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A Transgenic Mouse Model for Human Autosomal Dominant Cataract

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Abstract

Purpose—To characterize lenses from transgenic mice designed to express mutant and wild-type α A-crystallin subunits.

Methods—A series of transgenic mouse strains was created to express mutant (R116C) and wild-type human α A-crystallin in fiber cells of the lens. Dissected lenses were phenotypically scored for the presence and extent of opacities, fiber cell morphology, and posterior suture morphology. Gene transcripts derived from integrated transgenes were detected by reverse transcriptase-PCR. Distribution of expressed transgenic protein was determined by immunohistochemical staining of lens tissue sections. The abundance of endogenous and transgenic lens proteins was estimated by quantitative Western blot analysis.

Results—Expression of R116C mutant α A-crystallin subunits resulted in posterior cortical cataracts and abnormalities associated with the posterior suture. The severity of lens abnormalities did not increase between the ages of 9 and 30 weeks. With respect to opacities and morphologic abnormalities, lenses from transgenic mice that express wild-type human α A-crystallin subunits were indistinguishable from age-matched non-transgenic control mice. Similar phenotypes were observed in different independent lines of R116C transgenic mice that differed by at least two orders of magnitude in the expression level of the mutant transgenic protein.

Conclusions—The results show that lens opacities and posterior sutural defects occur when mutant R116C α A-crystallin subunits are expressed on the background of wild-type endogenous mouse α -crystallins. Low levels of R116C α A-crystallin subunits are sufficient to induce lens opacities and sutural defects.

The lens is composed of a monolayer of anterior epithelial cells overlaying a core of terminally differentiated and elongated fiber cells. At the equatorial region of the lens, fiber cells are formed continuously by differentiation of epithelial cells as they exit the cell cycle, increase in length, and synthesize large amounts of lens-specific proteins called crystallins.^{1–3} The α -crystallins comprise the most abundant class, contributing approximately 35% of the total soluble protein in vertebrate lenses.⁴ Two major α -crystallin subunits, α A- and α B-crystallins, are expressed as ~ 20-kDa subunits, in roughly a 3:1 molar ratio in the human lens. Based on their primary sequences, α -crystallins belong to the family of small heat shock proteins (sHSP).

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Like most members of the sHSP family, α A and α B subunits noncovalently associate to form heterooligomeric complexes of approximately 550 kDa. In addition to its structural function in lens transparency, α -crystallin is also thought to function as a chaperone-like protein.^{5,6} As a lens chaperone, α -crystallin may suppress the aggregation and precipitation of other proteins, acting as an anticataract protein in the lens. α -Crystallin has also been shown to associate with both the cytoskeleton of the fiber cells and the plasma membrane, although no biological function has been directly demonstrated for either interaction.^{7–10}

Mutations in α -crystallin are associated with autosomal dominant cataract (ADC) in humans.^{11,12} Congenital cataracts in family members with R116C missense mutation have been described as zonular central nuclear opacities, with subsequent development of cortical and posterior subcapsular cataracts as adults in their third decade of life.¹¹ However, detailed slit lamp or morphologic characterization of lens defects in affected family members has not been reported.

We and others have shown that the R116C mutation is associated with a reduction in chaperone-like activity.^{13–15} Given that the R116C missense mutation is associated with a dominant cataract phenotype that cannot be explained solely by a reduction in chaperone-like activity, we hypothesized that the mutation induces a deleterious gain of function to α -crystallin that could affect one or more pathways leading to cataracts.¹⁶ To facilitate a test of this hypothesis, we created a series of transgenic mouse strains designed to express mutant and wild-type human α A-crystallin in fiber cells of the lens. Because the human R116C α A-crystallin mutation leads to an autosomal dominant phenotype, we hypothesized that expression of the mutant α A-crystallin subunit on the background of wild-type α A- and α B-crystallins in the mouse lens would lead to a mild phenotype amenable to morphologic and biochemical analysis. Our results demonstrate that expression of the R116C mutant subunit of α A-crystallin results in posterior cortical cataracts and abnormalities associated with the posterior suture. Surprisingly, similar phenotypes are observed in different lines of mice that differ by at least two orders of magnitude in the expression level of the mutant transgenic protein. These results suggest that low levels of R116C mutant α A-crystallin subunits are sufficient to induce lens opacities and sutural defects.

MATERIALS AND METHODS

Production of Transgenic Mice

To prepare the mutant R116C human α A-crystallin (CRYAA^{R116C}) transgene construct, sequences encoding amino-terminal histidine-tagged (His-tag) human α A-crystallin were PCR-amplified from an expression plasmid¹⁴ using gene-specific primers: 5'-GGATCCAAGAAGGAGATATACATGGGA-3' and 5'-GGATCCAGAAGGTGGAAAGGAAAA-3'. The resultant PCR amplicon was then subcloned into the *Bam*HI site of the promoter vector pIVS2, which contains the mouse 409 bp α A-crystallin promoter.¹⁷ The CRYAA^{WT} transgene construct was produced using site directed mutagenesis to accomplish the nucleotide change necessary to rescue the arginine-116 wild-type sequence. Coding sequences and promoter elements in both transgene constructs were confirmed by DNA sequencing. After release from vector sequences by digestion with *Sma*I and *Acc*I, transgene fragments were injected into fertilized B6CBA F₁ hybrid embryos (Jackson Laboratory, Bar Harbor, ME) essentially as described.¹⁸ Pups were screened for the presence of the transgene by a PCR-based genotyping assay. Independent lines were established from transgenic founders using C57BL/6 breeding stock (Jackson Laboratories, Bar Harbor, ME).

