Mutations in βB3-Crystallin Associated with Autosomal Recessive Cataract in Two Pakistani Families

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PURPOSE. To identify the disease locus for autosomal recessive congenital cataracts in consanguineous Pakistani families.

METHODS. Two Pakistani families were ascertained, patients were examined, blood samples were collected, and DNA was isolated. A genome-wide scan was performed using >382 polymorphic microsatellite markers on genomic DNA from affected and unaffected family members. Two-point lod scores were calculated, haplotypes were formed by inspection, and candidate genes were sequenced. Real-time quantitative PCR techniques were used to determine the mRNA levels, and molecular modeling was performed to gain a better understanding of the significance of the disease-causing mutation.

RESULTS. In the genome-wide scan, maximum lod scores of 2.67 and 2.77 for family 60004 and 2.02 and 2.04 for family 60006 were obtained for markers D22S539 and D22S115, respectively. The linked region, 22.7 cM (10 Mb) flanked by markers D22S420 and D22S1163, contains the β-crystallin gene cluster including the genes CRYBA4, CRYBB1, CRYBB2, and CRYBB3. Sequencing of these genes showed a G→C transition in exon 6 of CRYBB3 resulting in a p.G165R change in the βB3-crystallin protein that cosegregates with the disease in both families. Real-time PCR analysis suggested that βB3-crystallin mRNA levels approximate those of other β-crystallins. Molecular modeling predicted changes in electrostatic potential that would be expected to reduce the stability of the fourth Greek-key motif, and hence the entire protein, dramatically.

CONCLUSIONS. For the first time, a mutation in CRYBB3 is reported in two consanguineous Pakistani families with autosomal recessive congenital cataracts. (Invest Ophthalmol Vis Sci. 2005;46:2100–2106) DOI:10.1167/iovs.04-1481

Congenital cataracts are one of the major causes of vision loss in children worldwide1,2 and are responsible for approximately one third of blindness in infants. Congenital cataracts can occur in an isolated fashion or as one component of a syndrome affecting multiple tissues. Nonsyndromic congenital cataracts have an estimated frequency of 1 to 6 per 10,000 live births. They vary markedly in severity and morphology, affecting the nuclear, cortical, polar, or subcapsular parts of the lens or, in severe cases, the entire lens, with a variety of types of opacity. Congenital cataracts can lead to permanent blindness by interfering with the sharp focus of light on the retina during critical developmental intervals.

Crystallins make up 95% of soluble lens protein and have an essential role in maintaining lens transparency. High concentrations of closely packed crystallins are required for transparency and focusing of light on the retina.3–4 Crystallins are subdivided into α, β, and γ crystallins, according to their elution on gel exclusion chromatography.5 α-Crystallins are molecular chaperones related to the small heat shock proteins.6 The β-crystallins share a common structure comprising four Greek-key motifs grouped into two domains. This family includes several very stable proteins, including protein S, a sporation-specific protein of the bacterium Myxococcus xanthus7; Spherulin 3a of the slime mold Physarum polycephalum8; CRBG-GEOCY of the sponge Geodia cydnonium9; and AIM1, a tumor-suppressor gene.10

β-Crystallins are the most abundant water-soluble proteins in the human lens.11 The basic β-crystallins have amino and carboxyl and the acidic β-crystallins have amino terminal extensions or arms.12,13 Mutations in β-crystallin genes have been associated with inherited autosomal dominant cataracts in humans, including a splice mutation (IVS3+1G→A) in the βA1/A3-crystallin gene resulting in zonular cataract with sutural opacities14; an IVS3+1G→C in the same gene associated with nuclear cataract with prominent sutures15; a pQ155X mutation in CRYBB2 in three different families with three different cataract phenotypes: cerulean cataracts,16 pulverulent nuclear opacification,17 and cerulean cataracts with prominent sutures18; and a nonsense mutation in CRYBB1 associated with autosomal dominant cataract.19

