

Report

A Nonsense Mutation in *CRYBB1* Associated with Autosomal Dominant Cataract Linked to Human Chromosome 22q

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Autosomal dominant cataract is a clinically and genetically heterogeneous lens disorder that usually presents as a sight-threatening trait in childhood. Here we have mapped dominant pulverulent cataract to the β -crystallin gene cluster on chromosome 22q11.2. Suggestive evidence of linkage was detected at markers D22S1167 (LOD score [Z] 2.09 at recombination fraction [θ] 0) and D22S1154 (Z = 1.39 at θ = 0), which closely flank the genes for β B1-crystallin (*CRYBB1*) and β A4-crystallin (*CRYBA4*). Sequencing failed to detect any nucleotide changes in *CRYBA4*; however, a G→T transversion in exon 6 of *CRYBB1* was found to cosegregate with cataract in the family. This single-nucleotide change was predicted to introduce a translation stop codon at glycine 220 (G220X). Expression of recombinant human β B1-crystallin in bacteria showed that the truncated G220X mutant was significantly less soluble than wild type. This study has identified the first *CRYBB1* mutation associated with autosomal dominant cataract in humans.

Crystallin genes encode >95% of the water-soluble structural proteins present in the vertebrate crystalline lens, accounting for >30% of its mass (for review, see Graw 1997). Biophysical studies have indicated that the unique spatial arrangement and short-range ordering of the crystallin proteins establish the optical transparency (Delaye and Tardieu 1983) and high refractive index (Fernald and Wright 1983, 1984) of the lens. At least 13 functional crystallin genes have been located in the human genome, and 11 major crystallin proteins have been isolated from the human lens (Lampi et al. 1997). The latter may be subdivided into two evolutionary distinct groups, comprising two α -crystallins, which are members of the small heat-shock family of proteins that function as molecular chaperones (Horwitz 1992), and nine β/γ -crystallins, which share a common two-domain structure composed of intercalating “Greek-key” motifs (Norledge et al. 1997). The β/γ -crystallins are structurally related to a

number of evolutionarily diverse proteins—including bacterial spore-coat protein S, slime mold spherulin 3a, and amphibian epidermis differentiation-specific protein—and to the absent-in-melanoma tumor suppressor (for review, see D’Alessio 2002).

Because of their abundant expression in the lens, crystallins represent compelling candidate genes for certain inherited forms of lens opacities, or cataracts, that usually present at birth (congenital) or during infancy and that represent a clinically significant cause of vision loss in childhood (Lambert and Drack 1996). So far, ≥ 10 mutations in six human crystallin genes have been associated with nonsyndromic forms of Mendelian cataract, linked to 2q (Héon et al. 1999; Stephan et al. 1999; Kmoch et al. 2000; Pande et al. 2000; Ren et al. 2000), 11q (Berry et al. 2001), 17q (Kannabiran et al. 1998; Bateman et al. 2000), 21q (Litt et al. 1998; Pras et al. 2000), and 22q (Litt et al. 1997; Gill et al. 2000; Vanita et al. 2001). Clinical examination of these crystallin-related cataracts by using a slit-lamp has revealed considerable inter- and intrafamilial variation with respect to the physical location and appearance of opacities in different developmental regions of the juvenile lens. Central pulverulent, or powdery, opacities have been linked with the genes *CRYGC* (MIM *123680), on 2q (Héon et al. 1999), and *CRYBB2* (MIM *123620), on 22q (Gill

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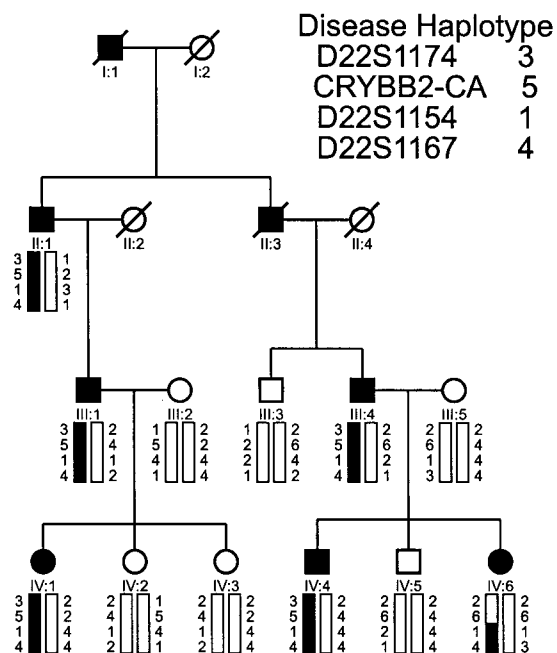


