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Exaggerated Eye Growth in IRBP-Deficient Mice in Early Development

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PURPOSE. Because interphotoreceptor retinoid-binding protein (IRBP) is expressed before being needed in its presumptive role in the visual cycle, we tested whether it controls eye growth during development.

METHODS. The eyes of congenic IRBP knockout (KO) and C57BL/6J wild-type (WT) mice ranging in age from postnatal day (P)2 to P440 were compared by histology, laser micrometry, cycloplegic photorefractions, and partial coherence interferometry.

RESULTS. The size and weight of IRBP KO mouse eyes were greater than those of the WT mouse, even before eye-opening. Excessive ocular enlargement started between P7 and P10, with KO retinal arc lengths becoming greater compared with WT from P10 through P30 (18%; P < 0.01). The outer nuclear layer (ONL) of KO retinas became 20% thinner between P12 to P25, and progressed to 38% thinner at P30. At P30, there were 30% fewer cones per vertical section in KO than in WT retinas. Bromodeoxyuridine (BrdU) labeling indicated the same number of retinal cells were born in KO and WT mice. A spike in apoptosis was observed in KO outer nuclear layer at P25. These changes in size were accompanied by a large decrease in hyperopic refractive error, which reached −4.56 ± 0.70 diopters (D) versus −9.98 ± 0.95 D (mean ± SD) in WT, by postnatal day 60 (P60).

CONCLUSIONS. In addition to its role in the visual cycle, IRBP is needed for normal eye development. How IRBP mediates ocular development is unknown. (Invest Ophthalmol Vis Sci. 2011;52:5804–5811) DOI:10.1167/iovs.10-7129
apoptosis that starts at P23 and peaks at P25. We conclude that IRBP has a role in controlling eye growth in development and a subsequent role in maintenance of retinal health.

**METHODS**

**Mouse Strains**

KO mice used in these experiments (originally created in 129/Ola embryonic stem cells) were backcrossed against C57Bl/6j for 10 generations. C57Bl/6j mice were used as wild-type (WT) controls. Food and water were provided ad libitum, with 12:12 light-dark cycling. Genotypes were verified with specific primers in all breeds, both KO and WT. The ERG phenotype in the KO was reduced by 40% of the WT a-wave magnitude at P30 as previously shown validating use of the current KO mice in this study. We adhered to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. The Emory IACUC approved these studies.

**Histology and Morphometrics**

We followed the recommendations of Howland and Howland for nomenclature of axes and planes in the mouse eye, as indicated in Figure 1. Histologic and morphometric procedures followed standard techniques. Sections were cut on a vertical (sagittal) plane through the optic nerve head (ONH) and the center of the cornea (Fig. 1). Paraaffin sections were used for bromodeoxyuridine (BrdU), TUNEL, cone-arrestin staining, and hematoxylin and eosin (H&E) staining. BrdU labeling (BrdU Labeling Reagent, Invitrogen, Carlsbad, CA) and TUNEL staining (DeadEnd Fluorometric TUNEL System, Promega, Madison, WI) followed the manufacturer’s instructions. Cone cell counting used an antibody specific to mouse cone arrestin (mCar/LUMJ). Other eyes were embedded in epon 802. Sections were cut at 1-μm thickness and stained with toluidine blue. Plastic sections were used for counting the number of nuclei in nuclear layers, measuring retinal arc length along a vertical great meridian (Fig. 1), measuring layer thicknesses (Longboard V7.5; ImagingPlanet, Goleta, CA). “ONL nuclei counts” are defined here as the number of nuclei on a perpendicular line crossing the thickness of the ONL. In this study, layer thicknesses and ONL nuclei counts were made at 250-μm steps from the optic nerve head to the far periphery in both inferior and superior halves of the retina. These increasingly eccentric measurements were made until there was no more retina to count. In counting other layers (inner nuclear layer [INL], ganglion cell layer [GCL], etc.) and cones, which had rarer numbers of objects, at each 250-μm step, a 100-μm wide column (also known as box or bin) running along the retinal arc was counted. All these measurements were averaged per section from each eye.