Herein, we report a mutation in CRYBB3 in two consanguineous Pakistani families with autosomal recessive congenital cataracts. Cataracts in both families 60004 and 60006 mapped to the long arm of chromosome 22, region 11, which contains the β-crystallin gene cluster, including the genes CRYBA4, CRYBB1, CRYBB2, and CRYBB3. Sequencing of these genes shows a G→C transition in exon 6 of CRYBB3 that cosegregated with the disease in both families and resulted in a p.G165R change in the βB3-crystallin protein. Real-time PCR analysis and molecular modeling predicts that βB3-crystallin is highly expressed and would be destabilized by the mutation. All previously described cataractogenic mutations in β-crystallins have been associated with autosomal dominant cataracts, suggesting that βB3-crystallin may have a function in addition to that of a structural protein.
Materials and Methods

Clinical Ascertainment

Twenty-five Pakistani families with nonsyndromic congenital cataract were recruited to participate in a collaborative study between the National Centre of Excellence in Molecular Biology, Lahore, Pakistan, and the National Eye Institute, Bethesda, Maryland, to identify new disease loci that cause inherited visual diseases. This study was approved by institutional review boards at the National Eye Institute, and the Centre of Excellence in Molecular Biology, Lahore, Pakistan. The participating subjects gave informed consent, consistent with the tenets of the Declaration of Helsinki.

The families described in this study, 60004 and 60006, were from the Punjab province of Pakistan. A detailed medical history was obtained by interviewing family members. Medical records of clinical examinations previously conducted with slit lamp biomicroscopy reported nuclear cataract in all affected individuals of 60004. Cataracts were either present at birth or developed in infancy. All affected individuals in family 60004 had undergone cataract surgery in the early years of life, and hence no pictures of their lenses were available. Individual 11 of family 60006 had nuclear cataracts with cortical ridges (Fig. 1), and her visual acuity was reduced to counting fingers. Blood samples were collected from affected and unaffected family members, and DNA was extracted using a nonorganic method.

Genotype Analysis

A genome-wide scan was performed with 382 highly polymorphic fluorescent markers from a linkage mapping set (Prism Linkage Mapping Set MD-10; Applied Biosystems, Inc. [ABI], Foster City, CA) having an average spacing of 10 cM. Multiplex polymerase chain reactions (PCRs) were performed as previously described. PCR products from each DNA sample were pooled and mixed with a loading cocktail containing size standards (HD-400; ABI) and loading dye. The resultant PCR products were analyzed on a 5% denaturing urea-polyacrylamide gel (Long Ranger; Fisher Scientific, Pittsburgh, PA) in a DNA sequencer (377; ABI) and analyzed with commercial software (GeneScan, ver. 3.1, and GENOTYPER, ver. 2.1; ABI).

Linkage Analysis

Two-point linkage analysis was performed with the FASTLINK version of MLINK from the LINKAGE Program Package (http://www.hgmp.mrc.ac.uk/; provided in the public domain by the Human Genome Mapping Project Resources Centre, Cambridge, UK). Maximum Lod scores were calculated with ILINK. Autosomal recessive cataract was analyzed as a fully penetrant trait with an affected allele frequency of 0.001. The marker order and distances between the markers were obtained from the Génethon database (http://www.genethon.fr/ provided in the public domain by the French Association against Myopathies, Evry, France) and the National Center for Biotechnology Information chromosome 22 sequence maps (http://www.ncbi.nlm.nih.gov/mapview/). For the initial genome scan equal allele frequencies were assumed, whereas for fine mapping allele frequencies were estimated from 125 unrelated and unaffected individuals from the Punjab province of Pakistan. Admixture analysis was performed using the HOMOG1 program (developed by Jurg Ott, Columbia University, New York, NY, and available at http://linkage.rockefeller.edu/soft/list2.html#h) comparing linkage to D22S315 at θs of 0.001, 0.01 0.02, 0.03, 0.04, 0.05, 0.06, 0.07, 0.08, 0.09, 0.1, 0.2, 0.3, and 0.4 with absence of linkage.