Figure 1 Pedigree and haplotype analysis of family ADC4, showing segregation of four microsatellite markers (in descending order from the centromere) on chromosome 22. Squares and circles symbolize male and female individuals, respectively; blackened symbols denote affected status.

et al. 2000). Crystalline, central-nuclear, and progressive-punctate opacities have been associated with distinct mutations in *CRYGD* (MIM *123690), on 2q (Stephan et al. 1999; Pande et al. 2000). Lamellar opacities have also been associated with *CRYGC* and *CRYGD* mutations (Santhiya et al. 2002). Sutural opacities, which affect the Y-shaped lines that mark the contact of fiber cell ends at the anterior and posterior poles of the fetal lens, have been linked with mutations in *CRYBA3/CRYBA1* (MIM *123610), on 17q (Kannabiran et al. 1998), and *CRYBB2*, on 22q (Litt et al. 1998). Cerulean (blue-dot) opacities, which progressively affect the cortex of the lens, have also been associated with a mutation in *CRYBB2* (Litt et al. 1997; Vanita et al. 2001). Finally, central or nuclear opacities have been associated with mutations in *CRYAA* (MIM *123580), on 21q (Litt et al. 1998), whereas posterior polar cataract has been linked with *CRYAB* (MIM *123590), on 11q (Berry et al. 2001). To gain further insight about the relationships between crystallin gene mutations and cataract morphology, we performed linkage analysis in a four-generation family that segregated autosomal dominant pulverulent cataract and subsequently identified a novel mutation in the gene for β basic 1 (β B1)-crystallin (*CRYBB1* [MIM *600929]), on 22q.

The cataract, which was bilateral in all cases, consisted of fine, dustlike opacities that mainly affected the central zone, or fetal nucleus, of the lens but also affected the cortex and the anterior and posterior Y-suture regions. Ophthalmic records confirmed that the opacities were present from birth and that there was no family history of other ocular or systemic abnormalities. After informed consent was obtained, 12 members of the pedigree (fig. 1) were genotyped with microsatellite markers from the (CA)_n map (Dib et al. 1996), at two crystallin loci known to be associated with pulverulent forms of cataract, on 2q (Héon et al. 1999) and 22q (Gill et al. 2000). From the fully annotated chromosome 22 database (Dunham et al. 1999) (see the Wellcome Trust Sanger Institute Web site), we selected three framework markers that closely flanked the four functional *CRYB* genes clustered on 22q (fig. 2). In addition, we selected a (CA)₁₅ dinucleotide repeat marker (*CRYB2-CA* [GenBank accession number X62390]) that had previously been closely associated with the *CRYBB2* gene (Marineau and Rouleau 1992). Alignment of this ~175-bp polymorphic sequence with the entire *CRYBB2* gene sequence by using the BLAST algorithm (Altschul et al. 1990) detected ~100% homology with a region of intron 3, confirming that *CRYB2-CA* was an intragenic marker. First, we excluded linkage of the cataract to *CRYBB2* and *CRYBB3* (MIM *123630) with markers *CRYB2-CA* (LOD score [*Z*] -1.24 at recombination fraction [θ] 0) and D22S1174 (*Z* = -1.16 at θ = 0); however, we detected positive *Z* values for markers D22S1167 (*Z* = 2.09 at θ = 0) and D22S1154 (*Z* = 1.39 at θ = 0), which flank *CRYBB1* and *CRYBA4* (MIM *123631) (fig. 2). Then, haplotype analysis (fig. 1)

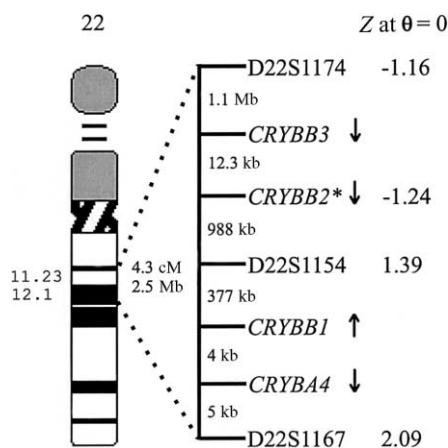


Figure 2 Idiogram of chromosome 22, showing the integrated genetic and physical order of microsatellite markers across the *CRYB* gene cluster. Physical distances between markers and genes are shown. Two-point *Z* values for linkage between the cataract and markers are indicated. An asterisk denotes the intragenic marker *CRYBB2-CA*. Arrows show the direction of gene transcription.