All procedures regarding animals in this study adhered to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research and were approved by the Washington University Institutional Review Board.

Reverse Transcriptase–Polymerase Chain Reaction

Transcriptional activity of transgenes was confirmed by reverse transcriptase-PCR (RT-PCR). A 253-bp fragment was amplified from oligo(dT)-primed cDNA using a pair of gene-specific primers designed to anneal to sequences associated with the His-tag epitope and nucleotides 206 to 225 in the α A crystallin coding sequence. Primer sequences were: 5'-AGGAGATATACATGGGACATCATCATC ATCATCATCA-3' (upstream) 5'-GAGGAAGATGACGAACTTGT-3' (downstream). For a size standard, control PCR reactions were performed using a human α A-crystallin cDNA as a DNA substrate.

Immunohistochemistry

Tissues collected from postnatal day 0 to 3 (P0–P3) mice were fixed overnight in 10% formalin/PBS for routine histology. For immunostaining, deparaffinized sections were treated with 3% hydrogen peroxide in methanol for 30 minutes to remove endogenous peroxidase. After blocking with 20% normal donkey serum for 30 minutes, slides were probed overnight at 4°C with primary antibody diluted into blocking solution. Primary antibody labeling was detected using a rabbit IgG ABC kit (Vectastain Elite; Vector Laboratories, Inc., Burlingame, CA) and 3,3'-diaminobenzidine as the peroxidase substrate. After color development, slides were counterstained with hematoxylin.

Immunoblot Analysis

Lens homogenates were prepared in buffer A (5 mM Tris, 1 mM EDTA, 5 mM β -mercaptoethanol; pH 8.0) and centrifuged at 22,000g for 30 minutes at 4°C to separate the water-soluble and water-insoluble phases. Proteins resolved by SDS-PAGE in 10% Bis-Tris gels (Invitrogen, Carlsbad, CA) were electroblotted to PVDF membrane (Hy-bond-P; GE Healthcare). Membrane blots were treated with anti-His-tag monoclonal antibody (Novagen, Madison, WI), by methods described previously.¹⁹ Immune complexes were detected with chemiluminescence (ECLplus; GE Healthcare) followed by scanning with a phosphorescence imager (Storm; Molecular Dynamics, Sunnyvale, CA).

For quantitative Western blot analysis, known quantities of purified recombinant human or mouse α A crystallins were analyzed on each gel/blot as standards. Each dilution series contained at least four quantities of recombinant protein standard. After electrophoresis and electroblot analysis, membranes were treated with primary and secondary antibody solutions. The primary antibody used for detection of α A-crystallin epitopes, which was generously provided by Usha Andley (Washington University, St. Louis), was used at a 1000-fold dilution. Chemiluminescence signal intensities were analyzed (ImageQuant; Molecular Dynamics). Quantities are reported as the mean $\pm$ SD obtained from at least three separate experiments.

Quantification of Lens Morphologic Defects

A scoring system for lens phenotypic changes was developed for quantitative assessment of the frequency and severity of morphologic defects. Phenotypes were measured in dissected lenses from three age groups: 9 to 11, 20 to 22, and 28 to 30 weeks. After removal through an incision in the posterior globe and measurement of wet weight, lenses were kept in warm physiological buffer for no more than 30 minutes during the course of phenotypic scoring. Lens images were then captured with a digital camera (Spot; Insight, Wembley, UK) attached to a dissection microscope (Stemi 2000; Carl Zeiss Meditec, Jena, Germany). In all cases,

phenotype scores were assigned in a range of 0 to 3, depending on the presence or severity of the defect. Scoring descriptors are given separately in the four tables in the Results section.

Statistical Analysis

Frequency tables were constructed comparing the distribution of severity of lens defects between transgenic and control mice for each level of gene expression. A χ^2 test was conducted with a significant statistic of $P < 0.05$ considered to indicate heterogeneity between the distributions. For the purposes of these analyses, the categories for grade II and III severity were collapsed due to the rarity of grade III defects within the control group. The influence of the level of gene expression on the severity of lens defects was tested in the transgenic group only, by using generalized linear modeling (GLM). A model was constructed to analyze the proportion of eyes that fell into each category of disease severity and included an interaction term for level of gene expression and severity of disease. We considered a significant ($P < 0.05$) interaction term to be evidence of an influence of the level of gene expression on severity of defect. The influence of gender and age on the frequency of lens defects was performed in a similar manner.

The subjective assessment of lens defects was conducted in an unmasked manner. We tested for bias in these assessments by randomly selecting 15 sets of eyes for assessment conducted in a masked manner. Agreement between these assessments and the original classification, which was tested with Spearman correlations and κ statistics, demonstrated a lack of bias in scores obtained by an unmasked observer.

