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Report

Alpha-B Crystallin Gene (*CRYAB*) Mutation Causes Dominant Congenital Posterior Polar Cataract in Humans

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Congenital cataracts are an important cause of bilateral visual impairment in infants. In a four-generation family of English descent, we mapped dominant congenital posterior polar cataract to chromosome 11q22-q22.3. The maximum LOD score, 3.92 at recombination fraction 0, was obtained for marker D11S898, near the gene that encodes crystallin alpha-B protein (*CRYAB*). By sequencing the coding regions of *CRYAB*, we found in exon 3 a deletion mutation, 450delA, that is associated with cataract in this family. The mutation resulted in a frameshift in codon 150 and produced an aberrant protein consisting of 184 residues. This is the first report of a mutation, in this gene, resulting in isolated congenital cataract.

Inherited cataract accounts for up to half of all congenital cataract, and the most common mode of inheritance is autosomal dominant congenital cataract (ADCC). Autosomal recessive and X-linked forms are also seen but are uncommon. Inherited cataract is clinically heterogeneous, and, thus far, 14 distinct loci in humans have been identified, for 11 phenotypically distinct forms of ADCC (Ionides et al. 1999; Francis et al. 2000).

Cataract mutations have been found in genes encoding lens connexins on 1q (Shiels et al. 1998) and 13q (Mackay et al. 1999), aquaporin 0 on 12q (Berry et al. 2000), cytoskeletal protein CP49 on 3q (Jakobs et al. 2000), and lens-developmental protein PITX3 on 10q (Semina et al. 1998). Mutations in five different crystallin genes that encode >90% of lens cytoplasmic proteins have been identified as a cause of dominant disease; these genes include *CRYAA* (Litt et al. 1998; Pras et al. 2000), *CRYBA* (Kannabiran et al. 1998), *CRYBB* (Litt et al. 1997), *CRYGC* (Héon et al. 1999), and *CRYGD* (Héon et al. 1998). Furthermore, mutations in *CRYAB* that encode alpha-B (αB)-crystallin have also been shown to cause desmin-related myopathy (Vicart et al. 1998), with the formation of protein inclusions comprising the intermediate filaments of the desmin class (Perng et al. 1999; Quinlan and van den IJssel 1999). We now report a novel mutation in *CRYAB* in a family with ADCC with posterior polar cataract (CPP2) and no other associated clinical phenotype.

Posterior polar cataract is a clinically distinctive opacity that is located at the back of the lens and that, because of its proximity to the optical center of the eye, can have a marked effect on visual acuity. Dominantly inherited posterior polar cataract has been linked to the haptoglobin locus on 16q (Richards et al. 1984), as has the dominant Marner congenital cataract (Marner et al. 1989). Both are described as progressive, but, in the Marner cataract, the opacities initiate in the nucleus rather than at the posterior pole of the lens, suggesting either allelic heterogeneity or that two distinct cataract genes exist at the 16q locus.

A four-generation family of English descent with autosomal dominant posterior polar cataract underwent a full ophthalmological examination. The opacity (fig. 1), which was bilateral in all cases, consisted of a single well-defined plaque that was confined to the posterior pole of the lens and that was 0.5–3 mm in diameter. Hospital records indicated that usually the opacity either

