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A unique form of autosomal dominant cataract explained by gene conversion between β -crystallin B2 and its pseudogene

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EDITOR—Using linkage analysis, a large Indian family with autosomal dominant sutural cataract and cerulean opacities was mapped to chromosome 22 and two cosegregating sequence changes (475C→T and 483C→T) were identified in the *CRYBB2* gene. The first was previously described in two genetically unrelated families with other inherited forms of cataract. The two sequence alterations are identical to the sequence of the *CRYBP1* pseudogene that is 228 kb apart. Furthermore, the pseudogene-like fragment within the *CRYBB2* gene is flanked by chromosomal junction sequences. Therefore, we conclude that gene conversion is the most likely mechanism leading to this mutation. Alternatively, dual point mutation would explain our findings. In addition, since the three families with Q155X mutations all show different types of cataract, we conclude that mutant *CRYBB2* causes cataract formation but other modifying factors determine the type of cataract.

Autosomal dominant congenital cataract (ADCC) is a clinically and genetically heterogeneous group of disorders that cause blindness. More than 13 independent loci have been mapped, and 10 different genes identified so far. Five of them are crystallin genes that are categorised into the α , β , γ , μ , and ζ subgroups. The crystallins constitute the main lens proteins, whereby β -crystallin B2 is the only abundant protein in the adult lens fibre in man.^{1, 2} Causative mutations have been recognised in the α -crystallin A gene (zonular central nuclear cataract),³ the β -crystallin A3/A1 gene (zonular cataracts with sutural opacities),⁴ the γ -crystallin C gene (Coppock-like cataract),⁵ and the γ -crystallin D gene (progressive juvenile onset punctate cataract).⁶ These and all other ADCC mutations identified so far are private mutations, with one exception. Litt *et al*⁷ described a nonsense mutation, Q155X, in the β -crystallin B2 gene leading to cerulean cataract. Exactly the same mutation was identified by Gill *et al*⁸ in familial Coppock-like cataract. Here, we report the identical mutation in a large Indian family exhibiting sutural cataract with punctate and cerulean opacities. In addition, we present evidence that this mutation in the β -crystallin B2 gene is an inde-

pendent event and most likely the result of gene conversion.

Identification and characterisation of the mutation

After obtaining informed consent, we performed linkage analysis in an Indian five generation family with 33 affected members, based on semi-automated genotyping with microsatellite markers from the Génethon linkage map; 48 members of this family, 25 of them affected, were selected for mapping (fig 1A). Assuming autosomal dominant inheritance with full penetrance and equal allele frequencies for each marker and using the LINKAGE program package, we calculated two point lod scores. After having excluded the autosomal dominant cataract loci on chromosomes 1, 2, 12, 13, 14, 16, 17, and 19, we detected linkage in our family to marker D22S315, with a lod score of $Z_{\max} = +8.500$ at $\theta_{\max} = 0.05$.

This region on chromosome 22 harbours four β -crystallin genes, *CRYBA4*, *CRYBB1*, *CRYBB2*, *CRYBB3*, and the pseudogene *CRYBP1*.⁹ We amplified the translated exons 2-6 of the *CRYBB2* gene by PCR as described previously.⁷ In addition, we designed primer sets for maximum discrimination between gene and pseudogene sequences: CRYBLg (5'-TGACCTTG TAGCTGGGCTTG-3'), CRYBLp (5'-TGACTTTGCAGCCAGGCTTG-3'), 596rg (5'-CACTGCATGTCGCGGATACG-3'), 596rps (5'-CCCTGCATGTCGTGGATGCA-3'). PCR products were purified with a Qiaquick PCR purification kit (Qiagen, Hilden, Germany) and sequenced directly using the Big-Dye-Terminator Cycle Sequencing Kit (PE Biosystems, Weiterstadt, Germany). Sequencing reactions were purified with a Dye-Ex Kit (Qiagen, Hilden, Germany) and run and analysed on ABI 310 and 377 sequencers (PE Biosystems, Weiterstadt, Germany).