All analyses were conducted on computer (SAS ver. 9.1; SAS Institute, Cary, NC).

RESULTS

Generation of Transgenic Mice Expressing Human α A Crystallin in Lens Fiber Cells

We developed a transgenic animal model to enable us to investigate potential mechanisms of autosomal dominant cataract formation associated with the R116C missense mutation in α A-crystallin. Transgene constructs were prepared by fusing the human α A-crystallin cDNA to a modified version of the 409-bp α A-crystallin gene promoter (Fig. 1A), which was shown to direct transgene expression predominantly to mouse lens fiber cells.¹⁷ To enable us to distinguish immunologically the human transgene product from high background levels of endogenous mouse α A-crystallin, we incorporated sequences to encode a seven-residue amino terminal poly-histidine epitope (His-tag). Our previous studies showed that addition of this affinity epitope did not measurably alter the functional properties of α A-crystallin.¹⁴ After preparation of the trans-gene fragment encoding the R116C-mutated human α A-crystallin (CRYAA^{R116C}), we used site-directed mutagenesis to change Cys-116 back to Arg-116 to create the wild-type version of our transgene construct (CRYAA^{WT}). Five independent founder transgenic mice were derived from the CRYAA^{R116C} transgene construct (Fig. 1B). These founders gave rise to CRYAA^{R116C} lines 8165, 8166, 8167, 8168, and 8170. In addition, two founder mice were produced from the CRYAA^{WT} transgene construct (Fig. 1B), giving rise to CRYAA^{WT} lines 10694 and 14934.

To verify expression of mRNA transcripts from integrated transgenes, we performed RT-PCR assays designed to distinguish α A mRNA transcripts derived from the transgene. Expression of the transgene was confirmed in all independent founder lines that were PCR-positive for the transgene. Control reactions not treated with reverse transcriptase were negative for the diagnostic RT-PCR product, indicating that the PCR signal did not originate from genomic DNA contamination of RNA extracts. Nontransgenic littermates were negative for the diagnostic RT-PCR product (Fig. 1C).

Lens expression of transgenic protein in founder lines was assessed by immunostaining using a monoclonal antibody to the His-tag epitope. As shown in Figure 2A, a band was observed at the expected size when lens extracts from CRYAA^{R116C} lines 8166, 8167, and 8170 as well as CRYAA^{WT} lines 10694 and 14934 were examined by Western blot. In contrast, we observed no detectable binding of this antibody to proteins extracted from lenses of nontransgenic littermates. No bands were observed in lanes containing lens extracts from CRYAA^{R116C} lines 8165 and 8168, although these lines were positive for transgene-derived RNA transcripts in the RT-PCR assay.

Consistent with the Western blot results, we observed substantial differences in the immunostaining intensity among transgenic lines when we used this antibody. Immunostaining results from lines 8165, 8168, and 8170 are shown in Figure 2B. Founder line 8170 demonstrated the most intense immunostaining, whereas immunostaining signals in founder lines 8165 and 8168 were very weak. Detection of immunostaining in founder line 8168 requires use of a fluorescently conjugated secondary antibody (data not shown). Strong immunostaining was also observed in CRYAA^{WT} line 10694. Quantitative Western blot analysis showed that the epitope-tagged human α A-crystallin comprised almost 9% of the total α A-crystallin (soluble fraction) in transgenic line 8170 ($254 \pm 32 \mu\text{g}$ transgenic α A out of $2.8 \pm 0.5 \text{ mg}$ total α A per lens). Similarly, wild-type human α A-crystallin made up approximately 4% of the total α A-crystallin pool in transgenic strain 10694 ($96 \pm 14 \mu\text{g}$ transgenic α A of $2.4 \pm 0.2 \text{ mg}$ total α A per lens). Transgene expression in lines 8165 and 8168 was too low for reliable measurement by Western blot analysis.

Because CRYAA^{R116C} lines 8166 and 8167 and CRYAA^{WT} line 14934 had relatively high expression levels, they were considered redundant to CRYAA^{R116C}-8170 and CRYAA^{WT}-10694, respectively, and were not extensively studied (Fig. 2A). Although transgene expression in CRYAA^{R116C} lines 8165 and 8168 was too low to quantify at the protein level (immunohistochemical staining or Western blot analysis), they were included for further study due to their inherently interesting phenotypes. Thus, three independent lines of CRYAA^{R116C} transgenic mice (8165, 8168, and 8170) and one line of CRYAA^{WT} transgenic mice (10694) were examined for the onset and progression of lens defects.

Lens Defects Associated with Expression of the R116C α A-Crystallin Transgene

A range of morphologic defects was observed in all three lines of CRYAA^{R116C} transgenic mice. Major abnormalities included posterior cortical cataracts and posterior suture defects. In contrast, CRYAA^{WT} transgenic animals were not significantly different from nontransgenic littermate controls with respect to these phenotypes.

To assess whether the onset or severity of lens abnormalities was influenced by age or transgene expression level, we used a numerical scoring system to measure phenotypes in animals stratified into three groups at the age of scoring: 9 to 11, 20 to 22, and 28 to 30 weeks. Roughly equal numbers of male and female mice were studied in each group.