Mutation Screening

Individual exons of CRYBA4, CRYBB1, CRYBB2, and CRYBB3 were amplified by PCR using primer pairs shown in Table 1. Amplification was performed in 20-μL reactions containing 80 ng genomic DNA, 10 pmol each of forward and reverse primers, 200 nM dNTP, 1X PCR buffer, 1.5 mM MgCl2, and 0.2 U Tag DNA polymerase (AmpliTaq Gold Enzyme; ABI). PCR amplification followed a touchdown protocol consisting of a denaturation step at 95°C for 8 minutes, followed by 40 cycles, each consisting of a denaturation step at 94°C for 30 seconds, an annealing step at 5°C for 30 seconds for the first 5 cycles (T is the specific temperature for each primer pair as shown in Table 1), T-2°C for 30 seconds for the next 5 cycles, T-4°C for 30 seconds for the next 15 cycles, and T-6°C for 30 seconds for the last 15 cycles, with each cycle consisting an extension step at 72°C for 60 seconds. The PCR products were analyzed on 2% agarose gel and were purified by a gel-extraction kit (200 nM; Qiagen). PCR amplification was performed on an automated sequencer (Prism 3100; ABI) and analyzed (Sequence Detector V 2.1 program; ABI). Reactions consisted of an incubation of 2 minutes at 50°C, a 10-minute denaturation at 95°C, 45 cycles of denaturation for 15 seconds at 95°C, and 1 minute of annealing and elongation at 60°C. All reactions were performed in a 20-μL reaction volume with 10 ng of cDNA template, 500 nM of primers and 200 nM of fluorescent-labeled probe. The amount of amplified PCR product was calculated from standard curves, and ββ3- and γ-crystallin mRNA levels were normalized against the GAPDH mRNA level.

Homology Modeling

Homology modeling of βB3 was performed with crystal coordinates for the bovine βB2-crystallin dimer (PDB: 1ibb) and human βB1-crystallin dimer (PDB: 1okr) as structural templates. Both dimers used as templates are composed of two monomers each in “open” or “closed” conformations, respectively. βB3-crystallin shows 56% se-
TABLE 2. Primers Used for Sequencing CRYBA1, CRYBB1, CRYBB2, and CRYBB3

<table>
<thead>
<tr>
<th>Forward Primer</th>
<th>Reverse Primer</th>
<th>Annealing Temperature (°C)</th>
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<tbody>
<tr>
<td>m.CRYBA1</td>
<td>TGGGCCATTCATCACTCCAGA</td>
<td>GAGCCCACTTCTCCCAACA</td>
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<tr>
<td>m.CRYBB1</td>
<td>CCACTTTCCGCTCTCTGCTTCA</td>
<td>AGAGATCTGGGCCCTTCTAGC</td>
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<tr>
<td>m.CRYBB2</td>
<td>GAGAATTTTTGCCCTTCTCTGCA</td>
<td>GAGCCGAGGCTGTTGCTG</td>
</tr>
<tr>
<td>m.CRYBB3</td>
<td>GCCCTGTACACTCTGGGCTCT</td>
<td>ACGGGCTGGTTGCTG</td>
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</table>

The first exon of β-crystallins is noncoding, so that primers are not given.

RESULTS

Sequence identity to both βB2- and βB1-crystallins in regions containing the domain structure. Thus, two structural models of the βB3 structure were built with two different structural templates. For each model, primary sequences were aligned by the method of Needleman and Wunsch, incorporated in the program Look, version 3.5.2 (available at http://www.bioinformatics.ucla.edu/genemine/), for three-dimensional structure prediction. Finally, the structures of βB3 dimers were built by the automatic segment-matching method in the program Look followed by 500 cycles of nonbound energy minimization. Both predicted βB3 structures were regularized by an energy minimization procedure in the presence of water on the final step using a commercial program (Sybyl rev. 6.9.2; Tripos Inc., St. Louis, MO). Electrostatic potential was calculated by an algorithm incorporated in the Swiss Institute of Bioinformatics, Geneva, Switzerland. The geometry of the predicted structures was tested with the program Procheck (available by anonymous ftp at ftp.biochem.ucl.ac.uk: provided in the public domain by the University College London, London, UK). The mutant structure of C-terminal domain of βB3-crystallin was refined by using 400 ps molecular dynamics simulation at 300 K (Sybyl rev. 6.9.2; Tripos). Electrostatic potential was calculated by an algorithm incorporated in the Swiss Institute of Bioinformatics, Geneva, Switzerland.

