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# Autosomal dominant zonular cataract with sutural opacities is associated with a splice mutation in the βA3/A1-crystallin gene

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**Purpose:** Congenital cataracts constitute a morphologically and genetically heterogeneous group of diseases that are a major cause of childhood blindness. Autosomal Dominant Zonular Cataracts with Sutural Opacities (CCZS) have been mapped to chromosome 17q11-q12 near the βA3A1-crystallin gene (CRYBA1). The βA3A1-crystallin gene was investigated as the causative gene for the cataracts.
**Methods:** The βA3/A1-crystallin gene was sequenced in affected and control individuals. Base changes were confirmed and assayed in additional family members and controls using NlaIII restriction digestion of PCR amplified DNA sequences. Base changes were assessed for their effects on splicing by information analysis.
**Results:** The cataracts are associated with a sequence change in the 5' (donor) splice site of intron 3: GC(g->a)tgagt. The sequence change also creates a new NlaIII site. This base change cosegregates with the cataracts in this family, being present in every affected individual. Conversely, this base change was not seen in 140 chromosomes examined in 70 unaffected and unrelated individuals. Information theory mutational analysis shows that the base change lowers the information content of the splice site from 6.0 to -6.8 bits, so that splicing would not be expected to occur at the altered site.
**Conclusions:** Taken together, these observations suggest that the observed mutation might be causally related to the cataracts in this family.

Congenital cataracts are a significant cause of visual disease, and without prompt treatment they can interfere with the sharp imaging on the retina necessary to develop normal visual cortical synaptic connections, resulting in irreversible visual loss. They form a heterogeneous group of diseases, with one-third being familial, most commonly inherited in an autosomal dominant fashion [1]. Dominantly inherited congenital cataracts are themselves genetically heterogeneous, with cataracts being mapped to 10 different loci including 1p36 [2], 1q21-q25 [3], 2q33-q35 [4], 13 [5],16q22 [6], 17p13 [7], 17q11-q12 [8], 17q24 [9], 21q22.3 [10],and 22q [11].

Autosomal dominant congenital cataracts are also phenotypically heterogeneous, including cerulean (17q24, 22q), anterior polar (17p13), and nuclear zonular or lamellar (1p36, 1q21-q25, 2q33-q35, 16q22, 17q11-q12, 22q) morphologies. Clinically identical cataracts can map to different loci [12], and cataracts segregating in the same family can show marked variability [13]. While the spatial distribution of opacity within the lens is generally expected to reflect expression patterns of the causative protein during development, precise explanations of the phenotypic appearance of specific hereditary cataracts has remained elusive. Sutural cataracts show selective opacification of the Y-sutures of the lens. They are infrequently reported, because they usually do not interfere with visual acuity and thus do not prompt patients to seek medical attention. Sutural cataracts can be inherited, most often in an autosomal dominant fashion, and Marner cataracts (CAM, 16q22) as well as Volkmann cataracts (CCV, 1p36) have sutural components, although they are primarily nuclear zonular in morphology.

High concentrations of closely packed crystallins are required for transparency and focusing of light by the lens. The major classes of ubiquitous crystallins are α-crystallin, molecular chaperones related to the small heat shock proteins, and the β- and γ-crystallins. The β- and γ-crystallins share an extremely stable common core structure comprising four twisted β-pleated sheets termed “Greek key motifs,” which are organized into two domains [14]. The β-crystallins also have amino and carboxy terminal extensions or “arms.” The β-crystallins associate into higher order assemblies while the γ-crystallins exist in solution as monomers. Both the β- and γ-crystallins tend to be more highly expressed at early developmental times in elongating fiber cells, so that they are found primarily in the lens nucleus [15-17].

Basti and coworkers [18] described a family with autosomal dominant cataracts having both a zonular component in the fetal nucleus and prominent opacities of both the anterior and posterior Y-sutures in the area enclosed by the zonular component. It differs from previously described families in that affected individuals uniformly showed significant sutural opacities. Linkage analysis localized the gene causing these cataracts to a region of chromosome 17q11-q12 including the βA3/A1-crystallin gene with a lod score of 3.91 [8]. Here we describe association of a splice site mutation at the end of the third exon of βA3/A1-crystallin with the cataracts in this family.