Sequencing of exon 6 showed a C→T mutation at nucleotide position 475 (Q155X). This stop mutation truncates the protein by 51 residues and has previously been described.^{7, 8} However, our sequencing of exon 6 showed an additional variant, a C→T substitution at nucleotide position 483. This silent polymorphism was found exclusively in patients. Since

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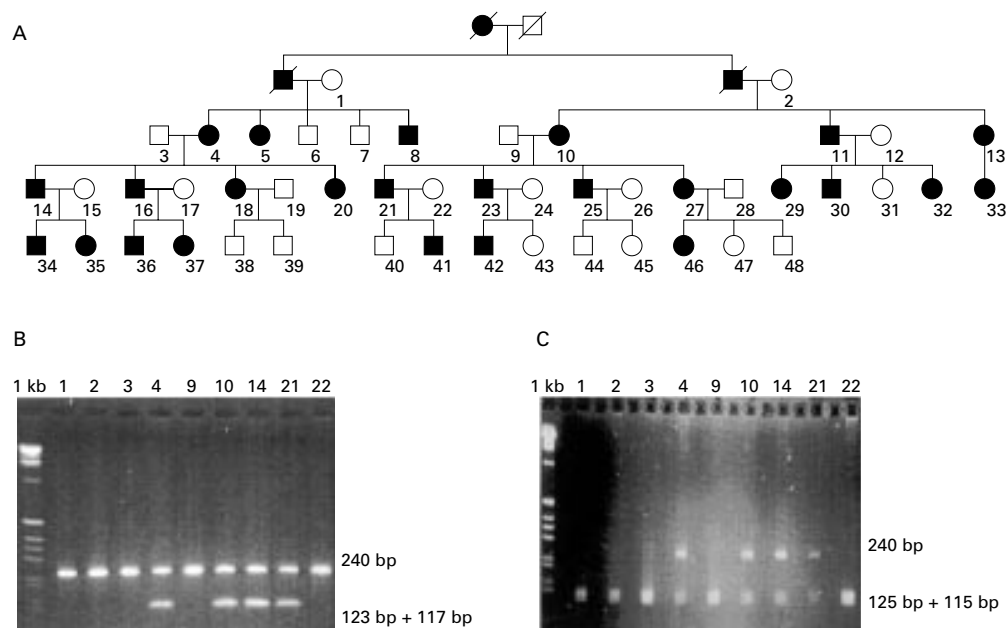


Figure 1 Pedigree of the family (A) and results of the restriction digestions of representative members. (B) *CRYBB2* mutation Q155X creates a *BfaI* site. Restriction digestion with *BfaI* shows two unresolved digestion products of 117 bp and 123 bp in addition to the undigested 240 bp fragment in affected subjects. Unaffected persons show only an undigested 240 bp PCR product. Numbers above lanes correspond to individual numbers in the pedigree. 1 kb ladder is used as size standard. (C) *CRYBB2* variant 483C→T destroys a *MspI* site. Restriction digestion with *MspI* shows the undigested 240 bp fragment in addition to two unresolved digestion products of 125 bp and 115 bp in affected subjects. Unaffected people show only two unresolved digestion products of 125 bp and 115 bp. Numbers above lanes correspond to subject numbers in the pedigree. 1 kb ladder is used as size standard.

the mutation Q155X creates a *BfaI* restriction enzyme site and the variant 483C→T destroys an *MspI* site, PCR products were *BfaI* and *MspI* (New England Biolabs, Frankfurt, Germany) digested and separated on agarose gels. The results showed complete cosegregation of mutation, variant, and disease in our family (fig 1B, C). Q155X and 483C→T were not found on 180 chromosomes of normal Indian subjects, excluding either from being a frequent polymorphism.

To address the question whether the mutations in our Indian family and the American family of Litt *et al*⁷ are derived from a single mutational event or represent recurrent mutations, we established the haplotypes at the *CRYBB2* locus, based on closely linked microsatellite markers. Their order and genetic distance is *TOP1P2* - 3 cM - *CRYBB2* - 1 cM - *D22S258*.¹⁰ The haplotypes were 133-173-180 in the Indian and 165-171-180 in the American family, indicating that the mutation Q155X arose independently on chromosomes with different haplotypes. This is confirmed by the "normal" cytosine at position 483 in the patients of Litt *et al*⁷ and Gill *et al*,⁸ as can be seen from the published sequences.

Gene conversion

Altogether these data point to a hot spot of mutation in exon 6 of the *CRYBB2* gene. Since the two sequence alterations in the Indian family, Q155X and 483C→T, are both cytosine to thymidine mutations, cytosine deamination as the mutational mechanism cannot be ruled out a priori. However, if one takes into account that the region harbours four closely related

β -crystallin genes and a pseudogene (fig 2A), two other mutational mechanisms need to be considered, unequal crossing over and gene conversion.

