Aey2, a New Mutation in the βB2-Crystallin-Encoding Gene of the Mouse

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PURPOSE. During an ethyl nitrosourea (ENU) mutagenesis screen, mice were tested for the occurrence of dominant cataracts. One particular mutant was found that caused progressive opacity and was referred to as Aey2. The purpose of the study was to provide a morphologic description, to map the mutant gene, and to characterize the underlying molecular lesion.

METHODS. Isolated lenses were photographed, and histologic sections of the eye were analyzed according to standard procedures. Linkage analysis was performed using a set of microsatellite markers covering all autosomal chromosomes. cDNA from candidate genes was amplified after reverse transcription of lens mRNA.

RESULTS. The cortical opacification visible at eye opening progressed to an anterior suture cataract and reached its final phenotype as total opacity at 8 weeks of age. There was no obvious difference between heterozygous and homozygous mutants. The mutation was mapped to chromosome 5 proximal to the marker D5Mit138 (8.7 ± 4.2 centimorgan [cM]) and distal to D5Mit15 (12.8 ± 5.4 cM). No recombinations were observed to the markers D5Mit10 and D5Mit25. This position makes the genes within the βA4/βB-crystalline gene cluster excellent candidate genes. Sequence analysis revealed a mutation of T→A at position 553 in the Crybb2 gene, leading to an exchange of Val for Glu. It affects the same region of the Crybb2 gene as in the Philly mouse. Correspondingly, the loss of the fourth Greek key motif is to be expected.

CONCLUSIONS. The Aey2 mutant represents the second allele of Crybb2 in mice. Because an increasing number of β- and γ-crystallin mutations have been reported, a detailed phenotype-genotype correlation will allow a clearer functional understanding of β- and γ-crystallins. (Invest Ophthalmol Vis Sci. 2001;42:1574–1580)

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The β- and γ-crystallins form the major part of the water-soluble proteins of the eye lens. The β- and γ-crystallins are recognized as members of a superfamily and have been considered for a long time to be present only in the eye and mainly in the ocular lens.1,2 However, just recently, expression of the βB2-crystallin mRNA and protein was reported also in brain and testis.3 The common characteristic of all β and γ-crystallins is the so-called Greek key motif. Crystallinology of bovine βB2- and γB-crystallins has shown that each of the β- and γ-crystallins is composed of two domains, each of them built up by two Greek key motifs.4,5 It is widely accepted that β- and γ-crystallins evolved in two duplication steps from an ancestral protein folded like a Greek key.6 Greek key motifs also have been reported for a few other proteins, such as a yeast killer toxin from Williopsis mrrakii (WmKT; 1 Greek key motif)7 the protein S from Myxococcus xantthus (4 Greek key motifs), spherulin 3c from Physarum polycephalum (2 Greek key motifs), the epidermis-specific protein from Cynops pyrrhogaster (EDSP or ED37; 4 Greek key motifs),8 and the putative human tumor suppressor protein AIM1 (12 Greek key motifs).9–8 The function of the Greek key motifs has not been elaborated in detail; however, computer-based analysis suggests that it may be responsible for particular protein-protein interactions similar to those seen with immunoglobulins.9 Similar to the immunoglobulins, the β- and γ-crystallins are folded in an all-β structure. Undoubtedly, the accumulation of hydrogen bonds in the symmetrical, twisted, antiparallel, β-sheet structure of each domain, and the hydrophobic interactions between them, contribute significantly to their stability. This overall stability can be understood as the sum of the contribution of independent folding units.10,11

Mutations in the β- and γ-crystallin-encoding genes (gene symbols are Cryb and Cryg, respectively) have been demonstrated to lead to lens opacification in mice12–17 and humans.18–24 In most cases, the formation of at least one of the Greek key motifs is affected by the mutation. In total, 13 genes belong to this superfamily in mammals.