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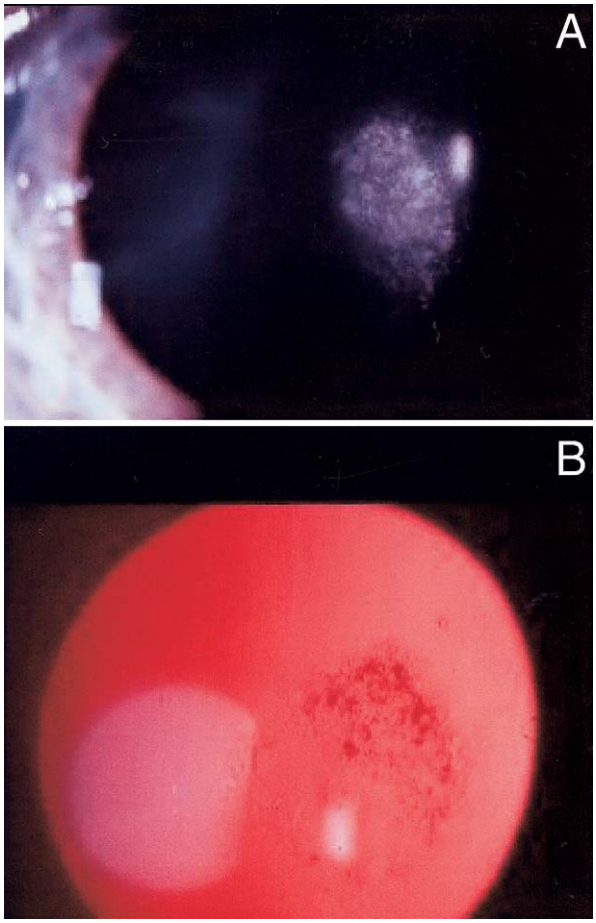

Figure 1 Direct illumination (*A*) and retroillumination (*B*) of posterior polar cataract observed in the 11-year-old boy (V:1) in the family with CPP2.

was present at birth or developed during the first few months of life but did not progress, with age, to other regions of the lens. There was no evidence of posterior lenticonus or high myopia, and there was no family history of other ocular or systemic abnormalities.

Linkage analysis was performed in this family, and, after the exclusion of a number of candidate loci for cataract, we obtained a positive two-point LOD score (3.92 at recombination fraction 0) for the marker D11S898, flanked by D11S4176 and D11S908, on chromosome 11q21.2-q22.3, encompassing the *CRYAB* locus. *CRYAB* encodes αB-crystallin (*CRYAB* [MIM 123590]), a member of the small heat-shock protein (sHSP) family of molecular chaperones (de Jong et al. 1998). *CRYAB* comprises three exons and encodes a protein of 175 amino acid residues. Sequence analysis of the *CRYAB* gene revealed, in exon 3, a deletion mutation, 450delA, that cosegregated with disease in the family (fig. 2). It resulted in a frameshift in codon 150 and produced an aberrant protein consisting of 184 residues (fig. 3). The mutation was not found in a panel of 100 normal unrelated individuals, excluding the possibility that it is a rare polymorphism.

In the lens, αB-crystallin is found associated with αA-crystallin, another sHSP, which shares 55% amino acid sequence identity with αB-crystallin. The two ~20-kD subunits together form soluble complexes comprising αA- and αB-polypeptides in a 3:1 ratio, with an average molecular mass of 600–800 kD. The α-crystallins are some of the most abundant soluble proteins in the lens and, along with the other lens crystallins, play an important role in establishing and maintaining the optical properties of the lens (Delaye and Tardieu 1983). Like other sHSPs, αA- and αB-polypeptides can act as molecular chaperones in vitro, preventing protein aggregation induced by heat and other stresses (Horwitz 1992).

A missense mutation, R120G, in *CRYAB* has already been reported to cause desmin-related myopathy and cataract. The mutation reported here is the first of its kind that has been shown to cause isolated congenital cataract. The effect of the mutation is the production of an aberrant protein of 184 residues with 35 novel amino acids at the C-terminus, starting at codon 154 of the protein, replacing several highly conserved residues (fig. 4). This may affect functionally important posttranslational modification reactions and may cause aberrant folding of the protein. It is known that both αA- and αB-crystallins undergo posttranslational modifications, including truncation of both the N terminus and the C terminus, deamidation, racemization, phosphorylation, methionine oxidation, glycation, disulfide formation, addition of O-GlcNAc, and the addition of 72 mass units to the C-terminal lysine of B-crystallin (Chiesa et al. 1987; de Jong et al. 1988; Roquemore et al. 1992; Smith et al. 1992; Groenen et al. 1994; Lin et al. 1997). Some of these activities, such as phosphorylation and specific cleavage, may be functionally important; others are likely the result of aging and detrimental stresses. As with modifications related to aging and stresses, the deletion mutation is also likely to alter the protein conformation, which, in turn, could alter the aggregate size and/or the function of α-crystallin in the cell. If there is aberrant aggregation of mutant protein, it may cause light-scattering and cataract and would explain the congenital phenotype of the mutation.