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## METHODS

*Pedigrees and Diagnosis:* Patients were ascertained and examined by (S. B.) at the L. V. Prasad Eye Institute in

Hyderabad. Patient studies and informed consent were approved by the L. V. Prasad Institutional Review Board, the National Eye Institute Review Board, and the National Institutes of Health Office of Protection from Research Risks. Clinical and Ophthalmologic examinations included detailed ophthalmic, medical, and family histories, dilated slit-lamp examination with photographs, Snellen visual acuity testing, intraocular-pressure measurement by applanation tonometry, and fundus examination. All affected family members showed a zonular cataract measuring 3.5-4 mm in diameter, an erect Y-shaped anterior sutural cataract, and an inverted Y-shaped posterior sutural cataract. Clinical findings in these patients are described in detail in [18] and [8].

*PCR Amplification of the βA3/A1-Crystallin Gene:* Human genomic DNA from 70 unaffected controls (50 of Indian descent and 20 from other populations) and the affected and unaffected family members shown in Figure 1 was amplified using polymerase chain reaction (PCR) primers from flanking sequences of exons 1-6 of the human βA3/A1-crystallin gene (HUMCRYBA1-6, GenBank accession numbers M14301, M14302, M14303, M14304, M14305, and M14306). The primers used are shown in Table 1. PCR was carried out for 35 cycles each consisting of a 45 seconds at 94 °C, 30 seconds at the annealing temperature shown in Table 1, and 30 seconds at 72 °C. The first and last primers shown were used together in a PCR to amplify the entire intervening βA3/A1-crystallin gene sequence. This fragment was then sequenced with the exon 6 reverse primer to examine the 5'-most 24 bases of exon 6, since these were not included in the amplified fragment for exon 6 using the specific forward and reverse primers.

*DNA sequencing:* PCR products were checked for correct size by agarose gel electrophoresis and purified by Wizard PCR Prep DNA Purification System (Promega, Madison, WI) followed by ethanol precipitation. They were sequenced bidirectionally using an ABI 377 Prism automated sequencer (ABI, Foster City, CA) using the original primers the an Amplitaq FS cycle sequencing kit (ABI) with dye-labeled terminators.

*Restriction endonuclease analysis:* PCR products were purified by phenol/chloroform extraction and ethanol precipitation, digested with NlaIII at 37 °C in NEB buffer 3 (New England Biolabs, Beverly, MA) for 2 h, and analyzed by ethidium bromide staining after electrophoresis on a 6% acrylamide gel in Tris-Borate-EDTA, pH 8.2.

*Information Theory Mutational Analysis:* The potential results of the G to A transition were estimated using information theory as described by [19], and [20]. The 10 bases constituting the donor and 27 bases constituting the acceptor splice sites were weighted according to sequence conservation and base frequency. These values are summed, providing a quantitative estimate of the ability of the sequence to serve as a splice site, reported in bits. This algorithm was implemented using the computer programs SCAN and RI, and the information contribution at each position of the site was depicted using the program WALKER [21,22].

Table 1. PCR primers for amplification of βA3/A1-crystallin exons.

| Primer | Sequence | Annealing temperature | Fragment size |
|---|---|---|---|
| exon 1 fwd | 5'-GGTCTTAGGAAGATCCCAAG-3' | 58 °C | 394 bp |
| exon 1 rev | 5'-AAGGAGAGGAAGGGCAAGGG-3' | | |
| exon 2 fwd | 5'-CGTGTGTGCTCTGTCTTCC-3' | 58 °C | 179 bp |
| exon 2 rev | 5'-GGTCAGTCACTGCCTTATGG-3' | | |
| exon 3 fwd | 5'-CAATCCTCCCTCCACCTC-3' | 54 °C | 520 bp |
| exon 3 rev | 5'-TCCTTCCTTCAGCTTTGG-3' | | |
| exon 4 fwd | 5'-CAAACACTACATGTCTTTGG-3' | 48 °C | 210 bp |
| exon 4 rev | 5'-CTTGCTACCCTCATATGC-3' | | |
| exon 5 fwd | 5'-TGCTTCCTTGTATAATCC-3' | 52 °C | 306 bp |
| exon 5 rev | 5'ACTATTGATGCAACCTCAGG-3' | | |
| exon 6 fwd | 5'-GGTTTGCTACCATTATCTTGG-3' | 52 °C | 183 bp |
| exon 6 rev | 5'-CATGCTTGAGGAATTATCG-3' | | |

Forward and reverse primers flanking exons 1-6 are shown with the primer sequence, the annealing temperature for the amplification reaction, and the expected fragment size of the PCR product. The product fragments were used for sequencing and restriction analysis (exon 3 only).