By alignment of the DNA sequences of the β -crystallin genes, it became clear that *CRYBB2* is much more similar to the closely linked pseudogene *CRYBP1* than to the other β -crystallin genes (fig 2B). This finding suggests a recent duplication of a common precursor gene where one of the duplicated genes was maintained (*CRYBB2*) and the other one accumulated point mutations, turning it into a pseudogene (*CRYBP1*). The physical distance of 228 kb between *CRYBB2* exon 6 and the homologous sequence in the pseudogene *CRYBP1* (fig 2A) is compatible with both unequal crossing over and gene conversion. To our surprise, the alignment of the crystallin gene sequences showed that the alterations Q155X and 483C→T in the *CRYBB2* mutant are identical to the "normal" sequence in the pseudogene (fig 2B). Thus, the two alterations, Q155X and 483C→T, define a fragment of at least 9 bp of pseudogene-like sequence in the *CRYBB2* gene. These 9 bp are flanked upstream by 28 bp and downstream by 67 bp where the gene and pseudogene are identical (fig 2B). At the nucleotides 29 bp upstream and 68 bp downstream that differ between gene and pseudogene, the patients showed homozygosity for the *CRYBB2* sequence. Therefore, the mutant allele carries more than 9 bp but less than 104 bp of pseudogene-like sequence (fig 2B).

In principle, recombinational events resulting from unequal crossing over or gene conver-

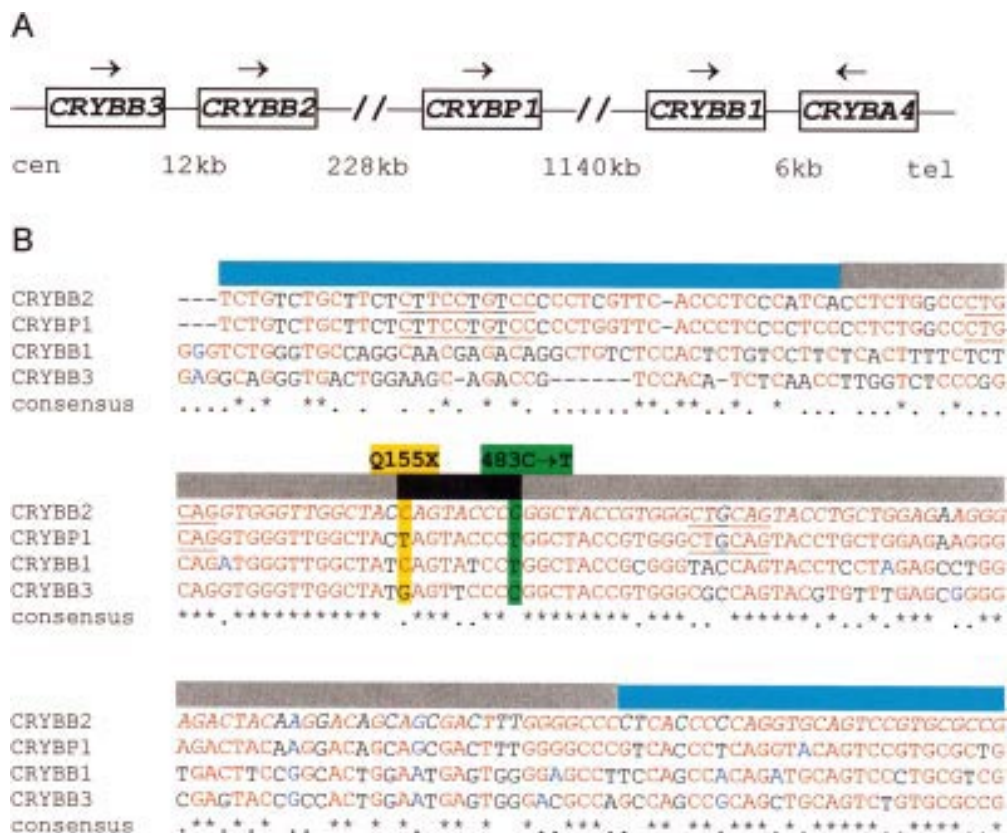


Figure 2 The crystallin genes on chromosome 22. (A) Position and orientation of crystallin genes and physical distances between them.⁹ (B) Alignment of CRYBB2 exon 6 (italicised) with the homologous sequences of CRYBP1, CRYBB1, and CRYBB3. The closest similarity is observed between CRYBB2 and CRYBP1. The sequence of the CRYBB2 gene with the Q155X mutation (highlighted yellow) and the 483C→T variant (highlighted green) is identical to the CRYBP1 pseudogene sequence. Sequences that promote recombination events are underlined. We identified one hypervariable minisatellite GGCAGGA(A/G)G with 1 bp mismatch and two chromosomal junction sequences ATGCAG with one mismatch each. Black and dark grey bars indicate sequence of probable and possible gene conversion, turquoise shows where gene conversion is excluded.

sion could explain this situation because the distance of 228 kb between gene and pseudogene (fig 2A) is compatible with both mechanisms. Unequal crossing over should lead to relatively large duplications or deletions. However, Southern blotting of *EcoRI* digested genomic DNA with an exon 6 probe did not show any differences between affected and unaffected subjects (data not shown).