In the β-crystallins, individual Greek key motifs are encoded by separate exons.25 The Cryb genes consist of six exons: The first exon is not translated, the second exon encodes the N-terminal extension, and the subsequent four exons are responsible for one Greek key motif each. Biochemically, the β-crystallins are characterized as oligomers (the molecular masses of the monomers ranges between 22 and 28 kDa) with native molecular masses ranging up to 200 kDa for octomeric forms. The N termini are blocked by acetylation.6,26

The family of β-crystallins can be divided into more acidic (βA-) and more basic (βB-) crystallins. Each subgroup is encoded by three genes (respectively, Cryba1, -2, and -4; and Crybb1, -2, and -3), however, Cryba1 encodes two proteins: βA1- and βA3-crystallins. This feature is conserved among all vertebrates. In mammals, the Cryb genes are distributed among three chromosomes (mouse: 1, 5, and 11; man: 2, 17, and 22; for recent review see Ref. 15 and references therein). Among the Cryb genes, the nucleotide sequence of Crybb2 was char-
Many). Mice with lens opacities were tested for a dominant mode of inheritance. Homozygotes were obtained by brother × sister mating.

In the course of analysis of mouse mutants obtained by a large-scale ethylnitrosourea (ENU) mutagenesis program, we identified several cataract mutations. Herein, we report one of them, which was mapped to mouse chromosome 5 and identified as the second allele in the mouse Crybb2 gene.

**Materials and Methods**

**Mice**

Mice were kept under specific pathogen-free conditions at the National Research Center for Environment and Health (GSF) and monitored within the ENU mouse mutagenesis screen project. The use of animals was in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research and with the German Law on Animal Protection.

Male C3HeB/FeJ mice were treated with ENU (160 mg/kg) at the age of 10 weeks according to Ehling et al. and mated to untreated female C3HeB/FeJ mice. The offspring of the ENU-treated mice were screened at the ages of 4 to 6 months for the presence of cataracts, and the offspring (second generation) were backcrossed to the wild-type C57BL/6J mice. The offspring of the ENU-treated mice were used for each autosome.

**Phenotypic Characterization**

The eyes of the mutants were examined continuously during postnatal life with the slit lamp. For documentation, lenses were enucleated under a dissecting microscope (MZ APO; Leica, Heidelberg, Germany) immediately after isolation of the DNA from the gel using an extraction kit (Qia-Quick; Qiagen, Hilden, Germany). Some of the PCR products, using the primers Crybb2-L2 and Crybb2-R1, were digested for 2 hours at 37°C by 5 U restriction endonuclease DdeI (MBI Fermentas, St. Leon-Rot, Germany), and the reaction products were analyzed on a 4% agarose gel.

**PCR and Sequencing**

For the molecular analysis, RNA was isolated from lenses of C3HeB/FeJ, T-stock, 129, JF-1, and C57BL/6j wild-type mice and from homozygous mutant mice at the age of 4 weeks. RNA from lens, brain, and testis were transcribed to cDNA by using a kit (Ready-to-Go; Pharmacia Biotech, Freiburg, Germany). Genomic DNA was isolated from tail tips or spleen of wildtype C3HeB/FeJ and C57BL/6j mice or homozygous mutants, according to standard procedures. For amplification of cDNA from Crybb4 and the three Crybb genes, primers were selected from the EMBL GenBank databases (provided in the public domain by the European Bioinformatics Institute at Hinxton, Cambridge, UK, at http://www.ebi.ac.uk/ and the National Center for Biotechnology Information at http://www.ncbi.nlm.nih.gov/, respectively; Table 1).

PCR was performed using a thermocycler (Hyvidia OmniGene; MWG BioTech, Ebersberg, Germany). The annealing temperatures are included in Table 1. PCR products were analyzed on a 1% agarose gel.

**RESULTS**

The Aey2 mutant was detected by slit lamp screening of the offspring of ENU-treated male mice. The mutation has a complete penetrance. The litter size in the matings of heterozygotes and of homozygotes indicated normal fertility and viability of the mutants.

The Aey2 mutant displays a progressive cataract. The cataractous changes were observed as early as at eye opening (i.e., 12 days after birth) as a diffuse opacity in the cortex and abnormally branched anterior suture (Fig. 1a). This type of opacity remained stationary until 8 to 11 weeks of age, after which a total opacity developed (Fig. 1b).

The histologic sections of mutant eyes at 4 and 11 weeks of age demonstrated striking irregularities in the anterior suture and a dark subcortical zone (Fig. 2a). A higher power view showed swollen and liquefied fibers in the anterior suture with dark particles (Fig. 2b). Abnormal dark bodies and dustlike particles were also present in the subcortical zone (Fig. 2c).