## RESULTS

The six exons of the βA3/A1-crystallin gene were amplified and their sequences determined, including all coding regions, all splice sites, and a small amount of each adjacent intron. Both the control and patient βA3/A1-crystallin sequences agreed with the published sequence [23], confirming the 6 base changes described by Lampi and coworkers [24], with the single exception shown in Figure 2. This shows the se-

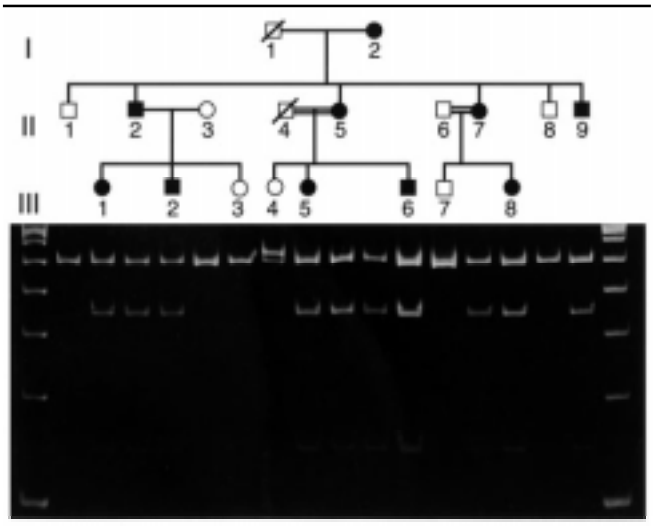


Figure 1. Pedigree and restriction endonuclease analysis for the G to A transition of the family described by Basti [18]. NlaIII digestions of PCR products are shown below each surviving individual in the pedigree. The first and last lanes are 100 bp ladders. The largest cleavage product, 488 bp, results from presence of the wild type sequence, while the cleaved products, 346 and 142 bp, indicate presence of the mutant sequence. In individual III-4 and to a lesser extent in individual II-8 a 520 bp band representing the PCR product partially digested with NlaIII is visible. No DNA sample was received from individual II-6, who has no corresponding lane on the gel. Individual III-7 was analyzed and the results are shown vertically below both II-6 and III-7.

quence at the 3' end of exon 3 of an unaffected control, comparing it with affected individual III-2 in the pedigree shown in Figure 1. The sequence in the unaffected individual is homozygous for a G at the first intronic base following exon 3, while the affected individual is heterozygous for a G to A transition at this base. This is also clearly seen in the reverse sequence, where the affected individual is heterozygous for the corresponding C to T transition. These results are summarized in Figure 3.

The G to A base change created a new NlaIII recognition site (CATG) not found in unaffected individuals. DNA from each member of the family was amplified using the primers flanking exon 3, resulting in a 520 bp fragment. When this fragment is subjected to digestion with NlaIII, it is cleaved to a 488 bp fragment in unaffected individuals (Figure 1). However, both the 488 bp fragment seen in unaffected individuals and 346 and 142 bp restriction fragments resulting from cleavage of the newly created NlaIII site are visible in samples from affected individuals. DNA samples from 50 unaffected Indian and 20 additional controls (140 chromosomes total) were examined and found to lack this NlaIII site (data not shown). These results are summarized in Figure 3.