TABLE 2. Primers and Probes Used for Real-Time PCR Analysis of Mouse CryBB3 and CryGC and Human CRYBB3

<table>
<thead>
<tr>
<th>Forward Primer</th>
<th>Reverse Primer</th>
<th>MGB Probe</th>
</tr>
</thead>
<tbody>
<tr>
<td>m.CRYBB3</td>
<td>TGGGCCATTCATCACTCCAGA</td>
<td>GAGCCCACTTCTCCCAACA</td>
</tr>
<tr>
<td>m.CryGC</td>
<td>CCACTTTCCGCTCTCTGCTTCA</td>
<td>AGAGATCTGGGCCCTTCTAGC</td>
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<td>m.GAPDH</td>
<td>GAGAATTTTTGCCCTTCTCTGCA</td>
<td>GAGCCGAGGCTGTTGCTG</td>
</tr>
<tr>
<td>h.CRYBB3</td>
<td>GCCCTGTACACTCTGGGCTCT</td>
<td>ACGGGCTGGTTGCTG</td>
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<tr>
<td>h.GAPDH</td>
<td>GCCCTGTACACTCTGGGCTCT</td>
<td>ACGGGCTGGTTGCTG</td>
</tr>
</tbody>
</table>

m, mouse; h, human.
of linkage of families 60004 and 60006 were 0.9849 and 0.9241, respectively, whereas conditional probabilities of linkage for the remaining families are <0.001. Visual inspection of the haplotypes of these two families supports the linkage analysis (Fig. 2) and localizes the cataract to a 22.7-cM (10.01-Mb) interval between D22S424 and D22S1163. A proximal recombination event at D22S424 in affected individual 11 of family 60006 and lack of homozgyosity at D22S424 in affected individuals 7, 8, and 11 of family 60004 set the proximal boundary of the linked region at D22S424. A distal recombination event at D22S1163 in affected individual 11 of 60004 (Fig. 2) sets the distal boundary at that marker, and lack of homozgyosity D22S280 and D22S283 in affected individuals 7 and 8 of family 60004 and for D22S283 in affected individuals 10 and 11 of family 60006 support this boundary.

Individuals 9, 10, and 13 in family 60004 and individual 8 in family 60006 were also heterozygous for the mutation, whereas all affected individuals were homozygous. The transversion in exon 6 results in a homozygous G→C transition: c.493G→C in affected individuals of both families. The transversion in exon 6 results in a nonconservative glycine-to-arginine substitution: G165R in the protein. This mutation cosegregates with the disease phenotype in both families, the parents in both families being heterozygous, whereas all affected individuals were homozygous. Individuals 9, 10, and 13 in family 60004 and individual 8 in family 60006 were also heterozygous for the mutation, whereas individual 07 was homozygous for the wild-type allele, in agreement with the haplotype analysis. The G→C transition in exon 6 of CRYBB3 was not seen in 125 unrelated and unaffected individuals (250 chromosomes) from the Punjab province of Pakistan.

To assure that B3-crystallin is expressed in the lens at levels sufficient to cause a cataract when mutated, real-time quantitative PCR was used to determine the B3- and γC-crystallin mRNA levels in the mouse and B3-crystallin mRNA levels in adult human lens. In the mouse lens, the B3- and γC-crystallin mRNA levels were approximately 47- and 43-fold that of GAPDH mRNA levels, respectively. B3-crystallin mRNA levels in the adult human lens are approximately 39% those of GAPDH mRNA, presumably reflecting its relative specificity for fiber cells (see the Discussion section).