**Refractive Power**

Unless stated otherwise, readings were taken on both eyes of each mouse and then averaged to generate a single measurement per mouse. Eyes were refracted with an infrared photorefractor modified for mouse eyes. Pupils were dilated with 1% (wt/vol) ophthalmic tropicamide in both eyes. Measurements were only taken when the pupil was larger than 2.0 mm. Three to six measurements per eye per mouse were recorded. Once the awake measurements were conducted, the mouse was anesthetized with ketamine and xylazine (80 mg and 16 mg per kg body weight) and the refractions were repeated in each eye. Longitudinal assessments of KO and WT were made at P30, P45, and P60.

We assessed axial length by partial coherence interferometry (PCI) and noncontact laser micrometry (LM) at P30, P45, and P60. The PCI was a custom-built system modified for the mouse eye and enabled in vivo measurements of anterior-posterior (A-P) axial length from the surface of the cornea to the RPE/choroidal peak. Ex vivo, laser micrometry was used to measure A-P, superior-inferior (S-I), and nasal-temporal (N-T) external eye dimensions, as previously described.

**Statistical Analyses**

All data are reported as mean and standard deviation (SD). Error bars in figures represent SD. Comparisons are made between two groups, KO and age-matched WT mice by two-tail, two-sample equal variance Student’s t-test (Excel; Microsoft, Redmond, WA). The number of mice in each group is presented in the Results section, table, or figure legends. A single asterisk represents significant differences at $P < 0.05$, and double asterisks denote significant differences at $P < 0.01$ throughout.

**RESULTS**

**Photorefraction and Ocular Growth Measures**

In a longitudinal study, photorefraction and axial length using partial coherence interferometry (PCI) was measured in the same mice at P30, P45, and P60 (Fig. 2). The eyes grew axially with corresponding changes in refractive power from P30 to P60 in both WT and KO mice. A-P length was significantly greater in the KO mouse at every age (e.g., 5.498 ± 0.051 mm vs. 5.286 ± 0.0506 mm at P60; Fig. 2), and KO mice were significantly less hyperopic at each age compared with WT mice (e.g., −4.56 ± 0.70 D vs. 9.98 ± 0.993 D at P60; Fig. 2). A-P, S-I, and N-T external dimensions (Fig. 1) of WT and KO mouse eyes were measured at P60 (Table 1). Each axis was longer in the KO mouse than the WT ($P < 0.05$, in each comparison), as measured by a noncontact laser micrometer. This indicated an enlarged globe in the KO mouse. Eye weights were consistent with eye size: the KO mouse eye was heavier by 28% ($P < 0.05$) than the WT. This was despite the same whole body weight in both groups (Table 1).

Given the severe myopic shift and the enlarged eye of the KO mouse, histologic cross-sections of KO mouse eyes should have a longer retinal arc length (Fig. 1) than WT. A representative histologic image of one half of a cross-sectional retinal arc from a P30 WT mouse is shown in Figure 3, along with a corresponding retinal arc from a P30 KO. From the ONH to the ora serrata, the arc length was 15% longer in KO mice compared with WT, as predicted. Further data corroborated this...
observation: the retina arc lengths from numerous litters during development are summarized in Figure 4. Mice from independent litters in each group (KO and WT) were measured at each age. Up to P7, arc lengths did not vary between KO and WT. However, from P10 on, the retina arc length averaged 18% larger in the KO compared with age-matched WT (Fig. 4). In the mouse, there is no space between the outermost tip of the retina and the ciliary body in either the WT or the KO. Any variation in size of the retina is reflected in a concomitant variation in size of the entire posterior segment. The KO retina and posterior segment are enlarged relative to WT. Retina arc length measurements in paraffin sections (data not shown) confirmed all the measurements in plastic sections.

Retinal Morphology Changes During Development

Retinal morphology was examined during development in KO and WT mice. At P10 and younger, the INL and ONL are not resolved and the elongated nuclear shape makes it difficult to count individual nuclei. However, it was possible to measure the thickness of retina layers (Fig. 5). The results showed little difference in the average thickness of the ONL of KO and age-matched WT from P2 to P10. At P12, the average ONL thickness of WT mice (n = 3) was 54.0 ± 3.29 μm while that of KO mice (n = 4) was 44.1 ± 2.44 μm (P < 0.03), an 18.3% difference. These data show that the initiation of retinal thinning in the ONL of the KO mouse started between P10 and P12. However, as described later, this thinning was not accompanied by apoptotic loss of nuclei.

At stages later than P10, nuclei were counted across the ONL of KO and WT mouse retinas. At P12 and P15, WT and KO mice had a comparable number of nuclei (Fig. 6). At P18, the number of nuclei per ONL was lower in KO by 23% compared with WT (P < 0.05; n = 3 mice for both strains), with similar differences persisting at P20 and P25 (Fig. 6).