Posterior Cortical Cataract—Cortical opacities were found with significantly greater frequency in CRYAA^{R116C} transgenic animals than in the nontransgenic littermate control ($P < 0.05$). In contrast, no differences in posterior cortical cataracts were found between transgenic mice expressing wild-type human α A-crystallin compared with the nontransgenic control ($P = 0.91$). The opacities tended to radiate outward from the posterior pole. The opaque areas covered variable amounts of the posterior aspect of the lens cortex. From a posterior perspective, the areas of opacity were highly variable and tended not to take on repetitive or patterned shapes (Figs. 3A–C). When the samples were viewed under dark-field illumination, a smooth transition was observed across the border between opaque and transparent regions.

Age-matched nontransgenic control lenses were routinely transparent and lacked the posterior opacities typical of CRYAA^{R116C} mice.

We quantified this phenotype by measuring the percentage of the posterior hemisphere covered by opacities (Table 1). Although frequently evident by 9 to 11 weeks, the area of opacity tended not to progress to involve larger portions of the lens with age (data not shown). In comparison with nontransgenic littermate controls, the frequency of cortical opacities was elevated in all three CRYAA^{R116C} transgenic lines ($P < 0.05$). We did not observe a statistically significant difference in the severity of cortical cataract in the high-expressing CRYAA^{R116C} transgenic line 8170 compared with the low-expressing CRYAA^{R116C} lines 8168 and 8165 ($P = 0.22$). No difference was observed in the appearance of background cortical opacities in lenses from CRYAA^{WT} transgenic and nontransgenic control mice, although the occurrence of opacities involving $\leq 20\%$ of the posterior lens surface was higher in this group than in nontransgenic littermates from the other three transgenic lines (Table 1).

When examined by bright-field microscopy, CRYAA^{R116C} superficial fiber cells that overlay areas of opacity appeared as enlarged or swollen fiber bundles that seemed to converge at the posterior suture (Figs. 3E, 3F). In contrast, lenses from nontransgenic littermates (not shown) or CRYAA^{WT} transgenic mice were smooth in appearance (Figs. 3G, 3H). As with posterior cortical opacities, the amount of superficial area with this swollen fiber appearance was significantly more extensive in lenses from CRYAA^{R116C} transgenic animals than in CRYAA^{WT} or nontransgenic control animals (Table 2). Areas of swollen fibers were more extensive in mice from the high expressing CRYAA^{R116C} line (8170) than in those from the low expressing lines 8165 and 8168 ($P = 0.03$; Table 2).

Suture Defects—Defects associated with the posterior sutures were a prominent morphologic abnormality observed in the CRYAA^{R116C} lenses. Large gaps were observed in the area where the posterior suture lines would normally be located (Figs. 4A–C). Sutural gaps were observed with significantly increased frequency in all three CRYAA^{R116C} transgenic lines compared with age-matched CRYAA^{WT} transgenic mice or nontransgenic littermates (Table 3). Associations between level of mutant transgene expression and severity of sutural defects approached significance ($P = 0.05$; Table 3).

In addition to sutural gaps, opacities frequently were found radiating outward from one or more branches of the posterior suture line (Fig. 4E). The posterior suture in the mouse lens normally forms as an inverted Y configuration, with no branches intersecting the three symmetrically arrayed suture lines. Posterior sutures in lenses from CRYAA^{R116C} transgenic animals frequently had irregular branching patterns (Figs. 4G, 4H). Sutures containing two or more branching lines were observed approximately four to six times more frequently in R116C transgenic lenses than in nontransgenic littermates (Table 4). Irregular branching patterns were observed at a higher frequency in the high expressing CRYAA^{R116C} transgenic line 8170 than from the low expressing lines 8165 and 8168 ($P = 0.03$; Table 4).

Cellular Defects—Abnormalities in lens fiber cell organization were noticeable in newborn (P1) lenses from CRYAA^{R116C} transgenic mice (Fig. 5). Marked fiber cell swelling and vacuole formation was observed in the posterior aspect of lenses from R116C transgenic lines 8170 and 8165, whereas these changes were less pronounced in transgenic line 8168 (Figs. 5B–D). Lenses from nontransgenic controls (Fig. 5A) or CRYAA^{WT} transgenic line 10694 (Fig. 5E) lacked these abnormalities. Posterior suture defects observed by bright-field microscopy in older lenses (10–30 weeks) could be observed as a cleavage in the tissue surface (Fig. 5F). Despite differences in fiber cell organization and structure, there was no measurable difference in lens growth, as the wet weights of age-matched lenses from transgenic mice were not significantly different from nontransgenic littermate control animals.

DISCUSSION

Mutations in α A- and α B-crystallins are associated with autosomal dominant cataracts in humans and animals. Previous studies from several laboratories showed that the R116C substitution has a relatively modest effect on the chaperone-like activity of α A-crystallin, resulting in an approximately two- to fourfold reduction, as measured by in vitro protein aggregation assays.^{13–15} Knockout mice null for α A-crystallin gene expression are cataract-free at birth and maintain clear lenses until approximately 7 weeks of age.²⁰ Therefore, it is unlikely that a reduction in the chaperone-like activity of α -crystallin can completely explain the congenital onset of cataract in humans with the R116C mutation.

