**CRYBB1** mutation associated with congenital cataract and microcornea

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**Purpose:** The molecular characterization of a UK family with an autosomal dominant congenital cataract associated with microcornea is reported.

**Methods:** Family history and clinical data were recorded. This phenotype was linked to a 7.6 cM region of chromosome 22q11.2-q12.2, spanning the β-crystallin gene cluster (Zmax of 3.91 for marker D22S1114 at θ=0). Candidate genes were PCR amplified and screened for mutations on both strands using direct sequencing.

**Results:** Sequencing of the coding regions and flanking intronic sequences of *CRYBB2* and *CRYBB1* showed the presence of a novel, heterozygous mutation X253R change in exon 6 of *CRYBB1*. SSCP analysis confirmed that this sequence change segregated with the disease phenotype in all available family members and was not found in 109 ethnically matched controls.

**Conclusions:** X253R is predicted to elongate the COOH-terminal extension of the protein and would be expected to disrupt β-crystallin interactions. This is the first documented involvement of *CRYBB1* in ocular development beyond cataractogenesis.

The vast majority of world blindness results from untreated cataract [1], with the number of people blind from cataract increasing by approximately 1 million per year [2]. The development of novel treatments to slow down the progression of cataract is hindered by a limited understanding of cataractogenesis.

Microcornea, defined by a horizontal corneal diameter of less than 11.00 mm [21,22], can be seen as an isolated anomaly or with microphthalmia (small eye). The association of cataract and microcornea only has been described in rare autosomal dominant pedigrees [23-29]. The molecular basis of cataract with microcornea in the absence of microphthalmia, anterior segment dysgenesis, or coloboma has not been elucidated (OMIM 116150). More complex ocular phenotypes such as Peters’ anomaly, sclerocornea, aniridia, iris coloboma, and ectopia pupillae are also associated with cataract and microcornea [24,28-30]. Some of these complex anomalies have an identified molecular basis. The combination of cataract and microphthalmia with or without microcornea was associated with a missense mutation in *CRYAA* in one case [12] and a homozygous mutation in *CRYBB2* in another [11,31]. Mutation in the DNA binding domain of the bZIP transcription factor (*MAF*; OMIM 177075) results in pulverulent cataract alone or cataract associated with microcornea or iris coloboma [29]. *PAX6* (OMIM 607108) mutations have also been identified in patients with cataract, microcornea, and anterior segment dysgenesis [32,33].

We report here the linkage of cataract with microcornea to a region of chromosome 22q11.2-q12.2, for which a novel mutation X253R in the *CRYBB1* gene was identified. These findings link ocular development and cataractogenesis.

**METHODS**

Clinical data: Respecting the Tenets of the Declaration of Helsinki, a three generation family from the UK with autosomal...
Figure 1. Haplotype analysis of a family with autosomal dominant congenital cataract and microcornea. A: Pedigree and haplotype analysis of family showing segregation of four microsatellite markers on chromosome 22, listed in descending order from the centromere (D22S1174; CRYB2-CA; D22S258; D22S1144). Blackened symbols indicate clinically affected individuals, unblackened symbols represent unaffected relatives. The disease haplotype is indicated as a red bar with alleles D22S1174 (1), CRYB2-CA (5), D22S258 (2), and D22S1144 (1). B: Integrated genetic (Marshfield) and physical (UCSC) order of microsatellite markers across the β-crystallin gene cluster in the chromosomal regions 22q11.2-q12.2 (arrows indicate direction of transcription). Two-point LOD scores (Z) at \( \theta = 0 \) for linkage between cataract with microcornea phenotype and markers are given. The asterisk indicates the position of CRYB2-CA (X62390) which is an intragenic marker of the CRYBB2 gene positioned in intron 3. C: Clinical photograph of individual II-6, aged 39, showing microcornea with a horizontal corneal diameter equal to 9 mm. The eye is aphakic following cataract surgery in infancy with opaque capsular remnants in the periphery of the pupillary aperture.
Table 1. Clinical data on family with cataract and microcornea