The mutation, which affects the C-terminal–extension region of the protein, is also likely to affect the chaperone function of αB-crystallin. Both αA- and αB-crystallin have polar, flexible C-terminal extensions that are thought to contribute to the solubility of these crystallins and that have been implicated in the chaperone-like activity of these crystallins (Carver and Lindner 1998). Substitution of Lys174–Lys175 of αB-crystallin by Leu-Leu significantly diminished chaperone-like activity; however, re-

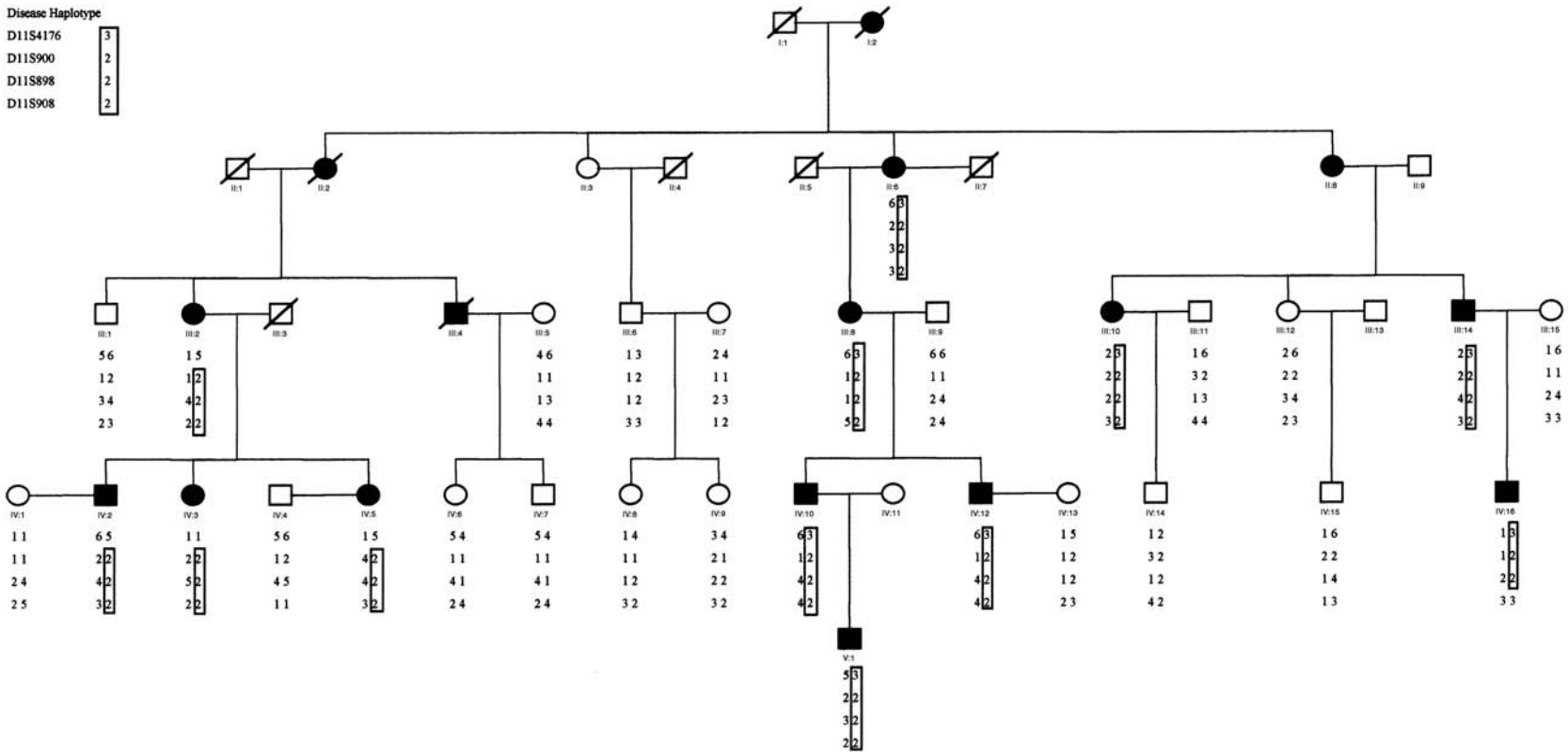

Figure 2 Family with CPP2. Square symbols denote males; circles denote females; affected individuals are denoted by black symbols. Autosomal dominant inheritance is suggested both by the presence of affected males and females in each generation and by male-to-male transmission.

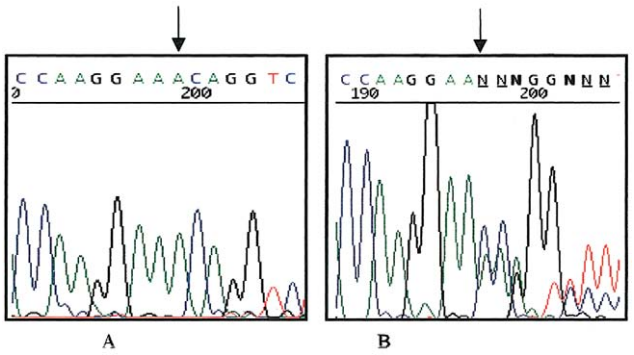

Figure 3 Sequence analysis of *CRYAB* in an unaffected individual (*A*) and an affected individual (*B*), both from the family with CPP2.

moval of the last five residues had little effect on chaperone-like activity (Plater et al. 1996). Likewise, in αA-crystallin, the introduction of tryptophan at the C terminus and removal of 17 C-terminal residues diminished chaperone-like activity (Takemoto 1994; Andley et al. 1996). The deleted 17 C-terminal residues in αA-crystallin include residues 156–167, which are highly conserved between αA- and αB-crystallins, a result that is suggestive of common functionality in chaperone-like activity (fig. 4). Since the 450delA mutation in *CRYAB* also leads to the replacement of these residues by novel residues, it is very likely to compromise the chaperone-like activity of αB-crystallin.