Between P25 and P30, the number of nuclei per ONL decreased further (38% decrease) in the KO compared with WT (Fig. 6). From P30 through P440 there was a gradual decrease in the ONL nuclear count in KO mice, which resulted in an ONL only three nuclei thick (Fig. 7) while the WT remained stable at 8 to 9 nuclei throughout the study period. This decrease at older ages is likely due to apoptotic photoreceptor cell death reflecting retinal degeneration as discussed later.

TABLE 1. P60 Laser Micrometer External Axes Data, Mouse Whole Body, and Eye Weight

<table>
<thead>
<tr>
<th>Strain</th>
<th>N</th>
<th>Whole Body Weight (g)</th>
<th>Eye Weight (mg)</th>
<th>Axial (mm)</th>
<th>Nasal-Temporal (mm)</th>
<th>Superior-Inferior (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT</td>
<td>18</td>
<td>19.7 ± 3.8</td>
<td>16.5 ± 1.6</td>
<td>3.231 ± 0.069</td>
<td>3.185 ± 0.079</td>
<td>3.162 ± 0.092</td>
</tr>
<tr>
<td>KO</td>
<td>13</td>
<td>20.1 ± 2.0</td>
<td>21.3 ± 0.8</td>
<td>3.532 ± 0.042</td>
<td>3.425 ± 0.069</td>
<td>3.400 ± 0.071</td>
</tr>
</tbody>
</table>

The IRBP KO mouse eyes are heavier, larger, and longer axially by external measures, P < 0.01, compared with WT. Mouse body weights at P60 are all the same between WT and KO. Data are presented as mean ± SD.
independent hypotheses might account for this reduced cell density. First, fewer cells might be born in the ventricular zone, leading to thinner layers in the KO. Second, increased cell death would reduce layer thickness. We used BrdU birth dating to test the first possibility and TUNEL staining to test the second.

As shown in Figure 10, BrdU labeling and immunostaining demonstrated that there was little difference in the number of dividing cells between the KO and WT retinas from P2 to P10. At early stages (P2 and P4), there were numerous dividing cells in the ventricular zone of the retina, but at matched ages there was no difference between the KO and WT mouse. At P7 and P10 there were few dividing cells but again no difference between WT and KO.

To test whether apoptotic cell death accounted for an eventual difference in the number of photoreceptor cells between KO and WT, TUNEL-positive cells in sections from P2 to P30 retina were counted. As shown in Figure 11A, there were no significant differences in the numbers of TUNEL-positive cells between KO and WT at P2 and P4. Unexpectedly, at P7 and P10 there was a trend toward more apoptosis in the INL of the WT, and a significant increase in TUNEL-positive cells in the WT mouse over the KO at P12 as well (Fig. 11A). This timing coincides with the extension of retinal arc length in the KO. As shown in Figure 11B, in the ONL from P15 through P22 there was a low level of apoptosis and no difference between KO and WT. Conversely, starting at P23 to P30 there were far more apoptotic cells in KO ONL. In the KO at P25, there were five times as many apoptotic nuclei (15.1 ± 2.46 cells per retina, n = 5; than WT (2.81 ± 1.75; n = 5) (P < 0.00001).

** Figure 3. ** Early lengthening and thinning of the IRBP KO mouse retina. Retina arc length and morphology at P30. (A) IRBP knockout (KO). (B) C57BL/6j (WT). The neural retina of the KO mouse at P30 directly abuts the ciliary body. There was no differential thinning of the retina at the tip abutting the pars plana. The arc length of the neural retina between the ONL and inner plexiform layer (IPL) was measured on the superior half of the eye. The WT arc length was 2397 ± 9262 µm, and the KO arc length was 2752 ± 1547 µm, or 15% longer. This increased arc length in the KO mouse is representative of the data. The KO retina exhibited a 40% thinning of the ONL and evidence of rod outer segment (ROS) disorganization. ROS, rod inner segment (RIS), and INL exhibited approximately 20% thinning. Scale bar, 250 µm.