The structure of B3-crystallin was modeled to elucidate the possible effects of the G165R mutation on its structure. Residue G165 is conserved in all β-crystallin sequences in the Protein Information Resource Database (http://pir.georgetown.edu/) provided in the public domain by Georgetown University, Washington, DC) showing >40% sequence identity to βB3 structure (data not shown). The G165R mutation has a negative Blosum80 score (~3) consistent with its nonconservative nature, exchanging a small amino acid for a moderate-sized positively charged amino acid with a hydrophobic stem. As can be seen in the multiple sequence alignment of the three β-crystallins used to model the three-dimensional structure of human B3-crystallin, G165 is conserved in human B3-, human βB1-, and bovine βB2-crystallins (Fig. 3).

The predicted human B3-crystallin structure (Fig. 4) has domains typical of the βγ-crystallin family, and the predicted B3-domain backbone structure superimposes on βB1- and βB2-crystallins with a root mean square (RMS) deviation of 0.5 Å. Each globular domain comprises 8 β-strands folded in two Greek-key motifs, forming a β-sandwich structure. The domain structures are connected by a linking peptide that can either be extended as in the βB2-crystallin structure derived from x-ray diffraction (forming a dimer) or curved if modeled on the βB1-crystallin structure derived from x-ray diffraction, in which the two domains fold back on each other to form a monomer.

Figure 4a shows the predicted effects of the G165R mutation based on the atomic model of the C-terminal domain of B3-crystallin. In the predicted B3-crystallin structure, Gly165 is located at the tip of the loop connecting β-strands 1 and 2 of the fourth Greek-key motif (see the red dot in Fig. 4b, inset). Maintenance of the hairpin fold involving the loop and β-strands 1 and 2 in each motif requires amino acid residues with small (e.g., alanine) or no (e.g., glycine) side chains, which can assume the acute torsional angles needed to form the Greek-key motif. Molecular dynamic simulation at 100-s intervals over a total time of 400 ps (Fig. 4a) shows the initial melting of the secondary structure of the short α-helix (the red cylinder on the right changes to a blue extended loop) and three of the β-strands (also converted to extended loops) of the G165R mutant, associated with expansion of the volume of the domain structure by almost 8% (920 Å³).

Ultimately, the G165R change would be expected to open up the hairpin fold and destabilize the fourth Greek-key motif, in addition to inserting the hydrophobic CH3 groups of arginine into a polar environment. In addition, in the predicted structure of B3-crystallin, residue Gly165 resides 8 Å and 11 Å from residues R167 and R195 respectively, which would, on substitution with R165, result in electrostatic repulsion, since they are exposed on the same side of the C-terminal domain surface (Fig. 4a). The G165R mutation increases the polarity of the C-terminal domain surface (depicted by increased blue color in Fig. 4d). Together with the repulsive interaction with Arg167, Arg195, and others shown in Figure 4a, this is predicted to result in a significant electrostatic energy change (~83 kJ/mol, as shown in Figures 4c and 4d).

