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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 $\theta = 0$), which closely flank the genes for βB1-crystallin (*CRYBB1*) and βA4-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 βB1-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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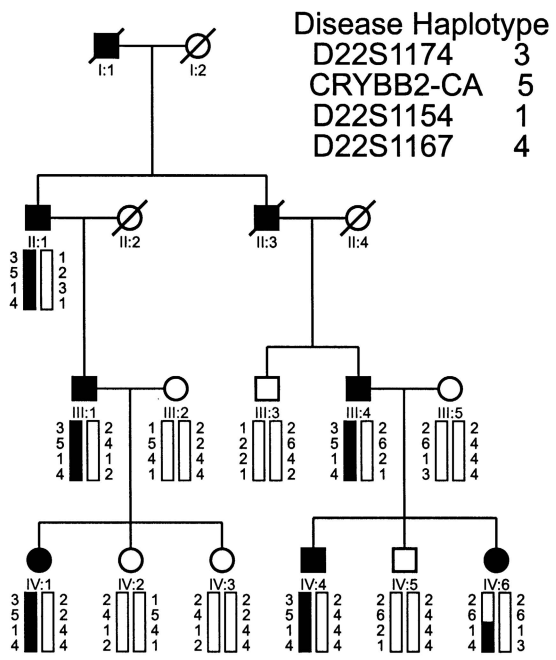

Figure 1 Pedigree and haplotype analysis of family ADC4, showing segregation of four microsatellite markers (in descending order from the centromere) on chromosome 22. Squares and circles symbolize male and female individuals, respectively; blackened symbols denote affected status.

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 (βB1)–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 $\theta = 0$); however, we detected positive Z values for markers D22S1167 ($Z = 2.09$ at $\theta = 0$) and D22S1154 ($Z = 1.39$ at $\theta = 0$), which flank *CRYBB1* and *CRYBA4* (MIM *123631) (fig. 2). Then, haplotype analysis (fig. 1)

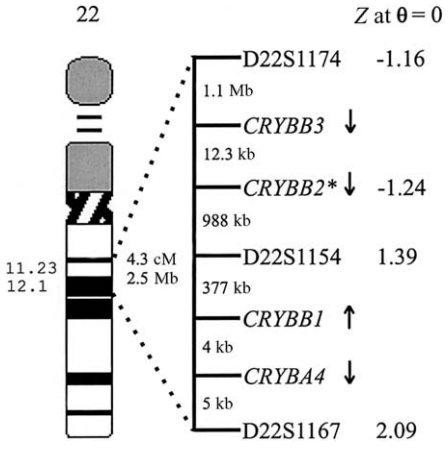

Figure 2 Idiogram of chromosome 22, showing the integrated genetic and physical order of microsatellite markers across the CRYB gene cluster. Physical distances between markers and genes are shown. Two-point Z values for linkage between the cataract and markers are indicated. An asterisk denotes the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

detected one affected female (IV:6) who was an obligate recombinant at markers CRYB2-CA and D22S1174, confirming that the disease gene was neither *CRYBB2* nor *CRYBB3;* however, no individuals recombinant for the cataract locus and for marker D22S1154 or marker D22S1167 were observed, further suggesting that either *CRYBB1* or *CRYBA4* was the disease gene. Finally, we excluded linkage of the cataract to 2q with markers D2S128 ($Z = -6.90$ at $\theta = 0$) and D2S157 ($Z = 0.41$ at $\theta = 0$), which lie close to the CRYG gene cluster.

Alignment of the cDNA sequences for *CRYBA4* (GenBank accession number NM_001886) (Lampi et al. 1997) and *CRYBB1* (GenBank accession number NM_001887) (David et al. 1996) with the fully annotated chromosome 22 sequence (GenBank accession number Z95115) (Dunham et al. 1999) confirmed that each gene comprised six exons and five introns, with the first exon being a noncoding exon. Sequence analysis of all six exons for *CRYBA4,* amplified using intron-specific primers (available on request), in two affected individuals detected no significant changes from wild type (data not shown); however, similar sequence analysis of *CRYBB1* in the same affected individuals identified a G→T transversion in exon 6 that was not present in wild type (fig. 3). This single-nucleotide change introduced a novel *Hph*I site—GGTGA(N)$_{8/9}$↓—and was predicted to result in a nonsense or chain-termination mutation, at codon 220, that changed a phylogenetically conserved glycine to a stop codon (G220X). Attempts to clearly resolve the complex heterozygous restriction-fragment pattern for the novel *Hph*I site on agarose gels proved unsatisfactory; therefore, we designed a T allele–specific PCR primer to identify individuals heterozygous for the G→T transversion. Primer extension analysis (fig. 3*C*) showed that the T allele cosegregated with affected individuals but not with unaffected family members. In addition, this single-nucleotide change was not detected in a panel of 102 normal unrelated individuals (data not shown), suggesting that it was the causative mutation, rather than a benign polymorphism in strong linkage disequilibrium with the disease.