The first 46 cataractous mice from the backcross (G3) were taken for genome-wide mapping. The results indicate linkage with markers on mouse chromosome 5. The following gene order was observed: D5Mit15—(12.8 ± 5.4 cM)—Aey2, D5Mit10, D5Mit25—(8.7 ± 4.2 cM)—D5Mit138. These results are in good agreement with the current report of the Chromosome Committee for mouse chromosome 5 (available at http://www.informatics.jax.org/bin/ccr/). A detailed haplo-

**TABLE 1. PCR Primers with Annealing Temperatures and Length of the Products**

<table>
<thead>
<tr>
<th>Designation</th>
<th>Lab Number</th>
<th>Sequence (5′ → 3′)</th>
<th>Accession Number</th>
<th>Annealing Temperature (°C); Fragment Size (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crybb4-L1</td>
<td>25026</td>
<td>GGC CCT TCT TGG ACC GTG C</td>
<td>AJ272228</td>
<td>53–60; 747</td>
</tr>
<tr>
<td>Crybb4-R2</td>
<td>26526</td>
<td>TGG CGG GGC ATG GGC</td>
<td>AJ272228</td>
<td>60; 517</td>
</tr>
<tr>
<td>Crybb1-L1</td>
<td>25028</td>
<td>GCA ATG CAG CAG CAG GAA CCA TTG C</td>
<td>AF106855</td>
<td></td>
</tr>
<tr>
<td>Crybb1-R1</td>
<td>25029</td>
<td>CTG TGG CAT CTC CAT GGT GTT GCG</td>
<td>AF106855</td>
<td></td>
</tr>
<tr>
<td>Crybb2-L1</td>
<td>24468</td>
<td>GTC GAC GCC AGA GAG TCC ACC</td>
<td>M60559</td>
<td>55; 728</td>
</tr>
<tr>
<td>Crybb2-R1</td>
<td>24469</td>
<td>GGC AGG AGG CAC ACT TTA TTC TTC</td>
<td>M60559</td>
<td>58; 215</td>
</tr>
<tr>
<td>Crybb2-L2</td>
<td>27037</td>
<td>GGC TAC GCT GGG CTC GAG TAC</td>
<td>M60559</td>
<td></td>
</tr>
<tr>
<td>Crybb3-L1</td>
<td>24469</td>
<td>GGC AGG AGG CAC ACT TTA TTC TTC</td>
<td>M60559</td>
<td></td>
</tr>
<tr>
<td>Crybb3-R2</td>
<td>25030</td>
<td>GTC GTT CTC CTC GAG GAG GGC</td>
<td>M60559</td>
<td>53–60; 738</td>
</tr>
<tr>
<td>Cryba4-L1</td>
<td>24468</td>
<td>GTC GAC GCC AGA GAG TCC ACC</td>
<td>M60559</td>
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<td>Cryba4-R1</td>
<td>24469</td>
<td>GGC AGG AGG CAC ACT TTA TTC TTC</td>
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<td>24468</td>
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<td>Crybb-R2</td>
<td>25030</td>
<td>GTC GTT CTC CTC GAG GAG GGC</td>
<td>M60559</td>
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</table>
The cluster encoding the four Cryb genes (Cryba4, Crybb1, Crybb2, and Crybb3; 59–60 cM from the centromere) was considered to contain good candidate genes for progressive cataract formation. The corresponding cDNAs were amplified both in the wild-type mice and in the homozygous mutants, by using cDNA templates derived by reverse transcription of RNA from lenses of 4-week-old mice.

The only sequence alteration cosegregating with the mutant phenotype was a T→A exchange at position 553 (exon 6) of the Crybb2 gene (Fig. 4). The mutation lead also to the appearance of a new Ddel restriction site in the mutant mice that was not present in the wild-type sequence of C3H and several other mouse strains. The presence of the mutation was validated by the presence of the Ddel restriction site in five homozygous mutants (Fig. 5). Therefore, the new allele should be referred to as Crybb2\textsuperscript{Aey2}.

The mutation is predicted to lead to a Val→Glu exchange at codon 187. At this position Val occurred in four of the six mouse \( \beta \)-crystallins. Only the acidic \( \beta A1/A3 \) and \( \beta A2 \)-crystallins had an Ile at this position. It is expected that this exchange prohibits the formation of the fourth Greek key motif in the mutants. However, final interpretations should include physicochemical data from corresponding recombinant proteins, together with a more sophisticated computer analysis.