### Table 3. Two-Point Lod Scores of Chromosome 22 Markers

<table>
<thead>
<tr>
<th>Marker</th>
<th>cM</th>
<th>Mb</th>
<th>0</th>
<th>0.01</th>
<th>0.05</th>
<th>0.1</th>
<th>0.2</th>
<th>0.3</th>
<th>0.4</th>
<th>Z&lt;sub&gt;max&lt;/sub&gt;</th>
<th>θ&lt;sub&gt;max&lt;/sub&gt;</th>
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<tbody>
<tr>
<td>D22S420</td>
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<td>−0.92</td>
<td>−0.29</td>
<td>0.06</td>
<td>0.06</td>
<td>0.02</td>
<td>0.01</td>
<td>0.06</td>
<td>0.1</td>
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<tr>
<td>D22S539</td>
<td>9</td>
<td>20.68</td>
<td>2.67</td>
<td>2.38</td>
<td>1.53</td>
<td>0.98</td>
<td>0.46</td>
<td>0.21</td>
<td>2.67</td>
<td>0</td>
<td>0</td>
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<tr>
<td>D22S315</td>
<td>16.2</td>
<td>24.34</td>
<td>2.77</td>
<td>2.49</td>
<td>1.62</td>
<td>1.06</td>
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<td>26.24</td>
<td>−∞</td>
<td>0.6</td>
<td>1.09</td>
<td>0.92</td>
<td>0.6</td>
<td>0.28</td>
<td>0.11</td>
<td>1.13</td>
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<td>−0.27</td>
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<td>35.02</td>
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<td>−2.46</td>
<td>−1.14</td>
<td>−0.22</td>
<td>−0.07</td>
<td>−0.02</td>
<td>−0.01</td>
<td>−0.01</td>
<td>0.4</td>
</tr>
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* Marker included in genome wide scan.
We report a mutation in \textit{CRYBB3} in two consanguineous Pakistani families with autosomal recessive congenital cataracts. Both families 60004 and 60006 mapped to chromosome 22q11. Family 60004 showed suggestive maximum lod scores of 2.67 and 2.77 and family 60006 showed maximum lod scores of 2.02 and 2.04 for markers D22S539 and D22S315, respectively. The 22.7-cM (10.01-Mb) linked region, flanked by markers D22S420 and D22S1163, contains the \( \beta \)-crystallin gene cluster, including the genes \textit{CRYBA4}, \textit{CRYBB1}, \textit{CRYBB2}, and \textit{CRYBB3}. Sequencing of these genes shows a \( G \rightarrow C \) transition in exon 6 of \textit{CRYBB3} that cosegregate with the disease in both families, resulting in a p.G165R change in the \( \beta \)-crystallin protein.

Of all the \( \beta \)-crystallins, \( \beta \)-3-crystallin is perhaps the least well studied. Together, \( \beta \)-3-crystallin and \( \beta \)-3-crystallin have been shown to make up approximately 6% of the protein mass of the young human lens, but individual quantitation was not possible.\textsuperscript{11} Hawse et al.\textsuperscript{31} have shown that \( \beta \)-3-crystallin transcripts are expressed in the lens epithelium and are decreased approximately 22-fold in cataract compared with clear lenses. However, although they are expressed in the lens epithelia, \( \beta \)-crystallin, including \( \beta \)-3-crystallin, expression increases substantially as epithelial cells elongate to form the fiber cells present in the lens nucleus.\textsuperscript{32,33} As crystallins are structural proteins, the significance of a mutation in a lens crystallin would be expected to increase with the expression level of that crystallin.

To investigate the significance of this \( \beta \)-3-crystallin mutation further, real time quantitative PCR shows that \( \beta \)-3-crystallin mRNA levels in the mouse are similar to those of \( \gamma \)-C-crystallin, suggesting that \( \beta \)-3-crystallin contributes significantly to the refractive properties and clarity of the lens. \( \beta \)-3-crystallin mRNA levels in the adult human lens are approximately 39% those of GAPDH mRNA. These results seem likely to relate to the massive induction of \( \beta \)-crystallin expression as fiber cells elongate, which results in high levels of GAPDH relative to the \( \beta \)-crystallins in the anterior epithelial cells relative to young fiber cells. Because there is an absence of mRNA in the adult human lens nucleus because of the anucleate nature of mature lens fiber cells and finite mRNA half-life, relatively low levels of \( \beta \)-crystallins are to be expected in the adult human.

The structure of human \( \beta \)-3-crystallin was modeled to understand better the effect of G165R mutation on its structure and folding. The predicted human \( \beta \)-3-crystallin structure (Fig. 4) has domains typical of the \( \beta \)-crystallin family, and the predicted \( \beta \)-3-domain backbone structure superimposes well on both the curved \( \beta \)-1 and the extended \( \beta \)-2-crystallins. This is to be expected, because both of these forms probably exist in equilibrium in the eye lens.\textsuperscript{33} Several specific effects of the G165R mutation are predicted based on the atomic model of \( \beta \)-3-crystallin. First, the inability of the arginine to assume the acute torsional angle at this position is predicted to open up the Greek-key motif, expanding the domain structure by approximately 8% and destabilizing it significantly. In addition, charge repulsion from nearby arginine residues and the increased charge density of the domain surface along with insertion of the relatively hydrophobic stem structure of Arginine into this polar environment are predicted to destabilize the domain by approximately \(~83\ kJ/mol\). For comparison, the free energy necessary to unfold the structurally similar domains of protein S is 21 kJ/mol and 31 kJ/mol for the C- and N-terminal domains respectively, suggesting that the electrostatic energy change resulting from the G165R mutation could be more than enough to destabilize the \( \beta \)-3-crystallin protein fold.

