [27,28] and almost 90% of the γ-crystallins synthesized in human lens are the products of these two genes [29]. CRYGD is one of only two γ-crystallins to be expressed at high concentrations in the fiber cells of the human embryonic lens and these cells subsequently form the lens nucleus fibers. Many identified mutations in CRYGD have been and have proven to be cataractogenic. We think that the heterozygous mutation was responsible for the congenital cataract in this family. This is because first, Kmoch et al. [30] has identified this mutation in a Czech 5-year-old boy who suffered from photophobia and decreased visual acuity due to symmetrical crystal deposition and grayish opacities in both lenses, and then proved that cataract was caused by deposition of defined crystallized protein, γD crystallin. Second, we sequenced 100 alleles of 50 unrelated control individuals and exclude the possibility of a rare polymorphism. Third, the single transversion in the heterozygous state (cDNA 109C>A)
predicted a R37S substitution at the protein level. The arginine residue is highly conserved, scoring 35 among 36 CRYGD homologs cloned from various species using the MAXHOM alignment [30]. This is the first report that the R36S caused autosomal dominant congenital cataract in China.

How does R36S mutation cause crystals in the lens nucleus and affect lens transparency? Kmoch et al. [30] analyzed this problem by protein crystallography and found that the crystal structure at 2.25 Å suggested that the R36S mutation in γ-crystallin has an unaltered protein fold and it was thought that the absence of the Arg36 charge led to the redistribution of the surface charges in the mutants which could decrease steric hindrances, promoting the permanent mutual contacts and aggregations of the protein molecules and decrease the solubility of the R36S mutated protein, leading to crystal formation. However, congenital cataract is phenotypically and genetically heterogeneous. The same mutation in cataract genes may result in different phenotypes under different ethnic background and environment, even the same mutation can cause different cataractous phenotypes in a family, such as the Mexican pedigree with congenital hereditary cataract reported by Zenteno [31]. In contrast to the Czech boy’s crystals [30], characterized by deposition of numerous birefringent, pleiochroic, and macroscopically prismatic crystals in the nucleus and cortex, our cases had many crystal dots in the lens with golden metal-like refractivity in the grey-white opaque nucleus. The punctuate crystal and pulverulent opacity affected the embryonal, fetal, and infantile nucleus of the lens, but the cortex was transparent. This, perhaps, is a phenotypic polymorphism of congenital cataract. It may indicate that gene expression or the formation of the protein structure for cataract might be influenced by many other factors, which include unidentified modifier genes and other sequence variations. Further studies will help us to better understand the mechanism of cataract formation and processing of gene expression.

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REFERENCES