The predicted effects of the G to A transition are shown in Figure 4. The sequence of the 5' (donor) splice site of control βA3/A1-crystallin exon 3 is shown in the top panel. The WALKER representation of the control splice site at position 474 is shown below this. It has an Ri (information content) score of 6.0 bits, much of it contributed by the G and T at positions +1 and +2, respectively. In addition, there is a weaker 4.5 bit splice site upstream at position 460. The mutant sequence is shown in the lower panel. The G to A base change at position 474 decreases the $R_i$ to -6.8 bits. The upstream potential splice site at position 460 is unaffected. All other potential splice sites in the sequence shown in Figure 3 have Ri values less than 2.4 bits and should not be recognized as splice sites.

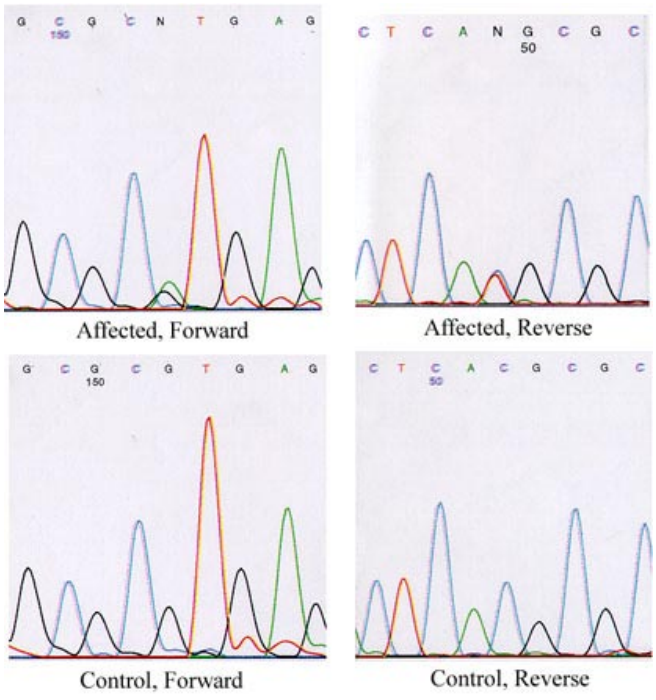


Figure 2. Sequence analysis of the 5' (donor) splice sites exon 3 of βA3/A1-crystallin in control and affected individuals. Forward reactions showing homozygous G at position 152 in an unaffected individual and heterozygosity for the G to A transition in an affected individual, and reverse reactions confirming the same change.

## DISCUSSION

Both direct sequencing of PCR products and restriction endonuclease digestion show that a G to A transition in the 5' (donor) splice site of exon 3 of the βA3/A1-crystallin gene on chromosome 17q is associated with autosomal dominant zonular sutural cataracts (CCZS). The location of the zonular sutural cataracts in the fetal lens nucleus is consistent with expression of βA3/A1-crystallin in the lens [15,25,26]. This base change was not seen in 100 chromosomes examined in 50 unaffected individuals.

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                                                  ----
  1 cctgggactc ccagaatcct ggcctctggg ccatgcttat gaaggaactg tggtggaatg
 61 ggagcccata agtccttggc ctgtccattg gtctagatat tatccctggc agaccatttc
121 tatcataaac caatcctccc tccacctcat ccctactctg ggcaaatgaa caccaaccag
181 tggcattaat tcaaacccct tcctgccctc tttacactgg aagagacctc atcaggcatc
241 ccaggttaca gctggtaaca gaaagcacag agtcagactg aagttaagct gttgacctgn
                                                                I  T
301 acctctgttt ttcctgatgt tctagctctc ttgcgccatt caatctcatt tcagATAACC
     I  Y  D   Q  E  N  F   Q  G  K   R  M  E   F  T  S  S   C  P  N
361 ATCTATGATC AGGAGAACTT TCAGGGCAAG AGGATGGAGT TCACCAGCTC CTGTCCAAAT
                                                              ----
                                                               a
     V  S  E   R  S  F  D   N  V  R   S  L  K   V  E  S  G   A
421 GTCTCTGAGC GCAGTTTTGA TAATGTCCGG TCCCTGAAGG TGGAAAGTGG CGCgtgagta
481 tggacttccg cagaaccgca gccccttatt tcaggtccct tcagacaggg gaccatagag
541 tggggatagg ggaagaagga tgttccccta ggctctagtc atttctcgga gacaggcctc
                ----
601 gaaagaaatc actacatgca taatttagta agccaaagct gaaggaagga acatgaggat
661 tcttagtgac ttgatactag atttcagggg ttggataaag aaatacatac aatggggtgg