Segregation of the G165R mutation with the disease phenotype, conservation of residue G165 among all \( \beta \)-crystallins, the molecular dynamic estimation of the effects of the mutation, the presence of significant levels of \( \beta \)-3-crystallin mRNA...
in the mouse and human lens, and the absence of the G→C transversion in exon 6 c.493G→C of CRYBB3 (NM_004076) in 125 unaffected Pakistani control subjects, together strongly suggest that the G165R mutation is responsible for autosomal recessive cataracts in these families and is not a benign polymorphism. Mutations in other β-crystallins previously have been associated with autosomal dominant congenital cataracts but not, to our knowledge recessive cataracts. The precise mechanism by which the p.G165R mutation in βB3-crystallin causes recessive cataract remains unknown. It seems likely, based on other known crystallin mutations, that synthesis of a deleterious or unstable crystallin expressed at high levels even in heterozygous patients would result in a dominant cataract. Thus, it is possible that the mutant βB3-crystallin either is degraded, or is for some reason not toxic to the lens cell, although a dosage effect cannot be ruled out. However, this seems unlikely, based on the clear lenses in parents of cataractous family members even though they are significantly older than their affected children. Conversely, redundancies among the β-crystallins suggest that mere absence of βB3-crystallin may not cause a cataract unless it is critical for formation of higher-order β-crystallin complexes. For example, in 2003, Klopp et al.36 reported a frame shift in exon 2 of CRYGA resulting in premature termination which was not associated a cataract phenotype. Although this has not been tested by knockout mice, such mutations have not been seen among unaffected control subjects tested for this or previous β-crystallin mutations. Recessive mutations are more generally associated with loss of an enzymatic or developmental function, raising the possibility that βB3-crystallin may play a role in the lens beyond that of a structural crystallin, or at least a role in complex formation for which other β-crystallins cannot substitute. Biochemical analysis of normal and mutant βB3-crystallin proteins expressed in vitro should help to elucidate the mechanism by which the p.G165R mutation in βB3-crystallin causes recessive cataract.

**Figure 3.** Multiple alignment of human βB3-crystallin with bovine βB2- and human βB1-crystallins used as templates for three-dimensional structure.

**Figure 4.** Structures of human βB3-crystallin (βB3) and the G165R mutant protein. (a) Diagram of the C-terminal domain of βB3-crystallin and the G165R mutant as predicted using a βB2-crystallin structural template. The α-helices and β-strands are shown as cylinders and ribbons, respectively. The superposition of wild-type (red) and the G165R mutant (blue) protein structures is shown. Side chains involved in charge interactions in the G165R mutant are shown for the wild-type (green) and mutant (white) proteins and for arginines (red) and glycine (green). (b) βB3-Crystallin (yellow) and mutant (red) proteins in the vicinity of the G165R mutation. Inset: schematic diagram of fourth Greek-key motif fold with the position of residue 165 shown (red dot). (c) The solvent accessible surface area of the normal C-terminal domain is colored according electrostatic potential: <-1.8 kT/e (red), neutral (white), and >1.8 kT/e (blue). (d) Surface model demonstrating the local change in electrostatic potential around residue 165 of the G165R mutant protein (color scheme as in c).
anism by which a mutant β-crystallin results in autosomal recessive cataract and should enhance our understanding of lens biology at a molecular level.

Acknowledgments

The authors thank the families for their participation in the study; the staff of Lyton Rehmatullah Benevolent Trust (LBBT) hospital, especially Mohammed Amer, for the identification of the families and expert clinical evaluation of affected individuals; and Farooq Sultan and Muhammad Awais for technical help.

References