Thus, the most likely explanation is that gene conversion between CRYBB2 exon 6 and its homologous CRYBP1 sequence led to the pseudogene-like alteration in the CRYBB2 gene. This assumption is in agreement with the shortness (≤ 104 bp) of the replaced fragment. It was estimated that gene conversions between human γ -globin genes are less than 300 bp in length¹¹ and between human β -globin genes less than 451 bp.¹² Our assumption of gene conversion is also supported by the presence of sequences that were described to promote gene conversion. Gene conversion is considered to be initiated at or near special sites.¹³ The critical region in our patients is flanked by the chromosomal junction sequence ATGCAG¹⁴ with one mismatch and also bears a hypervariable minisatellite GGCAGGA(A/G)G¹⁵ with one mismatch (fig 2B). Putative gene conversion between highly homologous genes, as in this family, was previously described at several

other loci, for example, the β -globin genes,¹⁶ the oxytocin vasopressin genes,¹⁷ or the steroid 21-hydroxylase gene and its pseudogene.¹⁸ Summarising all our data, we conclude that gene conversion between CRYBB2 and its pseudogene CRYBP1 is the most likely explanation for this mutation in exon 6 of the CRYBB2 gene.

Phenotypic variation and modifiers

There are now three families described that carry the CRYBB2 Q155X mutation. However, each of these shows a different phenotype. The clinical diagnosis in our Indian family is sutural cataract with punctate and cerulean opacities. The slit lamp examination (fig 3) showed prominent, dense, white opacification around the anterior and posterior Y sutures. The posterior Y sutures and the posterior pole of the lens were more severely affected than the anterior pole. It also showed greyish and bluish, sharply defined, elongated, spindle shaped, and oval punctate and cerulean opacities of various sizes arranged in lamellar form. The spots were bigger and more concentrated towards the peripheral layers. These did not delineate the embryonal or fetal nucleus. No pulverulent disc-like opacity was observed in the nuclear region. The sutural opacities appeared denser and whiter compared to the punctate and cerulean spots and were also more elongated and

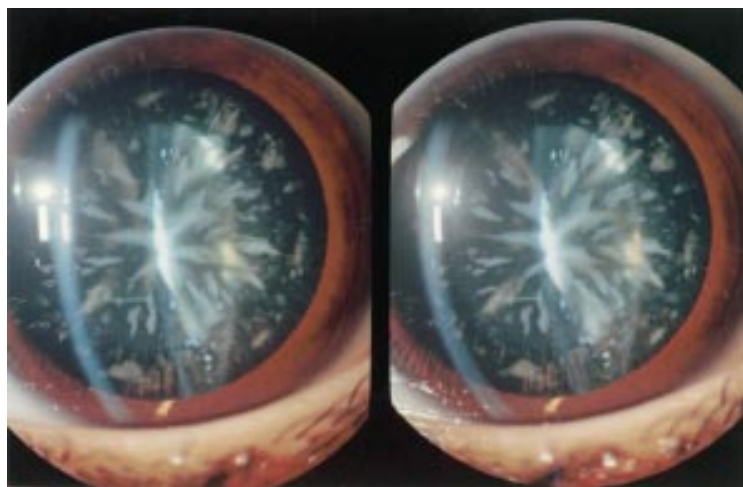


Figure 3 3-D photograph of the eyes of a 9 year old patient through slit lamp microscope. In the centre of the lens there is a dense, white, sharply defined sutural opacity with two of its branches showing fish tail-like division towards the periphery. A large number of sharply defined, elongated, spindle shaped, and oval punctate opacities are directed radially. Similar presence and distribution of opacities are seen posteriorly which are much larger and denser than the anterior ones.

larger in size. Phenotypic variation with respect to the size and density of the sutural opacities as well as the number and position of punctate and cerulean spots was observed among the affected members. Some subjects showed severely affected sutures with dense white opacifications spreading along the secondary divisions of the Y sutures. In some affected subjects the spots were present only as a single layer in the cortex while in the others the spots occurred in concentric layers involving the whole cortex.

