Two Chinese families with pulverulent congenital cataracts and \( \Delta G91 \) CRYBA1 mutations

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Purpose: To characterize the disease-causing mutations and related phenotypes in two Chinese families with autosomal dominant congenital cataract.

Methods: Family members were clinically characterized by a complete eye examination. Genome-wide linkage screening was performed in Family 1 using a 10K single nucleotide polymorphism approach followed by genotyping of microsatellite markers from the regions with highest support for linkage. The candidate gene, \( \beta A1 \)-crystallin (CRYBA1), was sequenced in both families.

Results: Lens examinations in three affected phakic members showed bilateral pulverulent nuclear cataracts in two subjects of Family 1 while another subject of Family 2 displayed bilateral pulverulent lamellar cataract. Linkage analysis in 14 individuals (eight affected, three unaffected and three of their spouses) of Family 1 gave a maximum logarithm of odds score of 2.41 for D17S1294 in chromosomal region 17q11.12 that includes the \( \beta A1 \)-crystallin (CRYBA1) gene. In both families in-frame deletions of three bp were detected in exon 4 of \( \beta A1 \)-crystallin (CRYBA1) leading to loss of a guanine residue (\( \Delta G91 \)). The mutations cosegregated completely with the cataract phenotype in both families but were associated with distinct haplotypes suggesting that they had occurred independently.

Conclusions: The previously described \( \beta A1 \)-crystallin (CRYBA1) \( \Delta G91 \) mutation was demonstrated in two Chinese families with distinct phenotypes of congenital cataract, suggesting a lack of genotype-phenotype correlation. The findings also raise the possibility that the \( \Delta G91 \) mutation arise in a relatively mutation-prone sequence of the \( \beta A1 \)-crystallin (CRYBA1) gene.

Congenital or juvenile cataract is a critical diagnosis in pediatric ophthalmology [1]. Its early recognition and surgical intervention are essential to avoid irreversible visual loss, especially in the case of complete cataract [1,2]. If untreated, normal retinal development will be impaired due to lack of sharp focus of light and sensory deprivation [2].

Congenital cataracts have an overall prevalence of 1 to about 6 per 10,000 live births [2], and comprise a group of clinically and genetically heterogeneous conditions. The patients are generally classified according to the type and location of the observed opacities including cataracts of anterior polar, posterior polar, nuclear, lamellar, pulverulent, aceuliform, cerulean, total, cortical, polymorphic, or sutural types [3]. Approximately one-third of patients with isolated congenital cataracts have a familial form of the disease preferentially with autosomal dominant inheritance (autosomal dominant congenital cataract [ADCC]) [4]. To date, 16 disease genes have been identified for ADCC and 10 additional loci are implicated from family studies [3,5,6]. Nine of the known disease genes are related to the normal formation and function of crystallins including \( \alpha A \)-crystallin (CRYAA) [7,8], \( \alpha B \)-crystallin (CRYAB) [9], \( \beta A1 \)-crystallin (CRYBA1) [10,11], \( \beta A4 \)-crystallin (CRYBA4) [6], \( \beta B1 \)-crystallin (CRYBB1) [12], \( \beta B2 \)-crystallin (CRYBB2) [13-15], \( \gamma C \)-crystallin (CRYGC) [16,17], \( \gamma D \)-crystallin (CRYGD) [18], and \( \gamma S \)-crystallin (CRYGS) [5].

Crystallins are essential for maintenance of lens transparency and refraction [19]. The super family of crystallins comprises crystallins of \( \alpha \)-, \( \beta \)-, or \( \gamma \)-crystallin types [20], among which the \( \beta \)-crystallins are most abundant in the lens. The \( \beta A1 \)-crystallin (CRYBA1) gene (also known as \( \beta A3 \)-crystallin) encodes the \( \beta \)-crystallin A3 isoform 1, a 215 aa protein with a molecular weight of 25 kDa. Following linkage in an affected family to chromosomal region 17q11-12 [10], \( \beta A1 \)-crystallin (CRYBA1) was first identified as a cause of cataract in a pedigree with autosomal dominant zonular cataract [11]. Consequently, \( \beta A1 \)-crystallin mutations were reported in several families [21-25]. In addition to three splice mutations at the donor splice site of intron 3 [11,21,22], \( \Delta G91 \) mutations have been described in three affected families of different ethnic backgrounds [23-25].
Herein, we report two unrelated Chinese families with ADCC in which a ∆G91 mutation of CRYBA1 were demonstrated. Phenotypic studies demonstrate that the ∆G91 mutation was associated with nuclear pulverulent cataract and lamellar pulverulent cataract in the respective families.