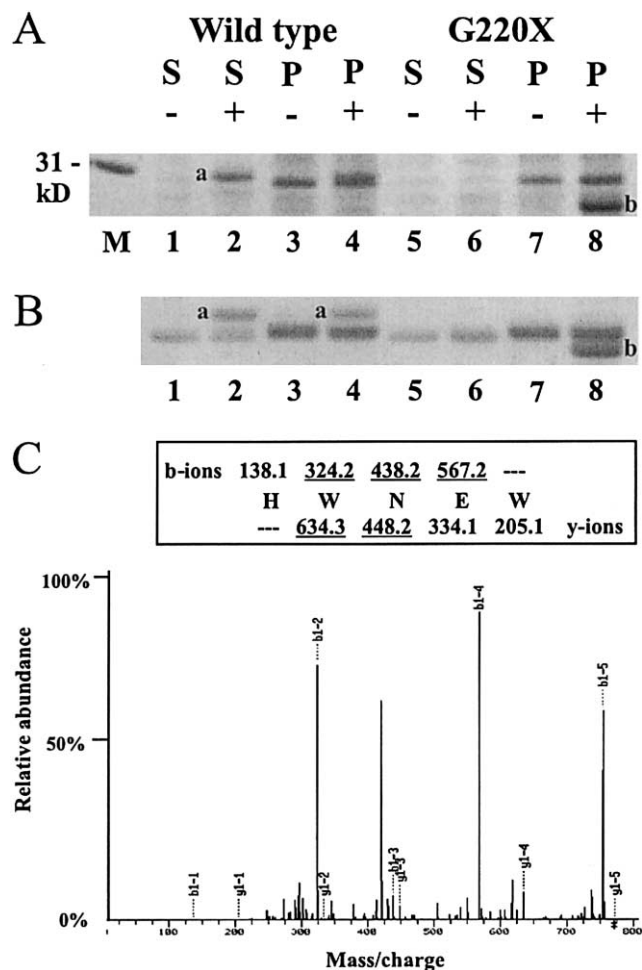


Figure 4 Expression of recombinant human β B1-crystallin in *E. coli*. *A* and *B*, SDS-PAGE (*A*) and immunoblot (*B*) analyses of wild-type β B1 and G220X- β B1 in the soluble (S) and insoluble pellet (P) fractions, before (–) and after (+) induction with IPTG. Lane M shows molecular mass-markers; lanes 1, 3, 5, and 7 show uninduced cell lysates, and lanes 2, 4, 6, and 8 show induced cell lysates; lanes 1, 2, 5, and 6 show soluble proteins, and lanes 3, 4, 7, and 8 show insoluble proteins. *C*, MS/MS spectrum, identifying the C-terminal peptide (HWNEW) derived from the G220X- β B1 mutant in band b excised from the gel (*lane 8*) shown in panel *A*. The inset shows masses of the expected fragment ions; intact N-terminal ions (b-ions) and C-terminal ions (y-ions) that were found in the spectrum are underlined.

most all of the wild-type β B1 was soluble. In contrast, there was no detectable induction of band a in the soluble fraction of *E. coli* transformed with G220X (figs. 4A and 4B, lane 6); however, a unique immunoreactive polypeptide of molecular size ~25 kDa (band b) was present in the induced pellet fraction from these bacteria (figs. 4A and 4B, lane 8). The β -crystallin antibody also cross-reacted with a closely migrating *E. coli* protein in all fractions analyzed (fig. 2B); therefore, we used mass-spectrometry techniques to unequivocally identify bands a and b as human β B1. Tryptic peptide masses derived from

band a (fig. 4A, lane 2), by using electrospray-ionization mass spectrometry (ESI-MS), were consistent with a molecular mass of 27,890 Da (data not shown), as described elsewhere for native β B1 (Lampi et al. 2001). Tryptic peptide masses derived from band b by ESI-MS were consistent with a truncated β B1 protein of molecular mass 24,046 Da (data not shown). Fragmentation of the peptides by tandem mass spectrometry (MS/MS) identified $\geq 75\%$ of the amino acid sequence of G220X- β B1 (Entrez-Protein accession number NP_001878) from excised band b (data not shown). No peptides corresponding to the C-terminal sequence of wild-type β B1 were detected by MS/MS; however, a peptide sequence matching the last five C-terminal residues (215 HWNEW 219) predicted by the G220X truncation (fig. 3D) was detected (fig. 4C).