The phenotype of this Indian family, sutural cataract with punctate and cerulean opacities, differs from all other reported forms of cataract. The American family of Litt *et al*⁷ has pure cerulean cataract, and the Swiss family of Gill *et al*⁸ shows Coppock-like cataract. The phenotype of our family overlaps with the American family, both showing cerulean opacities. However, the sutural cataract and the punctate opacities in our phenotype have not been reported in the American or Swiss families. Moreover, the prominent pulverulent central disc-like opacity involving the embryonal and fetal nucleus seen in the Swiss family is not present in the American or this Indian family. There is not even an overlap between the phenotype of the Swiss family and the other two families.

Hence, we conclude that the Q155X mutation causes cataract formation but the distinct type of cataract depends on modifying genetic and epistatic factors. The influence of modifiers would make it impossible to infer the mutant gene from the cataract phenotype. Consequently, ADCC patients would need to be analysed for much more than only one ADCC gene.

Cis acting major modifiers could explain the considerable phenotypic variability between families. Minor modifying factors, acting in *trans*, could cause the phenotypic differences within families. The minor modifier of our family is obviously not linked to the Q155X mutation, since there is considerable clinical variability within our patients who share an

identical haplotype. Interestingly, this excludes three further crystallin genes, *CRYBA4*, *CRYBB1*, and *CRYBB3* (fig 2A), from being the minor modifier. On the other hand, exactly these crystallin genes are candidates for being the major modifier.

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Novel mutations of *TMPRSS3* in four DFNB8/B10 families segregating congenital autosomal recessive deafness

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EDITOR—Congenital deafness occurs in approximately 1 in 1000 live births and 50% of these cases are hereditary. Non-syndromic deafness is classified according to its mode of inheritance as DFN, DFNA, and DFNB (X linked, autosomal dominant, and autosomal recessive, respectively). Non-syndromic recessive deafness accounts for ~80% of congenital hereditary deafness cases.¹ At least 30 DFNB loci have been mapped in the past few years by genetic linkage studies, but the causative gene has been identified for only eight of these loci²⁻⁴ (Hereditary Hearing Loss Homepage, <http://www.uia.ac.be/dnalab/hhh>).

Two of the previously reported loci for non-syndromic recessive deafness are DFNB8 and DFNB10, both located on chromosome 21q22.3 (MIM 601072 and 605316). The DFNB8 locus was originally identified in a large consanguineous Pakistani family, segregating childhood onset deafness,⁵ while DFNB10 was identified in a large consanguineous Palestinian family, in which deafness was congenital.⁶ Recently, the *TMPRSS3* gene was shown to be mutated in affected subjects of both families.⁷

TMPRSS3 belongs to a family of transmembrane serine proteases, also including *TMPRSS1*,⁸ *TMPRSS2*,⁹ and *TMPRSS4*.¹⁰ The *TMPRSS3* gene extends over 24 kb and comprises 13 exons. It has four alternative transcripts (*TMPRSS3 a, b, c, and d*), encoding putative peptides of 454, 327, 327, and 344 amino acids, respectively.⁷ *TMPRSS3a*, which contains all 13 exons, is the most abundant transcript and its expression could be detected in various tissues, including fetal cochlea.⁷ In addition to the serine protease and the transmembrane domains, *TMPRSS3* also encodes low density lipoprotein receptor class A (LDLRA) and scavenger receptor cysteine rich (SRCR) domains, which are potentially in-

involved in binding with extracellular molecules and/or the cell surface.⁷

Material and methods

To identify DFNB8/B10 linked families, we analysed a total of 159 consanguineous Pakistani families that segregate profound congenital deafness and are either large enough to support statistically significant linkage or have at least three affected subjects. Families were ascertained in schools for the deaf in Punjab and Karachi. IRB approval (OH93-DC-016) and informed consent were obtained for all participating family members. Genomic DNA was extracted from venous blood samples according to a standard protocol.¹¹ Linkage to known recessive deafness loci (DFNBs) and refinement of the DFNB8/B10 region in linked families was performed on an ABI-377 sequencer (PE Applied Biosystems) using marker information provided by the Hereditary Hearing Loss Homepage and by Berry *et al.*¹² Linkage analysis was conducted with the FASTLINK version of the LINKAGE program package.^{13,14} Five of the families showed potential linkage to the DFNB8/B10 locus on chromosome 21q22.3 (fig 1). Medical history and pure tone audiometry testing indicated that all five families segregate congenital, profound, non-syndromic sensorineural deafness.

Results and discussion

To detect mutations in the *TMPRSS3* gene in DFNB8/B10 families, we determined the gene sequence in two affected subjects from each family by PCR amplification from genomic DNA of the 13 exons, including intron-exon boundaries, and cycle sequencing of the PCR products using *TMPRSS3* specific primers. Primer sequences and PCR conditions are summarised in table 1. PCR products were