METHODS
Families and clinical examinations: The two families (Family 1 with eight affected members and three unaffected siblings and Family 2 with four affected members and two unaffected siblings) with ADCC were identified and clinically evaluated in Tianjin Eye Hospital, Tianjin, China (Figure 1). In 17 individuals (12 affected and 5 unaffected members), the ophthalmic investigations included best correct visual acuity, slit-lamp examinations, measurement of intraocular pressure, and direct funduscopy. In three affected phakic individuals, (IV:3 and IV:4 of Family 1 and III:3 of Family 2), the lens was examined by slit-lamp. Peripheral blood samples for DNA analysis were obtained from 22 individuals including 14 members of Family 1 and 8 members of Family 2 (Figure 1). Informed written consent was obtained from each member of the family or their parents for sample collection and molecular analysis and the research was conducted with local ethical approval according to the Declaration of Helsinki.

Genotyping and linkage analysis in Family 1: Genome-wide genotyping of single nucleotide polymorphisms (SNPs) was carried out in 10 members of Family 1 (Figure 1) using Affymetrix GeneChip Mapping 10K Set of microarrays (Affymetrix, Santa Clara, CA). Genomic DNA was extracted from peripheral blood leukocytes using standard methods and 250 ng genomic DNA was assayed according to the recommendations of the manufacturer (GeneChip Mapping 10K 2.0 Assay Manual). The experiments were carried out at the Affymetrix Core Facility BEA at Karolinska Institutet, Stockholm, Sweden. Genotypes were determined using GDAS 2.0 software (Affymetrix). Non-parametric linkage (NPL) analyses were carried out using Merlin software [26] whereby the eight affected family members were included. Parametric linkage analyses were performed with GENEHUNTER version 2.1 and errors were removed using Pedcheck software (version 1.0) [27]. Chromosomal intervals were chosen based on linkage peaks in the non-parametric analyses. Furthermore,
in some regions, SNPs were randomly removed to allow analysis by GENEHUNTER and to avoid association between markers. Parametric analyses were performed under an assumption of an autosomal dominant inheritance with a penetrance of 0.999 for heterozygotes and included the 10 SNP typed individuals. A disease allele frequency of 0.001 and a phenotype rate of 0.001 were used. Allele frequencies were calculated based on the typed individuals. Genetic positions of non-parametric linkage (NPL) or logarithm of odds (LOD) scores on a chromosome were indicated in relation to the deCODE map.

Subsequently, additional microsatellite markers were selected for genotyping in five linkage-peak regions in chromosomes 1, 3, 4, 15, and 17 (Table 1). The aims were to refine the linkage mapping to a single chromosome, to refine the critical interval on a candidate chromosome, and to support cosegregation between a detected mutation and disease-associated haplotype. Amplification and detection of microsatellites was performed as previously described [28]. Multi-point linkage analysis was performed using the LINKAGE software package of SimWalk2, Version 3.35, under the assumption of an autosomal dominant trait with a disease-allele frequency of 0.0001 and a penetrance of 99%. The allele frequencies for each marker were assumed to be equal as well as the recombination frequencies in males and females.

Genotyping in Family 2: In Family 2, genotyping was carried out for eight microsatellite markers located close to the CRYBA1 gene in 17q11.2 (D17S921, D17S805, D17S1294, D17S1293, D17S966, D17S1299, D17S1868, and D17S787) with the aim of supporting cosegregation between detected mutation and disease-associated haplotype as well as to evaluate the possibility of a founder effect between Families 1 and 2. Pedigrees and haplotype data were generated using Cyrillic (version 2.1) software and confirmed by inspection.

Mutation screening of CRYBA1 in Families 1 and 2: The CRYBA1 gene was screened for mutations by sequencing of the six coding exons and flanking exon-intron boundaries using newly designed primers (Table 2). After amplification, the PCR products were purified and sequenced using the ABI BigDye™ Terminator cycle sequencing kit v3.1, according to the manufacturer’s instructions. The sequencing products were run and analysed in an ABI 3730 Genetic Analyzer (Perkin Elmer, Forster City, CA). Sequencing in both directions was carried out on DNA samples from three affected (II:1 and IV:4 in Family 1, II:1 in Family 2) and two unaffected (III:1 in Family 1, II:2 in Family 2) individuals. Exons with detected variations were sequenced in all family members, and candidate mutations were sequenced in 100 reference individuals to verify whether they represent disease-associated mutations.