<table>
<thead>
<tr>
<th>Individual</th>
<th>Age (years)</th>
<th>Age at cataract surgery</th>
<th>Best corrected Snellen visual acuity</th>
<th>Horizontal corneal diameter</th>
<th>Axial length</th>
<th>Glaucoma</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Right</td>
<td>Left</td>
<td>Right</td>
<td>Left</td>
</tr>
<tr>
<td>I-1</td>
<td>70</td>
<td>5 years</td>
<td>6/60</td>
<td>6/60</td>
<td></td>
<td></td>
</tr>
<tr>
<td>I-2</td>
<td>46</td>
<td>3 months</td>
<td>6/24</td>
<td>CF</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>I-4</td>
<td>32</td>
<td>17 years</td>
<td>6/36</td>
<td>6/60</td>
<td></td>
<td></td>
</tr>
<tr>
<td>II-6</td>
<td>39</td>
<td>3 months</td>
<td>6/60</td>
<td>LP</td>
<td>9</td>
<td>9</td>
</tr>
<tr>
<td>II-8</td>
<td>37</td>
<td>3 months</td>
<td>6/24</td>
<td>6/60</td>
<td>11</td>
<td>11</td>
</tr>
<tr>
<td>II-10</td>
<td>35</td>
<td>3 months (R) 22 years (L)</td>
<td>HM</td>
<td>6/18</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>III-1</td>
<td>22</td>
<td>6 months</td>
<td>6/6</td>
<td>6/36</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>III-2</td>
<td>19</td>
<td>3-4 months</td>
<td>6/9</td>
<td>3/60</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>III-4</td>
<td>18</td>
<td>3-4 months</td>
<td>6/9</td>
<td>6/24</td>
<td>11</td>
<td>11</td>
</tr>
<tr>
<td>III-5</td>
<td>10</td>
<td>5 months</td>
<td>6/24</td>
<td>6/18</td>
<td>11</td>
<td>11</td>
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<tr>
<td>III-8</td>
<td>1</td>
<td>2-3 months</td>
<td>6/96</td>
<td>LP</td>
<td>11.5</td>
<td>11</td>
</tr>
</tbody>
</table>

In the “Snellen visual acuity” column, persons who could not be assessed with the Snellen scale were categorized as count fingers at 3 m (CF), sees hand motions (HM), or light perception (LP). The “Age” column indicates the age when ocular measurements were recorded. Individual II-4 had axial length measurements carried out preoperatively at age 32 (microphthalmos is defined as an axial length <18.5 mm). In individual III-8, axial length measurements were taken at 4 weeks of age; the normal axial length for a neonate of this age is 16.7-17.6. All axial length and corneal diameter measurements were in mm.
of the Royal Air Force (UK) and so had passed a full ophthalmological assessment as part of his employment. A-scan ultrasonography measurements showed normal axial length of the eyes for age [35,36]. The mean axial length in affected individuals (excluding III-8) was 22.04 mm, and 22.20 mm in unaffected individuals. The axial lengths of the four-week-old patient III-8 (18.11 mm right eye and 17.91 mm left eye) were within the normal range for a neonate (normal: 16.7-17.6 mm) [37]. The morphology of the cataract was assessed in one individual at birth (III-8). The cataract was dense and nuclear but included cortical fibers (riders) and anterior and posterior polar opacities. Previous medical records showed that a similar cataract morphology was present at birth in all affected family members. No photographic documentation was available. Patient III-5 also had a single fine irido-lenticular strand in each eye. The majority of affected family members underwent surgery in infancy. Surgery was delayed in some cases, despite the early diagnosis, either because of initial postoperative complication (II-10) or for family reasons (II-4). The presence of nystagmus and level of postoperative visual impairment suggests that visual deprivation was significant in the critical period of visual development. Younger affected individuals had improved visual outcomes. Five affected individuals developed glaucoma postoperatively at different ages, three of which (II-10, III-1, and III-2) required glaucoma filtration surgery to control their intraocular pressure.

Molecular genetic data: Thirteen candidate genes were excluded by haplotype and linkage analysis (data not shown). The family could not be excluded from the β-crystallin cluster on chromosome 22q11.2-12.1 (Figure 1A). This region includes the β-crystallin genes CRYBB1, CRYBB2, CRYBB3, and CRYBA4 (Figure 1B), and the CRYBB2 pseudogene (CRYBB2P1). Linkage analysis using the MLINK program of the LINKAGE package version 5.2 gave a maximum two-point LOD score (Z_{Max}) of 3.91 for marker D22S1114 at \( \theta = 0 \) (Table 2). A homozygous mutation in CRYBB2 was previously associated with cataract and microphthalmia, but no pathological sequence variations were detected in CRYBB2 (exons 2-6) in this family. The previously reported nonsense heterozygous mutation (G220X) in exon 6 of CRYBB1 was excluded using NlaIV restriction endonuclease digestion. Direct sequencing of the complete coding sequence of CRYBB1 (exons 2-6) and flanking intronic regions identified a novel c.827T>C change (nucleotide change based on sequence NM_001887; gi:21536279; Figure 2B), changing the stop codon (TGA) to an arginine (CGA). SSCP analysis confirmed that this sequence alteration, X253R, segregated with the phenotype in the family (Figure 2A). X253R is predicted to result in translational read through at the stop codon causing elongation of the COOH-terminal chain until the next in-frame stop codon in the downstream 3′-UTR. This would result in elongation of the COOH-terminal chain by an additional 26 amino acid residues. Multiple sequence alignments of 4 mammalian CRYBB1 genes demonstrates that the coding region of exon 6 and the stop codon are highly conserved (data not shown). Analysis of 109 ethnically matched control individuals failed to detect this sequence variation and was not detected in 50 patients with age related cataracts or 50 patients with congenital cataracts.