Because Crybb2 expression also has been reported recently in the brain and testis,\textsuperscript{3} we tested this expression also in our wild-type C3H and cataractous mice. We observed conflicting results with different sets of primers. The primer pair Crybb2-L1 and -R1 amplified specifically the full-length cDNA of Crybb2 in the lens only. No transcripts were detected, either in brain or testis (Fig. 6a). The detection limit was determined for \( 10^{-18} \) moles (Fig. 6b). However, using another 5' primer, Crybb2-L2, located inside the Crybb2 transcript, a transcript was observed in brain and testis of the same size as in the lens (Fig. 6c). The sequence analysis confirmed this short amplification product as part of Crybb2. The absence of the entire Crybb2 transcript but presence of a shorter 5' end indicates the presence of a novel splice product of Crybb2 in brain and testis that is missing the N-terminal part. This is in line with the
observation by Mugabo et al.\textsuperscript{3} who presented just the C-terminal part of $\beta$B2-crystallin, but not the entire protein. The presence of the mutation in brain and testis was confirmed by Aey2 mutants. They were caused by a disturbed denucleation process in those fibers, which were completely degraded in the normal inner lens fiber, suggesting impaired chromatin degradation. (b) Higher magnification of the central anterior region of the lens demonstrates the irregular fiber cells in the outer cortical fiber cells and shows a distinct zone of discontinuity between the outer and inner fiber cells. The cellular structure of the inner fiber cells was markedly destroyed. The epithelial cells remained unaffected. (c) The remnants of the fiber cell nuclei were still present in the lens bow of the Aey2 lens. Partially degraded nuclei are obvious behind the zone of discontinuity from the outer cortex to the cataractous inner cortex. c, cornea; i, iris; lb, lens bow; le, lens epithelium; r, retina; s, anterior suture. Staining, methylene blue and basic fuchsin. Bar, 100 $\mu$m.

\textbf{Figure 2.} Histologic analysis of lenses of the Aey2 mutant at the age of 11 weeks. The section through a lens of an 11-week-old mouse is shown. \textit{Arrows:} Irregular remnants of fiber cell nuclei. (a) Striking abnormality of the anterior suture, a layer of dustlike particles in the anterior cortex and intercellular clefts in the lens nucleus appeared in FIGURE 3. Linkage analysis of the Aey2 mutation localized on chromosome 5. (a) Heterozygous mutant mice were outcrossed to wild-type C57BL/6J mice, and the heterozygous carriers were backcrossed to wild-type C57BL/6J mice. Among the offspring, only the cataractous mice were analyzed for their parental genotypes with respect to a variety of microsatellite markers. Results are given for those on chromosome 5. The total number of progeny scored for each locus is to the right of the boxes, including the calculated distances between the loci (in cM). The number of progeny that inherited each haplotype is below the boxes. (b) A partial chromosome map shows the location of the Aey2 mutation in relation to relevant markers and to the candidate genes Cryba4 and Crybb1, -2, and -3. Numbers to the left of the chromosome indicate the genetic distance (in cM) from the centromere, as given by the Chromosome Committee report. At right, the actual linkage data are summarized.

\textbf{Figure 3.} Haplotype analysis of the Aey2 mutation localized on chromosome 5. Heterozygous (C3HeB/FeJ) / (C57BL/6J) and cataractous homozygous for C57BL/6J and cataractous.
the presence of the DdeI restriction site as in the lens cDNA (Fig. 6c).

**DISCUSSION**

A new cataract mutation was observed among the F1 offspring of ENU-treated male mice. This progressive opacity, preliminarily referred to as **Aey2**, was mapped to mouse chromosome 5, close to the cluster of the $\beta A4$-, $\beta B1$-, $\beta B2$-, and $\beta B3$-crystallin–encoding genes. By molecular analysis of all four genes, the mutation was finally demonstrated to be caused by an A$\rightarrow$T exchange within exon 6 of the **Crybb2** gene. The mutant allele was therefore designated **Crybb2**$^{\text{Aey2}}$. The corresponding alteration at the amino acid level (Val187Glu) is thought to affect the formation of the fourth Greek key motif of the $\beta B2$-crystallin. Additionally, the progressive character of the cataract is in line with the expression profile of **Crybb2**.