RESULTS

Clinical characteristics of the two autosomal dominant congenital cataract families: Both families demonstrated autosomal dominant inheritance of cataract predisposition with affected members in four successive generations and male to male transmission (Figure 1). According to the medical records, all affected individuals had presented with bilateral and symmetrical pulverulent nuclear cataracts in early childhood but were without progressive development of lens opacities. Taken together with the negative history of other systemic abnormalities, the disease was classified as autosomal dominant congenital cataract (ADCC) in both families. In the three phakic individuals, slit lamp examinations were performed to characterize the lens phenotypes. Individual IV:3 and IV:4 in Family 1 presented powdery opacities with a diameter of approximately 5 mm in the nucleus whereas the proband III:3 in Family 2 showed perinuclear-shaped pulverulent opacities which were restricted to the lamellae with a transparent embryonic nucleus (Figure 2). Furthermore, horizontal and pendular nystagmus was observed in five individuals in Family 1 in four cases together with strabismus (Table 3).

Table 1. Results from genome-wide linkage screening in Family 1

<table>
<thead>
<tr>
<th>Chromosome number</th>
<th>Affymetrix 10 K chip Max NPL</th>
<th>Linkage-peak interval flanking SNPs</th>
<th>Microsatellite markers selected from linkage region for genotyping and further mapping</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4.9</td>
<td>rs3122425 to rs2341709</td>
<td>D1S2130, S2134, S1669, S1665, S1728, S551, S1588</td>
</tr>
<tr>
<td>3</td>
<td>4.9</td>
<td>rs1822813 to rs2138212</td>
<td>D15S2409, S1766, S1285, S2406, S2459, S3045, S2460, ATA28H11</td>
</tr>
<tr>
<td>4</td>
<td>4.6</td>
<td>rs2866796 to rs1388070</td>
<td>D4S2623, S2394, S1644, S1625, S1629</td>
</tr>
<tr>
<td>15</td>
<td>4.9</td>
<td>rs1964562 to rs2380165</td>
<td>D15S653, S652</td>
</tr>
<tr>
<td>17</td>
<td>4.6</td>
<td>rs718993 to rs1320283</td>
<td>D17S921, S805, S1294, S1293, S966, S1299, S1868, S787</td>
</tr>
</tbody>
</table>

The maximum non-parametric linkage score (NPL) and logarithm of odds score (LOD) were summarized in this table. Five linkage-peak regions were identified in chromosomes 1, 3, 4, 15, and 17. The flanking single nucleotide polymorphisms (SNPs) of each linkage-peak interval were shown in “flanking SNPs” column. Additional microsatellite markers were selected for genotyping in the five regions.
Genome-wide linkage screening: Genome-wide SNP typing using Affymetrix 10 K SNP arrays was carried out in Family 1. Non-parametric linkage analysis identified regions of NPL scores between 4.5 and 4.9 in chromosomes 1, 3, 4, 7, 15, and 17 using an “affected-only” approach. Subsequent parametric linkage analysis revealed maximum LOD score of 1.8 in chromosomes 1, 3, 4, 15, and 17, while only nonsignificant LOD scores were obtained for chromosome 7. The maximum NPL and LOD scores and target intervals of the five significant chromosomal regions are summarized in Table 1. In the next step microsatellite markers representing the five candidate regions were analyzed (Table 2). This excluded chromosomes 1, 3, 4, and 15 while close linkage without recombination was observed between ADCC and markers from chromosome 17 (D17S1294, D17S1923, D17S966, D17S1299, and D17S1868). From multi-point linkage analysis, a maximum LOD score of 2.41 was obtained for D17S1294, which is located close to CRYBA1, a gene known to be involved in ADCC (Figure 3).