We have demonstrated that the R116C mutant of α A-crystallin differed from the wild-type protein by having a dramatically increased (approximately 10-fold) membrane binding capacity.^{14,16} Because the amount of α -crystallin associated with lens membranes is known to increase with age and cataract,^{7,8} we hypothesized that accelerated membrane binding could be a deleterious gain of function associated with mutant R116C α A-crystallin subunits.^{14,16} Lenses from humans with the R116C mutation are not available to support a biochemical test of our hypothesis. Therefore, we designed a transgenic mouse model, using the rationale that the cataract mechanism could be studied in a transgenic model if the gain-of-function alteration in the R116C mutant protein were dominant with respect to the endogenous pool of mouse lens crystallins. Furthermore, we reasoned that the expression level of a transgenic protein would be far lower than in mice that were true heterozygotes for the R116C allele, anticipating that transgenic lenses would demonstrate a level of disease suitable for biochemical and morphologic study. Although our goal is to create a model system to study biochemical mechanisms of lens defects associated with the R116C mutation, we note that our transgenic approach does not fully recapitulate the status of genetic heterozygosity in affected patients. As an additional arm of our study, we produced transgenic mice to express the wild-type allele of human α A-crystallin. These control animals were included in our study, to examine whether phenotypes in the R116C transgenic animals result simply from ectopic overexpression of a human crystallin in the mouse lens.

Three independent lines of transgenic mice expressing R116C-mutated human α A-crystallin were studied. Two of these lines (8165 and 8168) expressed low levels of transgenic protein, whereas one line (8170) expressed abundant quantities. Quantitative Western blot analysis from whole lens homogenates demonstrated that the transgenic human α A-crystallin comprised approximately 9% of the total α A-crystallin pool in transgenic line 8170. Despite these marked differences in the transgene expression levels, we observed similar lens phenotypes in all three independent CRYAA^{R116C} founder lines. In contrast, no differences in the frequency or severity of lens defects were found between CRYAA^{WT} transgenic mice and their age-matched nontransgenic control animals. These results indicate that even low-level expression of the R116C mutant α A-crystallin transgene can have a profound impact on lens morphology and cataract. Because marginal or no enhancement of mutant phenotypes was observed in line 8170 (which expresses the highest level of transgenic protein) compared with lines 8165 and 8168 (which express low levels of transgenic protein), it appears that extremely small amounts of R116C mutant α A-crystallin subunits are sufficient to induce lens abnormalities. No evidence of truncated or incorrectly spliced RNA transcripts was observed when lens RNA extracts from these lines were examined by RT-PCR. In addition, no bands of unexpected size were observed on Western blot analysis of lens extracts from these lines, suggesting that the phenotypes do not arise from translation of an alternate coding sequence. Therefore, we consider it unlikely that the phenotypes in lines 8165 and 8168 could arise from expression of alternate translation products derived from the integrated transgene.

Although it is theoretically possible that a mutant phenotype can reflect a transgene integration effect, we consider it unlikely that the virtually identical phenotypes we observed in all three founder lines could have resulted from transgene integration independently into the same locus.

Although significant differences were observed in the frequency of lens abnormalities between *CRYAA*^{R116C} transgenic mice and their nontransgenic controls, we were surprised at the relatively high number of defects in nontransgenic wild-type mice. Lens defects are known to occur in normal laboratory strains of mice such as C57BL/6, but the frequency of spontaneous cataract, detectable by slit lamp ophthalmoscopy, is reported to be very low until animals are at least 6 months of age.^{21,22} In the present study, most of the nontransgenic wild-type lenses observed with morphologic abnormalities or opacities in the dissected lens, as well as control *CRYAA*^{WT} transgenic animals, were assigned into the least severe of three categories for the corresponding phenotype. Therefore, it is possible that most of the defects we quantified in wild-type lenses would have escaped detection by routine slit lamp microscopy in the living animal. To our knowledge, a detailed study of lens defects in the C57BL/6 inbred strain at ages out to 30 weeks has not been performed on freshly dissected lenses. Therefore, it is not possible to calibrate our findings on dissected lenses against a comparable data set obtained using slit lamp ophthalmoscopy. The relatively higher frequency of minor opacities in transgenic and nontransgenic littermates in the *CRYAA*^{WT} line is puzzling, as this line was established using the same source of *B6CBA F₁* hybrid embryos and the same breeding program as for the other transgenic lines. Because all eye dissections in this study were performed by the same investigator using a standardized procedure, we consider it unlikely that the higher frequency of minor opacities in this line could have resulted from dissection artifacts. Because no difference was observed in the abundance of these minor opacities in *CRYAA*^{WT} transgenics and their nontransgenic littermates, it is unlikely that expression of the transgene contributed to the high background. Further study will be necessary to uncover the factor(s) that contribute to the relatively higher frequency of minor opacities in this particular transgenic line. These minor defects are quite distinct from microphthalmia and associated lens abnormalities noted to occur with increased frequency in inbred and congenic strains of *C57BL* mice.²¹