**FIGURE 4.** Characterization of the **Aey2** mutation within the **Crybb2** gene. The cDNA sequence of the mouse **Crybb2** gene is shown (EMBL accession number, M60559). The amino acid sequence is above the DNA sequence. The mutation is indicated in **bold**; the exchanged amino acid is noted **below** the sequence. The DdeI restriction site at position 553 is **underlined**.

![DdeI restriction site](image)

**FIGURE 5.** **Crybb2** digest by DdeI. The cDNA of the **Crybb2** gene was amplified, and the PCR fragment was analyzed by agarose gel electrophoresis with (+) and without (−) subsequent digest by DdeI. The fragment from the wild-type C3H, C57BL/6J (C57BL), T-stock (T), 129, and JF-1 (JF) mice were not digested (a), but the cDNA from lenses of five **Aey2** mutants elicited the expected digest pattern (b).

![DdeI digestion](image)

**FIGURE 6.** RT-PCR for **Crybb2** expression in lens, testis, and brain. cDNA from lenses, testis, and brain from 3-week-old wild-type male mice were amplified for **Crybb2** expression by RT-PCR. −, negative control; M, marker. (a) Amplification of full-length **Crybb2** revealed amplification only in the lens, not in the testis or brain. (b) One femtomole of cloned entire **Crybb2** cDNA was used as a template for PCR reactions (lane 1). Stepwise 1:10 dilution of the template DNA (lanes 2–7) revealed a detection limit of $10^{-18}$ moles for the PCR reaction conditions reported in (a). (c) Amplification of the 215-bp fragment of the 3' end of **Crybb2** cDNA detected corresponding transcripts in lens, brain, and testis of wild-type C3H and mutant **Aey2** mice. The amplification product of the **Aey2** mutants were digested in all tissues, indicating that it represented the 3' end of the **Crybb2** transcripts.
The observation of the progressive cataract formation and the characterization of the molecular lesion within the βB2-crystallin-encoding gene demonstrates very strong similarities to the Philby cataract in the mouse. This mutation leads also to a progressive dominant cataract,5,11–13 which is caused by an in-frame deletion of 12 bp in the 3′ end of the Crybb2 gene.12

The Philby mouse cataract develops after the first week of postnatal life. At that time, abnormal particles appear in the anterior cortex that extend by the 10th day in the anterior subcapsular area.5,14 Two weeks after birth, enlargement of the persisting bow nuclei becomes prominent. During the fourth postnatal week, posterior lens fibers are swollen, and degenerating cells and large intercellular spaces are present in the superficial cortex. Between 5 and 7 weeks, epithelial cells at the equator become tall, and the number of their mitotic figures are markedly reduced. The lens cells in the posterior cortex degenerate, causing widening of the posterior suture. At this final stage of Philby mouse cataractogenesis, the lenticular nucleus becomes markedly opaque.5,15 The increasing severity of the phenotype is temporally correlated with the expression of the Crybb2 gene.5,12

Biochemical studies indicate that the cataractous process in the Philby mouse is associated with a variety of osmotic changes. At 20 days of age, there is an increase in lens water along with an alteration in electrolyte levels. Lenticular sodium rapidly increases, and potassium levels decrease. Concomitant with cataract formation is an increase in total lenticular calcium and a decrease in lens dry weight, in reduced glutathione, and in adenosine triphosphate.5,16 Associated with this altered membrane permeability in the Philby mouse lens, a changed pattern of membrane glycoproteins5,17 and membrane lipids5,18 has been reported.

The cause of these biochemical changes may be the different biophysical properties of the altered βB2-crystallin, as outlined by the absence of heat-stable characteristics.5,16 Moreover, the altered form of the βB2-crystallin in the Philby mouse lens is present primarily in the heavy-molecular-weight fraction, indicating that the altered βB2-crystallin in the Philby lens can interact with other β-crystallins in the lens.5,19 It is a matter for speculation whether the interactions of the altered βB2-crystallin with other lens proteins cause a rapid aggregation of the cellular proteins, leading to the formation of the heavy-weight material and resulting finally in the cataract.