Table 3. Results from ophthalmic investigations of family members affected by autosomal dominant congenital cataract

<table>
<thead>
<tr>
<th>Number</th>
<th>Sex</th>
<th>Age (years)</th>
<th>Age at cataract operation (years)</th>
<th>BCV</th>
<th>Nystagmus</th>
<th>Strabismus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Family 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>II:1</td>
<td>F</td>
<td>68</td>
<td>58</td>
<td>FC</td>
<td>0.05</td>
<td>yes</td>
</tr>
<tr>
<td>III:2</td>
<td>M</td>
<td>40</td>
<td>26</td>
<td>0.1</td>
<td>0.1</td>
<td>yes</td>
</tr>
<tr>
<td>III:3</td>
<td>M</td>
<td>36</td>
<td>22</td>
<td>0.05</td>
<td>0.1</td>
<td>yes</td>
</tr>
<tr>
<td>III:4</td>
<td>M</td>
<td>30</td>
<td>16</td>
<td>0.4</td>
<td>0.2</td>
<td>yes</td>
</tr>
<tr>
<td>IV:1</td>
<td>F</td>
<td>15</td>
<td>2</td>
<td>0.6</td>
<td>0.6</td>
<td>no</td>
</tr>
<tr>
<td>IV:2</td>
<td>F</td>
<td>12</td>
<td>2</td>
<td>0.7</td>
<td>0.6</td>
<td>no</td>
</tr>
<tr>
<td>IV:3</td>
<td>M</td>
<td>14</td>
<td>*</td>
<td>0.1</td>
<td>0.1</td>
<td>yes</td>
</tr>
<tr>
<td>IV:4</td>
<td>M</td>
<td>10</td>
<td>*</td>
<td>0.1</td>
<td>0.1</td>
<td>yes</td>
</tr>
<tr>
<td>Family 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>II:1</td>
<td>M</td>
<td>40</td>
<td>2</td>
<td>0.3</td>
<td>0.5</td>
<td>no</td>
</tr>
<tr>
<td>III:1</td>
<td>F</td>
<td>20</td>
<td>2</td>
<td>0.6</td>
<td>0.7</td>
<td>no</td>
</tr>
<tr>
<td>III:2</td>
<td>M</td>
<td>15</td>
<td>2</td>
<td>0.6</td>
<td>0.6</td>
<td>no</td>
</tr>
<tr>
<td>III:3</td>
<td>F</td>
<td>10</td>
<td>*</td>
<td>0.3</td>
<td>0.1</td>
<td>no</td>
</tr>
</tbody>
</table>

“Age (years)” column indicates the age when ocular measurements were recorded. In the “Age at cataract operation (years)” column, the asterisk indicates that cataract surgery has not been carried out. Lens phenotypes were documented by dilated slit lamp examination. In Family 1, individuals II:1, III:2, 3, IV:3, and 4 had horizontal and pendular nystagmus. Moreover, strabismus were also observed in individuals II:1, III:3, IV:3, and 4. Abbreviations are: BCV, best corrected vision; and FC, finger count/30 cm.

Figure 2. Lens phenotypes and detection of identical CRYBA1 mutations in Families 1 and 2. Shown in the top panel are direct slit-lamp photographs showing typical opacities of pulverulent nuclear cataract in affected member IV:4 in Family 1. Member III:3 of Family 2 shows lamellar pulverulent cataract with a few dot-like opacities restricted to the lamellae together with a transparent normal embryonic nucleus. In the bottom panel are shown deletions of 3 bp in exon 4 of CRYBA1 in the two families as illustrated by the forward and reverse sequences shown, corresponding to Family 1 and Family 2, respectively. These mutations lead to loss of a glycine residue at amino acid position 91 (ΔG91).
Detection of CRYBA1 mutations: In-frame deletions of 3 bp (GAG) were identified in both families in exon 4 of CRYBA1. These deletions affect nucleotides 276-278 or 279-281 and are predicted to result in loss of a glycine residue at amino acid position 91 (ΔG91; Genbank NM_005208). The mutations completely cosegregated with the cataract phenotype in both families but were not found in any unaffected members or in 100 unrelated normal individuals. SNPs were also observed including rs1047790 and rs2286047 in Family 1 and rs1047790 in Family 2. To determine whether the mutations had occurred independently in the two families, or whether they could represent a founder effect, the disease-associated haplotypes of the CRYBA1 region were determined and compared. For this purpose, microsatellite markers from the CRYBA1 region were genotyped in Family 2 as well. Taken together with the SNP rs1047790 in exon 5 of CRYBA1, the ΔG91 mutations were shown to be linked to different haplotypes in the two families (Figure 1), which argues against a founder effect.

DISCUSSION

Our two families with distinctive ADCC phenotypes harbored ΔG91 mutations of the CRYBA1 gene. The cosegregation of mutation and cataract phenotype together with its absence in reference individuals support its pathogenic importance. By analyzing allele sharing for SNPs between affected members in Family 1, chromosome 17 was correctly identified as a candidate location. However, several additional chromosomes were also suggested and supported by NPL scores close to five. Similar results were obtained in parametric linkage analysis, which gave maximum LOD scores of 1.8 in five of these chromosomes. Subsequent analysis of regional microsatellites permitted assignment to the CRYBA1 region in 17q11 with exclusion of the other suggested chromosomes. The correct assignment was confirmed by demonstration of a ΔG91 mutation.