Cataracts in human carriers of the R116C allele have been characterized as zonular central nuclear opacities in newborns and cortical and posterior subcapsular in adults.¹¹ It is possible that the cortical and posterior subcapsular defects we observe in our *CRYAA*^{R116C} transgenic mice mirror those described in humans. However, we did not see a high frequency of central nuclear opacities in the transgenic mice at any age. In addition, we did not observe age-related changes in the regions of the lens affected by cataract in our transgenic model. A possible limitation of our transgenic approach is that the R116C mutation may have a different level of penetrance in true heterozygotes compared with transgenic carriers of the mutant allele. It is intuitively attractive to assume that equal quantities of wild-type and R116C mutant α A crystallin subunits are expressed in heterozygotes, and that a relatively high ratio of mutant to wild-type α A crystallin subunits would result in a more severe cataract phenotype. However, this notion has yet to be tested.

In the process of differentiation, fiber cells elongate and migrate bidirectionally until their tips reach a point where they encounter fiber cells from the opposite hemisphere of the lens. When elongation is completed, fiber cell tips detach from the epithelium or capsule²³ and overlap with tips of opposing fiber cells.²⁴ The overlap of tips from opposing cells forms a seam referred to as a suture line.²⁴ During differentiation, all fiber ends must reach a precise migratory destination for the purpose of forming and maintaining a particular suture pattern. Improper or disorganized fiber end migration leads to the formation of irregular and/or excess suture branches, reduced lens optical quality,^{25–27} and cataract.^{26,28}

During elongation, fiber cells maintain contact with the epithelial layer and lens capsule, respectively, through interactions involving the tips of the fiber cells.^{29,30} Although the mechanisms controlling the rate of migration along these substrates is not understood, recent evidence suggests that complexes involving actin bundles, integrins, and N-cadherins facilitate interactions with the capsule.³⁰ In most vertebrate lenses, distinct patterns of fiber cell curvature give rise to a Y suture at the anterior aspect and an inverted Y suture at the posterior. Sutural defects may occur when the elongation, migration, and detachment of fiber ends is disrupted.³¹

Most of the phenotypic abnormalities observed in the R116C transgenic animals are localized to the posterior suture. Others have shown that sutural defects are commonly associated with specific types of cataract, including posterior sub-capsular cataracts in the RCS rat³⁰ and Δ FosB transgenic mouse strain.³² In humans, sutural defects have been associated with both autosomal dominant^{33,34} and X-linked³⁵ gene defects. Recently, sutural cataracts have been described in three separate families that carry mutations in *BFSP2*, the gene encoding beaded filament structural protein 2.^{33,36,37} BFSP2 combines with BFSP1 (filensin) to form beaded filaments, a lens-specific intermediate filament.^{38,39} These proteins assemble into a filamentous structure during lens fiber cell elongation and differentiation.⁴⁰ Although α -crystallin binding can influence assembly of intermediate filament from purified BFSP1 and BFSP2 subunits in vitro,⁴¹ it is presently unknown whether the binding event affects intermediate filament function in vivo.

Given that α -crystallin is known to bind intermediate filaments and mutations in beaded filament proteins are associated with dominantly inherited sutural cataracts, it is intriguing to consider whether the sutural defects we observe in our mutant α -crystallin transgenic mice result from some functional alteration of intermediate filaments during lens development and fiber cell differentiation. Further study is needed to determine whether interactions between intermediate filaments and α -crystallin complexes containing mutant R116C subunits are functionally different from wild-type α -crystallin.

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Figure 1.

α A-crystallin transgene structure and function. (A) α A-crystallin transgene for microinjection, including the mouse α A-crystallin 409-bp promoter, intervening sequence (IVS 2), seven-residue poly-histidine epitope (7X-HIS), CRYAA coding sequences, 3'-untranslated sequences, and SV40 fragment containing the polyadenylation signal. (B) Detection of the transgene by PCR genotyping. Results from five CRYAA^{R116C} lines (8165, 8166, 8167, 8168, and 8170) and two CRYAA^{WT} lines (10694 and 14934) are shown. Human α A-crystallin cDNA carried in a plasmid (CRYAA) and nontransgenic littermate DNA (Tg-) provided positive and negative controls, respectively. (C) RT-PCR analysis of gene expression in transgenic mice. Results are shown for three CRYAA^{R116C} lines (8165, 8168, and 8170) and

one CRYAA^{WT} line (10694). Reverse transcriptase (RT) treatment is indicated for each sample. Human α A-crystallin cDNA carried in a plasmid (CRYAA) provided a control PCR template. The presence of the DNA template in this control reaction is indicated. (**B**, **C**, *arrows*) bands that are diagnostic of the α A transgene or its transcript, respectively.

Figure 2.