721 gacagtggca gtgatcaaga ttttgcccct
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Figure 3(A). βA3/A1-Crystallin exon 3 sequence. βA3/A1-Crystallin exon 3 and surrounding intronic sequence, numbered as in GenBank, accession number M14303. Bases in exon 3 are in capitals. Amino acid sequences are shown in single letter code above the exon sequence. Sequences of the exon 3 forward and reverse primers (Table 1) are in green text. The 5' (donor) splice site for exon 3 at base 474 is in blue text, as is the potential splice site at base 460. The G to A transition disruption the 5' splice site at position 474 is shown in blue above the sequence. The NlaIII sites at 618 and 476 (within the amplified fragment) are marked with hyphens above the sequence.

```
Exon      3' (acceptor) splice site          5' (donor)  splice site
                                                W   K
Exon 2:                                      ...TGG AAG       gtaa...
                    I   T                       S   G   A
Exon 3:   ...tcag    ATA ACC.....................AGT GGC GC    gtga...
                    W   I                       S   A
Exon 4:   ...gcag  C TGG ATT.....................TCA GCT       gtga...
                      N   H                     S   G   A
Exon 5:   ...acag    AAT CAT.....................AGT GGG GC    gtaa...
                    W   V                       Q   *
Exon 6:   ...ccag  C TGG GTT.....................CAG
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Figure 3(B). . Summary of splice sites and adjacent exon and intron sequence for exons 2-6 of the βA3-crystallin gene. Introns are shown in small letters and exons in capitals. Amino acids of the first and last two codons are shown in single letter code above the DNA sequence. Exons are aligned by the first and last complete codon, with additional bases shown extending to the right and left. From this figure it can be seen that splicing from exon 2 to exons 3 or 5 would result in conservation of the reading frame, while splicing between exons 2 and either 4 or 6 would alter the reading frame. The in-frame stop codon following the 5' (donor) splice site for intron 3 is shown in blue as the first full codon following exon 3.

Both animal and human studies indicate the importance of the lens crystallins in establishing and maintaining lens transparency [27]. Cataracts have been shown to result from mutations in mouse βB2-crystallin [28], mouse γE-crystallin, [29], and guinea pig ζ-crystallin [30]. Human cataracts have been associated with pseudoγE-crystallin [31], βB2-crystallin [32], and αA-crystallin [10]. In each of these cases, the mutant crystallin is thought to have altered stability, solubility, or ability to oligomerize, and is predicted to precipitate from solution, resulting in lens opacity.

Besides their roles in transmission and focusing light, some lens crystallins also have biochemical or enzymatic activity. α-Crystallin has been shown to function as a molecular chaperone [33], probably serving to stabilize partially denatured crystallins within the lens cells. Similarly, βB2-crystallin has autokinase [34] and βA3/A1-crystallin has been suggested to have autoproteolytic activity [35]. However, it would seem that with the possible exception of α-crystallin, cataracts associated with mutations in these proteins might be more likely to result from disruption of their roles as structural lens proteins rather than loss of a particular enzymatic function.

The βA3/A1-crystallin gene is thought to encode both βA1- and βA3-crystallin from two in frame AUG codons [36]. These two proteins differ only in the presence of an additional 17 amino acids in the βA3-crystallin amino terminal arm. βA3- and βA1-crystallins are equally stable and both associate into dimers and higher order assemblies [37], as does an intermediate βA3-crystallin with an amino terminal arm shortened by 8 amino acids [38]. Presumably both forms of the amino terminal arm would occur on the mutant protein, although the effect the shorter arm might have on stability or association of the mutant crystallin is unclear.