Taken together with the present study, CRYBA1 mutations have been reported in eight ADCC families. These CRYBA1 mutations were found in two locations, the splice donor site of intron 3 and nucleotides 276-281 in exon 4 [11,23-25]. In general, mutational clustering could reflect founder effects, mutational hot spot sequences, or a functional importance for the ADCC phenotype. In this study, ΔG91 was linked to different haplotypes of the CRYBA1 region, suggesting that the mutations in Family 1 and 2 have occurred independently. This finding is supported by the three published kindreds with ΔG91 that are from different geographical regions, which suggests their independent occurrence. Furthermore, the ΔG91 mutation can result from a 3 bp deletion affecting either the GAG at nucleotide 276-278 or the GAG at nt 279-281, while kindreds...
with ΔG91 do not necessarily share the same mutational event. The functional consequences of ΔG91 have been described by Reddy et al. [25]. In experimental systems, the mutant protein was found to cause defective folding and reduced solubility [25], βA1-crystallin consists of four “Greek-Key” β-sheet motifs with four β-strands termed a, b, c, and d [29]. The impaired folding is suggested to result from broken hydrogen bonds between c2 and d1 strands following the loss of glycine 91 next to a tyrosine corner that stabilize the protein at the connection between b and c β-strands [24].

Studies of affected families segregating the same mutations allow for genotype-phenotype comparisons. Although such comparisons in ADCC will be hampered because of the limited numbers of studied individuals, some observations deserve mentioning. A ΔG91 mutation of CRYBA1 has so far been identified in one Swiss [23], one British [25], and one Chinese family [24]. In our study, the two phakic individuals in Family 1 showed pulverulent nuclear cataract with powderly and white opacities while the proband of Family 2 had pulverulent lamellar cataract without involvement of embryonic nucleus or surrounding cortex. In the previously reported British family, bilateral dense opacities of lamellae were observed in seven phakic individuals [25]. In the Swiss family, one phakic subject presented with a symmetrical nuclear opacity (radial diameter of 5 mm) but was without involvement of the anterior or posterior Y-sutures [23]. Similarly, bilateral nuclear cataracts with a well-defined and dense opacity in the embryonic nucleus were observed in the Chinese family [24]. According to the most recent classification [3], patients with pulverulent cataracts have powderly opacities in the lens. In pulverulent nuclear or pulverulent lamellar cataracts, the opacities are restricted to the nucleus or lamella, respectively [3]. The opacity observed in Family 1 had a size of 5 mm, indicating that both fetal and embryonic nuclei were involved. The phenotype of Family 1 partly overlaps with that of the previously-reported Swiss and Chinese families. Both of these families showed nuclear cataracts however, pulverulent opacities were not reported. Family 2 has pulverulent opacities restricted to the lamellae and are perinuclear-shaped with a transparent embryonic nucleus (Figure 2). In addition, a few dot-like opacities deposited in anterior lamellae were observed. In contrast to the British family reported by Reddy et al [25], Family 2 did not show dense opacities of lamellae.

Five affected members of Family 1 developed horizontal and pendular nystagmus at early ages before cataract surgery although the exact ages of onset are unknown due to delayed ophthalmic examination. It has been shown that children who lose central vision in both eyes before the age of two years develop nystagmus and that its severity depends on the extent of visual loss [30]. Given the central location of the opacity, the nystagmus is more likely secondary to the ADCC.

Together, the variation in cataract phenotype resulting from a ΔG91 mutation in CRYBA1 argues against a genotype-phenotype association. Instead, environmental factors or genetic variations in modifier genes could influence the phenotypic expression through inactivation or elimination of the mutant protein. Additionaly, the different phenotypes observed in ΔG91-carrying patients could result from mutation expression at different time-points during lens development [25]. A transient impairment might lead to a lamellar cataract [3] while nuclear cataract would develop over a longer period and at earlier stages.

In summary, we demonstrated ΔG91 mutations associated with distinct phenotypes in two Chinese families with ADCC. The findings suggest that ΔG91 occurs in a mutation prone sequence of CRYBA1 and that the phenotypic presentation of ADCC involves additional factors to the exact CRYBA1 mutation.

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REFERENCES