Immunostaining for transgene expression. (A) Western blot analysis. Recombinant His-tagged human α A (CRYAA) and soluble fractions of lens homogenates from CRYAA^{R116C} and CRYAA^{WT} transgenic mice were subjected to immunoblot analysis with monoclonal antibody to the His-tag epitope. Lens homogenate from a nontransgenic littermate (Tg-) was included as a negative control. Homogenate samples contained approximately 20 μ g protein. (B) Immunostaining of eye sections from CRYAA^{R116C} transgenic lines (8165, 8168, and 8170), CRYAA^{WT} transgenic line (10694) and nontransgenic control (Tg-). Whole globes from newborn mice were fixed and sectioned by standard techniques. After immunostaining, sections were counterstained with hematoxylin. Scale bar, 100 μ m.

Figure 3.

Morphologic defects in transgenic lenses. Posterior-view images of lenses taken with dark-field (A–E, G) and bright-field illumination (F, H), from transgenic CRYAA^{R116C} (A–C, E, F), transgenic CRYAA^{WT} (G, H), and nontransgenic (D) mice at the age of 10 (A, D) and 28 (E–H) weeks. At 10 weeks, lenses from CRYAA^{R116C} transgenic lines 8168 (A), 8165 (B), and 8170 (C) demonstrated posterior cortical opacities. In contrast, no opacities were observed in age-matched nontransgenic control lenses (D). A representative lens from a 28-week old CRYAA^{R116C}8170 mouse (E) showed posterior cortical cataract that corresponded in pattern and geographical location to an array of swollen fibers at the superficial layer of the lens (F).

Age-matched CRYAA^{WT} lenses (**G**), which appeared transparent by dark-field illumination, had a smooth posterior lens surface (**H**). Scale bar: 100 μ m.

Figure 4.

Posterior suture defects in CRYAA^{R116C} transgenic lenses. Prominent gaps at the posterior suture are observed in all three CRYAA^{R116C} founder lines (A–C), whereas age-matched nontransgenic lenses had normal posterior sutures (D). Posterior sutural cataracts as well as posterior subcapsular opacities that converge at the posterior sutures are frequently observed in CRYAA^{R116C} lenses (E). Other suture-related defects included highly branched patterns and deviation from the normal inverted-Y suture shape (G, H). Age-matched non-transgenic control lenses (F) infrequently showed these types of sutural abnormalities. Scale bar, 100 μ m.

Figure 5.

Fiber cell swelling and vacuoles in CRYAA^{R116C} transgenic lenses. (A–E) Whole globes were paraformaldehyde fixed and sectioned for hematoxylin-eosin staining by standard techniques. Lens collected at P0 to P1 from CRYAA^{R116C} strains 8165, 8168, and 8170 are shown along with CRYAA^{WT} strain 10694 and a nontransgenic littermate (Tg–). Arrows: regions showing fiber cell swelling and vacuole formation. (F) Histologic section of lens from CRYAA^{R116C} strain 8170 collected at P51 and stained with hematoxylineosin. Arrow: margin of a posterior sutural gap. Scale bar, 100 μ m.

Table 1
Cortical Cataracts in Transgenic Mice Compared with Age-Matched Nontransgenic Control Mice

Transgenic Line	Genotype	Lenses (<i>n</i>)	Grade of Opacity			
			Grade 0	Grade I	Grade II	Grade III
Line 8168 (R116C)	Tg [*]	54	20 (37)	21 (39)	11 (20)	2 (4)
	Control	50	37 (74)	9 (18)	4 (8)	0 (0)
Line 8165 (R116C)	Tg [*]	74	29 (39)	24 (32)	14 (19)	7 (10)
	Control	46	32 (69)	10 (22)	4 (9)	0 (0)
Line 8170 (R116C)	Tg [*]	72	17 (23)	26 (36)	20 (28)	9 (13)
	Control	62	34 (55)	18 (29)	10 (16)	0 (0)
Line 10694 (wild type)	Tg	62	18 (29)	38 (61)	4 (7)	2 (3)
	Control	70	21 (30)	42 (60)	7 (10)	0 (0)

Lenses were examined ex vivo by stereomicroscopy under dark-field illumination. Cataracts were graded according to the percentage of posterior lens surface covered by opacity. Grades were 0, no opacity; I, < 20% coverage; II, 20%–50% coverage; III, > 50% coverage. Tg, transgenic animals; controls were age-matched nontransgenic littermates. Opacity scores were not significantly different among lenses examined at the ages of 9 to 11 weeks, 20 to 22 weeks, and 28 to 30 weeks. Therefore, data from all age groups were pooled. Data are number of lenses, with percentage of total group in parentheses. For the purpose of statistical analyses, grades II and III are combined. Test results for interaction between level of gene expression and severity of cortical cataract (transgenic group only) were not significant ($P = 0.22$).

* Distribution of severity of lens defect differed between transgenics and control mice ($P < 0.05$).