The precise effect of the G to A transition on the mRNA and protein structure of βA3/A1-crystallin is currently under investigation. Initial attempts to analyze ectopic (illegitimate) transcripts of βA3/A1-crystallin mRNA from leaky transcription in transformed lymphoblasts have been unsuccessful (data not shown). In βA3/A1-crystallin, the first two exons encode sequence of the amino terminal arm, while exons 3-6 encode Greek key motifs 1-4, respectively [23]. The G at position +1 of the 5' (donor) splice site is highly conserved, and mutation of this base would be expected to disrupt the splice site [39]. This is indicated by the fall in $R_i$ from 6.0 bits to -6.8 bits, as shown in Figure 4. In a study of over 100 splice sites, the minimum value of Ri in a functional splice site was 2.4 [20].

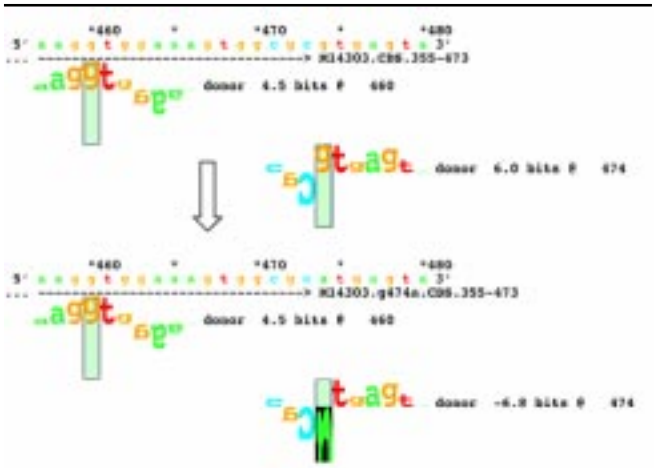

Figure 4. Walker diagram of the exon 3 5' (donor) splice site and surrounding sequence. The Control sequence is at the top, showing the splice site at position 474 and the potential splice site at position 460. Bases in each splice site are shown in the corresponding WALKER diagram, with contribution to the average sequence conservation of the splice site indicated by the positive or negative height of the base. The arrow points to the mutant sequence with the G to A base change, showing the change from a positive contribution by the G to a negative contribution of the A.

The G to A transition probably would have one of two possible effects: skipping of exon 3 with splicing of exon 2 to exon 4 or possibly exons 5 or 6, or recruitment of a cryptic splice site, or possibly a combination of both. If exon 3 were simply skipped with splicing of exon 2 to exon 4, the result would be a deletion that does not maintain the reading frame and results in premature termination after addition of 4 amino acids (leu-asp-trp-leu) to the 32 amino acids encoded by the first 2 exons, basically the amino terminal arm. If the illegitimate splice site at position 460 within exon 3 is utilized, the first 35 amino acids of exon 3 would occur in a normal fashion, followed by the same 4 additional amino acids and then premature termination. If an illegitimate splice site within intron 3 is used or intron 3 is included, the amino acids encoded by exon 3 including the terminal alanine would be conserved. However, the very next codon within the retained intron 3 would be a UGA stop site, mimicking a missense mutation at the protein level. This would cause truncation of the βA3/A1-crystallin immediately after the first Greek key motif. If exon 3 is skipped and exon 2 is spliced directly to exon 5, the coding frame would be maintained, with the resulting crystallin consisting of the amino terminal arm and a single (carboxy) domain. Finally, if exon 2 is spliced directly to exon 6, the resulting protein would consist of the amino terminal arm followed by 18 “random” amino acids due to the frameshift caused by the aberrant splice.

If an illegitimate splice site in exon 3 or intron 3 is used, the effect on the protein would be to terminate the βA3/A1-crystallin peptide near the end of the first Greek key motif, adding the 4 amino acids leu-asp-trp-leu to the carboxy terminal. This would disrupt the first Greek key motif, since the Greek key motifs are formed with the fourth strand of the first motif being provided by the second motif and vice versa [40]. This means that without the second motif, which is encoded by the fourth exon, it is not possible to form even a single Greek key structure. The lack of the fourth chain required to form the Greek key motif makes it unlikely that the protein would be folded correctly after synthesis. Not only would this improperly folded crystallin be unstable and serve as a nidus for precipitation of other damaged proteins, but it might also interfere with appropriate associations by the remaining β-crystallins. The net result of any of these events would probably be a dominantly inherited cataract. This suggests that the βA3A1-crystallin splice mutation might cause the cataracts in this family. This possibility is being further investigated.

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