The G220X mutation was predicted to truncate wild-type βB1-crystallin by 33 amino acids (fig. 3*D*). To assess the functional effects of this premature chain termination, we compared the inducible expression of wild-type and G220X forms of recombinant human βB1 in *Escherichia coli.* SDS-PAGE analysis detected a unique polypeptide (band a) of molecular size ~28 kDa in the induced soluble fraction from *E. coli* transformed with wild-type βB1 (fig. 4*A,* lane 2). Band a was also present in the induced pellet fraction from these bacteria but was difficult to distinguish from a closely migrating *E. coli* protein (fig. 4*A,* lane 4). Immunoblot analysis, using a polyclonal antibody to rat β-crystallin (David et al. 1987), confirmed that band a was present in the induced soluble and insoluble fractions from bacteria transformed with wild-type βB1 (fig. 4*B,* lanes 2 and 4); however, since approximately eight times as much of the insoluble sample was analyzed as compared with the soluble sample, it was estimated that al-

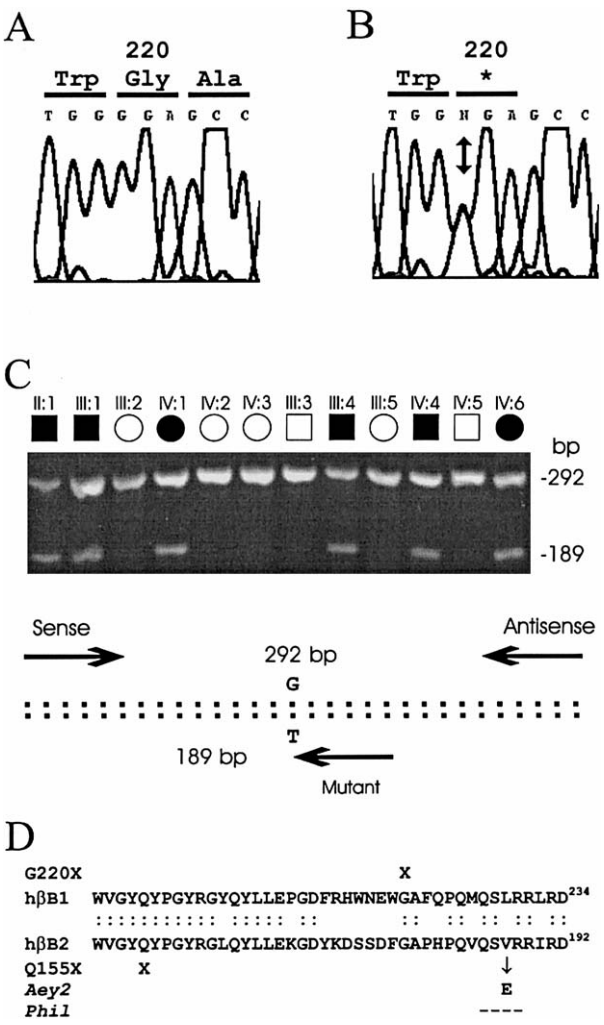

Figure 3 Mutation analysis of *CRYBB1. A,* Sequence chromatograms of wild-type allele, showing translation of glycine (GGA) at codon 220. *B,* Sequence chromatograms of mutant allele, showing a G→T transversion that changed glycine 220 to a stop codon (TGA). *C,* Mutant allele–specific primer extension analysis. Exon 6 was amplified in the presence of three PCR primers (as indicated by arrows): a sense anchor primer located in intron 5, an antisense primer located in the 3′ UTR, and a nested antisense primer specific for the G→T mutation in codon 220. Unaffected individuals have only the wild-type G allele (292 bp), whereas, affected individuals also have the mutant T allele (189 bp). *D,* Amino acid alignment of the fourth Greek-key motif of βB1-crystallin (codons 193–234) with that of βB2-crystallin (codons 151–192). Colons indicate identical amino acids. "X" indicates premature-chain-termination mutations in human βB1 (G220X) and βB2 (Q155X). The locations of a missense substitution (V187E) and an in-frame deletion (*hyphens*) in βB2 from the *Aey2* (Graw et al. 2001) and *Philly* (Chambers and Russell 1991) mouse mutants, respectively, are also indicated.