Table 2

Abundance of Swollen Cortical Fibers in Transgenic Mice Compared with Age-Matched Nontransgenic Control Mice

Transgenic Line	Genotype	Lenses (<i>n</i>)	Grade of Opacity			
			Grade 0	Grade I	Grade II	Grade III
Line 8168 (R116C)	Tg	54	11 (20)	18 (33)	17 (32)	8 (15)
	Control	50	16 (32)	19 (38)	13 (26)	2 (4)
Line 8165 (R116C)	Tg*	74	7 (9)	23 (31)	31 (42)	13 (18)
	Control	46	20 (44)	16 (35)	8 (17)	2 (4)
Line 8170 (R116C)	Tg*	72	4 (5)	13 (18)	38 (53)	17 (24)
	Control	62	6 (10)	28 (45)	28 (45)	0 (0)
Line 10694 (wild type)	Tg	62	7 (11)	30 (48)	22 (36)	3 (5)
	Control	70	14 (20)	31 (44)	22 (32)	3 (4)

Lenses were examined ex vivo by stereomicroscopy under bright-field illumination. Swollen fiber phenotypes were graded according to the percentage of posterior lens surface covered by swollen fibers. Grades were 0, no swollen fibers; I, < 20% coverage; II, 20%–50% coverage; III, > 50% coverage. Tg, transgenic animals; controls were age-matched nontransgenic littermates. Scores were not significantly different among lenses examined at the ages of 9 to 11 weeks, 20 to 22 weeks, and 28 to 30 weeks. Therefore, data from all age groups were pooled. Data are number of lenses, with percentage of total group in parentheses. For the purpose of statistical analyses, grades II and III are combined. Test results for interaction between level of gene expression and severity of swollen cortical fibers (transgenic group only) were significant ($P = 0.03$).

* Distribution of severity of lens defect differed between transgenic and control mice ($P < 0.05$).

Table 3
Suture Defects in Transgenic Mice Compared with Age-Matched Nontransgenic Control Mice

Transgenic Line	Genotype	Lenses (<i>n</i>)	Grade of Sutural Defect			
			Grade 0	Grade I	Grade II	Grade III
Line 8168 (R116C)	Tg [*]	54	12 (22)	15 (28)	17 (31)	10 (19)
	Control	50	29 (58)	16 (32)	5 (10)	0 (0)
Line 8165 (R116C)	Tg [*]	74	12 (16)	24 (32)	25 (34)	13 (18)
	Control	46	35 (76)	7 (15)	3 (7)	1 (2)
Line 8170 (R116C)	Tg [*]	72	25 (34)	7 (10)	31 (43)	9 (13)
	Control	62	41 (66)	12 (19)	9 (15)	0 (0)
Line 10694 (wild type)	Tg	62	52 (84)	6 (10)	2 (3)	2 (3)
	Control	70	53 (76)	11 (16)	3 (4)	3 (4)

Lenses were examined ex vivo by stereomicroscopy under dark-field illumination. Sutural defects were graded according to the maximum width of a gap at the posterior suture. Grades were 0, no gap; I, gap width is < 15 μ m; II, gap width is 16 to 30 μ m; III, gap width is > 30 μ m. Tg, transgenic animals; controls were age-matched nontransgenic littermates. Scores were not significantly different among lenses examined at the ages of 9 to 11 weeks, 20 to 22 weeks, and 28 to 30 weeks. Therefore, data from all age groups were pooled. Data are number of lenses, with percentage of total group in parentheses. For the purpose of statistical analyses, grades II and III are combined. Test results for interaction between level of gene expression and severity of suture defects (transgenic group only) were not significant ($P = 0.05$).

* Distribution of severity of lens defect differed between transgenic and control mice ($P < 0.05$).

Table 4
Sutural Branching in Transgenic Mice Compared with Age-Matched Nontransgenic Control Mice

Transgenic Line	Genotype	Lenses (<i>n</i>)	Grade of Sutural Branching Defect			
			Grade 0	Grade I	Grade II	Grade III
Line 8168 (R116C)	Tg [*]	54	9 (17)	25 (46)	19 (35)	1 (2)
	Control	50	20 (40)	25 (50)	5 (10)	0 (0)
Line 8165 (R116C)	Tg [*]	74	19 (25)	28 (38)	22 (30)	5 (7)
	Control	46	24 (52)	12 (26)	10 (22)	0 (0)
Line 8170 (R116C)	Tg [*]	72	18 (25)	26 (36)	21 (29)	7 (10)
	Control	62	32 (52)	26 (42)	4 (6)	0 (0)
Line 10694 (wild type)	Tg	62	34 (55)	23 (37)	5 (8)	0 (0)
	Control	70	29 (41)	34 (49)	6 (9)	1 (1)

Lenses were examined ex vivo by stereomicroscopy under bright-field illumination. Sutural branching defects were graded according to the number of branch lines extending from the normal inverted Y posterior suture. Grades were 0, no branch lines; I, 1 branch line; II, 2 branch lines; III, > 2 branch lines. Tg, transgenic animals; controls were age-matched nontransgenic littermates. Scores were not significantly different among lenses examined at the ages of 9 to 11 weeks, 20 to 22 weeks, and 28 to 30 weeks. Therefore, data from all age groups were pooled. Data are number of lenses, with percentage of total group in parentheses. For the purpose of statistical analyses, grade II and III are combined. Test results for interaction between level of gene expression and severity of suture branching (transgenic group only) were significant ($P = 0.03$).

* Distribution of severity of lens defect differs between transgenic and control mice ($P < 0.05$).