Of the two other functional basic β -crystallin genes clustered on 22q, only *CRYBB2* has previously been associated with autosomal dominant cataract in humans (Litt et al. 1997; Gill et al. 2000; Vanita et al. 2001). *CRYBB2* and *CRYBB1* share ~55% sequence identity at the amino acid level and encode ~14% and ~9%, respectively, of the total crystallins present in the newborn human lens (Lampi et al. 1997). On the basis of the crystallographic structure of β B2-crystallin (Norledge et al. 1997), the G220X nonsense mutation in *CRYBB1* was predicted to result in a truncated β B1 protein that lacked ~33% of the fourth Greek-key motif (codons 220–234; fig. 3D) and the entire C-terminal arm (codons 235–252). Previously, a similar chain-termination mutation (Q155X) in *CRYBB2* (Litt et al. 1997; Gill et al. 2000; Vanita et al. 2001) was predicted to remove the last 51 amino acids of the β B2 protein, effectively deleting ~90% of fourth Greek-key motif (codons 155–192; fig. 3D) and the entire C-terminal arm (codons 193–205); however, no expression studies were reported. Remarkably, the Q155X mutation in *CRYBB2* has arisen in three geographically distinct families. Clinical descriptions of the cataract morphology varied from cerulean opacities, in an American family (Litt et al. 1997), to central and/or zonular pulverulent opacities, in a Swiss family (Gill et al. 2000), and sutural cerulean opacities, in an Indian family (Vanita et al. 2001). The G220X mutation in *CRYBB1* identified here was also associated with fine punctate opacities located in the central and sutural regions of the lens. The partial overlap in cataract morphology associated with the G220X and Q155X mutations may reflect the codistribution of β B1- and β B2-crystallins, respectively, in the high-molecular-mass octamer (M_r ~150 kDa) fraction and the lower-molecular-mass dimer (M_r ~46 kDa) and trimer/tetramer (M_r ~71 kDa) fractions present in the human lens (Ajaz et al. 1997; Ma et al. 1998).

Whereas bacterial expression of the G220X- β B1 mutant showed that the truncated protein, lacking a C-terminal arm and intact fourth Greek-key domain, was

almost totally insoluble, site-directed deletion of C-terminal residues from rat β B2 (Trinkl et al. 1994), chicken β B1 (Coop et al. 1998), and human β B1 (Bateman et al. 2001) did not significantly impair the ability of the recombinant proteins to be solubilized from bacteria or to form dimers in vitro. In contrast, a predicted in-frame deletion of four amino acids (del¹⁸⁵QSVR¹⁸⁸) from the fourth Greek-key motif, leaving the C-terminal arm intact, rendered β B2 in the *Philly* mouse lens thermally unstable and prone to abnormal aggregation (Chambers and Russell 1991). These observations suggest that partial loss of the fourth Greek-key motif, rather than truncation of the C-terminal arm, is primarily responsible for G220X- β B1 insolubility in bacteria. Significantly, the *Philly* deletion was proposed to impair critical hydrogen bonding between the third and fourth Greek-key motifs of β B2, ultimately destabilizing the entire C-terminal domain. Similar instability of the G220X- β B1 mutant in the human lens is likely to provide a potent trigger for cataract formation.

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Electronic-Database Information

Accession numbers and URLs for data presented herein are as follows:

BLAST, <http://www.ncbi.nlm.nih.gov/BLAST/>
 Entrez-Protein, <http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=Protein> (for CRYBB1 [accession number NP_001878])
 GenBank, <http://www.ncbi.nlm.nih.gov/Genbank/> (for *Homo sapiens* CRYBB1 mRNA [accession number NM_001887], *H. sapiens* CRYBA4 mRNA [accession number NM_001886], BAC clone containing CRYBB1 and CRYBA4 [accession number Z95115], and CRYB2-CA [accession number X62390])
 Online Mendelian Inheritance in Man (OMIM), <http://www.ncbi.nlm.nih.gov/Omim/> (for CRYAA [MIM *123580], CRYAB [MIM *123590], CRYBB1 [MIM *600929], CRYBB2 [MIM *123620], CRYBB3 [MIM *123630], CRYBA3/CRYBA1 [MIM *123610], CRYBA4 [MIM *123631], CRYGC [MIM *123680], and CRYGD [MIM *123690])
 Wellcome Trust Sanger Institute, The, <http://www.sanger.ac.uk/> (for human chromosome 22)

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