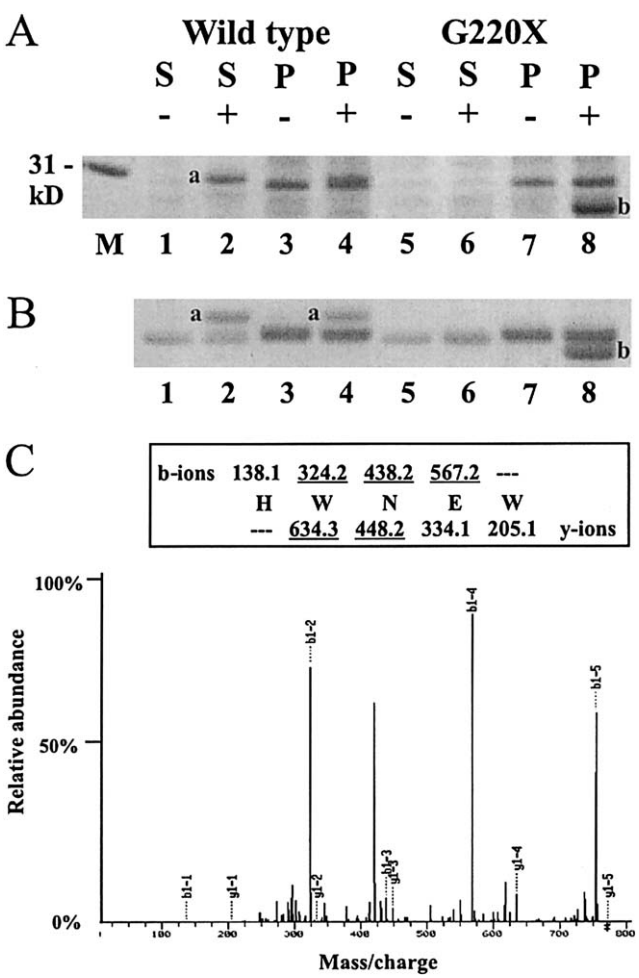

Figure 4 Expression of recombinant human βB1-crystallin in *E. coli*. *A* and *B*, SDS-PAGE (*A*) and immunoblot (*B*) analyses of wild-type βB1 and G220X-βB1 in the soluble (S) and insoluble pellet (P) fractions, before (−) and after (+) induction with IPTG. Lane M shows molecular mass-markers; lanes 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-βB1 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 βB1 was soluble. In contrast, there was no detectable induction of band a in the soluble fraction of *E. coli* transformed with G220X (figs. 4*A* and 4*B*, 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. 4*A* and 4*B*, lane 8). The β-crystallin antibody also cross-reacted with a closely migrating *E. coli* protein in all fractions analyzed (fig. 2*B*); therefore, we used mass-spectrometry techniques to unequivocally identify bands a and b as human βB1. Tryptic peptide masses derived from band a (fig. 4*A*, 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 βB1 (Lampi et al. 2001). Tryptic peptide masses derived from band b by ESI-MS were consistent with a truncated βB1 protein of molecular mass 24,046 Da (data not shown). Fragmentation of the peptides by tandem mass spectrometry (MS/MS) identified ⩾75% of the amino acid sequence of G220X-βB1 (Entrez-Protein accession number NP_001878) from excised band b (data not shown). No peptides corresponding to the C-terminal sequence of wild-type βB1 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. 3*D*) was detected (fig. 4*C*).

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 βB2-crystallin (Norledge et al. 1997), the G220X nonsense mutation in *CRYBB1* was predicted to result in a truncated βB1 protein that lacked ~33% of the fourth Greek-key motif (codons 220–234; fig. 3*D*) 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 βB2 protein, effectively deleting ~90% of fourth Greek-key motif (codons 155–192; fig. 3*D*) 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 βB1- and βB2-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-βB1 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 βB2 (Trinkl et al. 1994), chicken βB1 (Coop et al. 1998), and human βB1 (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 (del^{185}QSVR^{188}) from the fourth Greek-key motif, leaving the C-terminal arm intact, rendered βB2 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-βB1 insolubility in bacteria. Significantly, the *Philly* deletion was proposed to impair critical hydrogen bonding between the third and fourth Greek-key motifs of βB2, ultimately destabilizing the entire C-terminal domain. Similar instability of the G220X-βB1 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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