The Philby mutation affects the same region of the protein (close to the carboxyterminus) as does the Crybb2<sup>α<sup>mp1</sup></sup> mutation. This region is considered to be essential for the correct formation of the tertiary structure of the βB2-crystallin, even if previous structural examination of the C-terminal region focused on the amino acids from 173 to 185, but did not include Val187.5,15 Therefore, it is very likely that the mechanisms involved in both cataractogenic processes are very similar. However, the lenses of the Aey2 mutant showed a slower progression of the opacification, which was terminated between 8 and 11 weeks of age. As in the Philby mouse, dustlike particles were present in the anterior cortex. They seemed to be breakdown products of the cell nuclei, which do not undergo the regular degradation process that occurs in the normal lens fiber cells. This slower progress in cataractogenesis may be due to a smaller molecular lesion in the Aey2 mice than that in the Philby mouse.

A corresponding human counterpart to the mouse mutants at the Crybb2 locus is the cerulean cataract detected in a large family as a dominantly inherited disorder. This disease was mapped to the region of human chromosome 22 that includes two β-crystallin-encoding genes (CRYBB2 and -3) and a pseudogene (CRYBB2<sup>p</sup>).40 Recently, a G→A transition has been reported at position 155 in CRYBB2. It affects the first base of a codon, usually coding for a Glu residue, but the mutation creates a stop codon and truncates the βB2-crystallin by 51 amino acids.19

Another human cataract mutation affecting a CRYB locus was described as a semidominant zonular cataract with sutural opacities. Padma et al.10 reported a linkage of the corresponding disease gene to human chromosome 17q11-12 in a three-generation family. Because the CRYBA1 gene is localized in this region, it was considered to be a good candidate gene. Recently, Kannabiran et al.21 reported that this particular form of a cataract is caused by the absence of exons 3 and 4 in the corresponding mRNA (resulting in a protein with only the C-terminal globular domains).

Also in this human mutation, a homologous semidominant mutation in the mouse has been reported recently.2 The heterozygous Crybb<sup>10</sup> mutants exhibit a progressive cataract, reaching its final stage at 8 weeks of age, whereas in the homozygotes the total cataract has already developed at eye opening. The mutation affects the splice site at the beginning of exon 6 and leads to two different cDNA forms and also to two different proteins. One of them is thought to affect the formation of the fourth Greek key motif.2

The β-crystallins form a superfamily of proteins together with the γ-crystallins, because of their common structural Greek key motifs.1,7 They are distinct from the α-crystallins in sequence, structure, and function. Physicochemical data point out that the monomeric γ-crystallins and the oligomeric β-crystallins can build similar folding structures that allow a dense package of these proteins without loss of transparency.5,41 Also in the γ-crystallin-encoding genes, a broad variety of mutations are currently being characterized in mice,13,14,16,17,42 and humans.20,22–24,43 Most of them also affect the Greek key motifs, by the loss of the corresponding sequence, by predicted changes of their folding properties, or by changes of their steric coordinations.

Changes of the structural characteristics of lens proteins can be rescued, at least in part, by the chaperone activity of the α-crystallins, which are also abundant in the lens.44 However, the prevention of denaturation and/or renaturation is restricted to physicochemical effects on the already-formed proteins, such as thermal or oxidative stress. The α-crystallins are not able to bring a protein with an altered primary structure (i.e., altered amino acid sequence) to its “regular” folding. This inability was demonstrated recently in a mutated form of a γ-crystallin.45

From the morphologic observations reported in the current study and in the references cited herein, it becomes evident that inherited congenital cataracts have some similar features, even if they are distinct in their details. The most common aspect is the presence of the fiber cell nuclei throughout the entire lens. They may be shifted to the anterior or posterior pole, but usually they are not correctly degraded and are still present, at least in a pyknotic form. This feature reflects a disturbance of the differentiation program of the lens fiber cells. The onset of the first cataractogenic characteristics usually correlates with the expression profile of the gene, which is affected by the deleterious mutation. However, it would be interesting to elaborate the underlying molecular and biochemical mechanisms in detail.

In conclusion, the present article describes a second allele of the mouse Crybb2 gene, providing a further excellent animal model for the homologous disease in humans. Moreover, together with recent reports in the literature concerning mutations affecting β and γ-crystallin-encoding genes in human and mouse, it demonstrates the importance of the β- and γ-crystallin superfamily in the functional integrity of the eye lens.
Acknowledgments

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