In conclusion, we have identified, in αB-crystallin, a novel mutation causing posterior polar cataract. We speculate that the cataract in this family may result from an increased tendency of the mutant polypeptide to aggregate and/or from loss of chaperone-like activity. This mutation may help delineate the functional domains of αB-crystallins, which are important both as structural proteins contributing to the refractive index of the lens and for their protective role as molecular chaperones suppressing aggregation of denatured proteins. Furthermore, biophysical studies of the mutant αB-crystallin may elucidate the mechanism of cataract formation.

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Electronic-Database Information

The accession number and URL for data in this article are as follows:

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim/ (for CRYAB [MIM 123590])

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Figure 4 Protein alignment depicting similarity of human αB- and αA-crystallin with those of mouse, cow, rabbit, and chicken. Human αB-crystallin shows the highest homology, over its entire length, to αB-crystallins of rabbit, cow, mouse, and chicken, with identity scores of 98%, 97%, 97%, and 76%, respectively. Human αB-crystallin has 55% amino acid identity with αA-crystallin of human and of cow. Amino acids that exhibit complete identity across species are boxed. The aberrant protein produced as a result of the deletion mutation, 450delA, is shown with its 35 novel residues (*blue*) after the 149th residue of the wild- type αB-crystallin (*arrow*). The amino acid residue mutated (R120G [*red*]) in desmin-related myopathy is also shown.

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