**DISCUSSION**

This study identified a novel mutation in the CRYBB1 gene and provides the first molecular basis for cataract with microcornea (OMIM 116150) in the absence of microphthalmia, anterior segment dysgenesis, or coloboma. This work demonstrates that CRYBB1 plays a role not only in cataractogenesis but also in ocular development. The mutational mechanism resulting in cataract with microcornea is also unique, as the previous mutations reported in the β-crystallins were nonsense chain terminating mutations in exon 6 of CRYBB1 and CRYBB2 [9,11,14,16].

βB1-crystallin is a major subunit of the β-crystallins and comprises 9% of the total soluble crystallins in the human lens [38]. The major sequence difference between oligmeric β-crystallin and monomeric γ-crystallin is that β-crystallin have long terminal extensions. There is interest in the structural role played by sequence extensions which have been suggested to modulate protein association [39]. Removal of residues at the COOH-terminus does not appear to have a major effect on dimer formation or protein folding of bovine βB2-crystallin [40]. The novel mutation, X253R detected in this family is predicted to elongate the COOH-terminal extension and would be expected to disrupt β-crystallin interactions and truncation. As previous mutations in β-crystallins associated with cataract are chain terminating mutations, this work is the first association of COOH-terminal extending mutations with cataract.

### Table 2: LOD scores for linkage between cataract with microcornea and 22q11.2-q12.2 markers

<table>
<thead>
<tr>
<th>Marker</th>
<th>0.0</th>
<th>0.05</th>
<th>0.10</th>
<th>0.20</th>
<th>0.30</th>
<th>0.40</th>
<th>Z Max</th>
<th>( \theta ) Max</th>
</tr>
</thead>
<tbody>
<tr>
<td>D22S1174</td>
<td>3.61</td>
<td>3.32</td>
<td>3.02</td>
<td>2.35</td>
<td>1.60</td>
<td>0.76</td>
<td>3.61</td>
<td>0</td>
</tr>
<tr>
<td>CRYB2A</td>
<td>2.41</td>
<td>2.23</td>
<td>2.04</td>
<td>1.63</td>
<td>1.17</td>
<td>0.63</td>
<td>2.41</td>
<td>0</td>
</tr>
<tr>
<td>D22S258</td>
<td>3.01</td>
<td>2.76</td>
<td>2.51</td>
<td>1.94</td>
<td>1.31</td>
<td>0.61</td>
<td>3.01</td>
<td>0</td>
</tr>
<tr>
<td>D22S1144</td>
<td>3.91</td>
<td>3.60</td>
<td>3.27</td>
<td>2.56</td>
<td>1.74</td>
<td>0.84</td>
<td>3.91</td>
<td>0</td>
</tr>
</tbody>
</table>

Two-point LOD scores for linkage in microsatellite markers across the β-crystallin gene cluster in the chromosomal regions 22q11.2-q12.2. The maximum two-point lod score was achieved for D22S1114 at \( \theta = 0 \). \( Z_{Max} \); the maximum lod score achieved, is given for each marker. Significant linkage was found with microsatellite marker D22S1144 with pair-wise lod score exceeding 3.0.
ract, offering new insight into the development of the lens is intrinsically linked to the development of the anterior segment and this family highlights the complexity present in these early developmental cataractogenesis and crystallin biology.

The development of the lens is intrinsically linked to the development of the anterior segment and this family highlights the molecular complexity involved in these early developmental processes. A transcriptional cascade is involved in early lens development through Pax6 expression, followed by the expression of Mafs, Sox6, and Proxl, resulting in the initiation of lens differentiation and crystallin expression [41-46]. Mutations in these genes result in a variety of ocular phenotypes combining cataract, anterior segment dysgenesis, and specific iris defects [29,32,33], while mutations in crystallins mainly have a phenotype restricted to the lens [9-20]. In this family the mutation, X253R in CRYBB1 results in congenital cataract with microcornea. Individual III-5 had a single iridolenticular strand in each eye suggesting a slight element of anterior segment dysgenesis but not as severe as that associated with PAX6 and MAF mutations [29,32,33]. This is in keeping with experimental work which shows that βB1-crystallin expression occurs downstream of PAX6 and MAF [41,42,47].

The novel X253R mutation in the CRYBB1 gene is predicted to elongate the COOH-terminal extension and would be expected to disrupt β-crystallin interactions and as such, offers new insight into cataractogenesis and anterior segment development.

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REFERENCES