** Figure 4. ** Retina arc length in developing mice. The arc length of the neural retina was measured from one pars plana to the other through the ONH on the vertical meridian. The arc length was measured at the interface of the ONL and the inner plexiform layer (IPL). Measurements were made at the indicated postnatal ages ranging from P2 to P30. The results showed a substantially longer arc length in the KO mouse starting at P10, suggesting that the posterior segment of the KO mouse eye is larger than the WT eye. The number of mice per group varied, but was always three or more. At P2, n = 3, both KO and WT. At P4, n = 3 WT, and n = 4 KO. At P7, n = 4 both WT and KO. At P10, n = 4 both WT and KO. At P12, n = 3 WT, and n = 4 KO. At P15, n = 3 WT, and n = 4 KO. At P20, n = 5 WT, and n = 4 KO. At P25, n = 4 WT, and n = 5 KO. At P30, n = 3 for both WT and KO. **P < 0.01.

** Figure 5. ** Very early postnatal ONL thicknesses are normal in IRBP KO mouse. Thickness measurements were made at increasing eccentricities of 250-µm increments from the ONH to the edges of the retina. These were averaged across the retina surface. The number of mice per group varied, but was always three or more. For all WT groups the sample size was three mice per group. For the KO groups, the sample size was four mice per group except P7, which had a sample size of three mice. Error bars represent standard deviations. ONL thicknesses were almost identical comparing WT to KO from P2 through P10.
than in the WT mouse. ** 4 WT, n = 5 KO). From P18 on, there were fewer nuclei across the ONL of the KO mice. These were averaged across the retina surface. Three mice from independent litters were measured at each age in each group (KO and WT). From P18 on, there were fewer nuclei across the ONL of the KO than in the WT mouse. **P < 0.01.

In summary, there was more apoptosis at P7–P12 in the WT in the INL. This reflects normal pruning in the WT mouse eye and appears to not occur in the KO. About two weeks later, there was a burst of apoptosis peaking at P25 in the ONL of the KO, coinciding with substantial degeneration of the ONL in the KO.

**DISCUSSION**

Our data suggest that IRBP is required in development for proper eye growth and required subsequently for retinal health. The absence of IRBP results in an extraordinary myopic shift that is consistent with developmental abnormalities in eye size and shape (Fig. 2, Table 1). There is a simultaneous axial elongation. Elongation may not be the sole cause of the differences in refraction, but we noted no differences in lens thickness or diameter or weight comparing KO to WT (data not shown). We did not measure surface curvatures or anterior chamber depth. The origin of excess axial length that contributed to the myopic shift is observed early in development. Comparing WT and KO, the critical change in eye growth occurred in a distinct and short time period in early postnatal development. Until P7, eye growth was the same in the WT and KO. After P7 and before P10, the KO mouse eye enlarged significantly more than in the WT (Figs. 4, 6, and 10). This time period is coincident with insufficient pruning of the INL in the KO mouse (Fig. 11) but is well before photoreceptor degeneration in the KO strain, which we now show here to begin shortly after weaning at P23 and to peak at P25 (Fig. 11B).

The IRBP KO mouse may help us to learn of relationships between prevision and later vision-dependent eye development. There is great interest in how vision-dependent adaptive processes and vision-independent genetically-determined eye growth might interact. If or how these two processes interact are open questions. It remains unclear how the myopic shifts (measured at P30–P60) and globe enlargement (starting at P10) in the IRBP KO mouse are caused. But because IRBP deficits affect both emmetropia and early eye size, it is possible that further study of IRBP might reflect on putative interactions of vision-dependent and vision-independent eye growth. Future work is needed to test biochemical pathways affected by the absence of IRBP.
Spreads to cover the enlarged globe in form deprivation. Also, in tree shrew the retina thins and ble, the ONL counts (which are not subject to the same sis in the KO ONL at these ages (P7–P18). Though artifactual both accounted for by spreading, with no evidence of apopto- eye grows. The KO ONL was thinner by mensuration at P12, the KO retina maintains a 20% longer arc length, as the mouse approximately 20% in a burst between P7 and P10 (Fig. 4). Thereafter, approaches. The KO retina elongated on a vertical arc by approx- were demonstrated by histologic and morphometric ap- It is hypothetically possible that signaling molecules are shared between the two processes for vision-dependent and vision-independent eye growth. For example, it is possible that local intraocular “growth control affectors” (the neurotransmit- ter dopamine and the morphogen retinoic acid [RA] are exem- plars) that originate within the local retina must pass through the interphotoreceptor space (IPS; also known as the subreti- nal space) to reach the RPE and to send information to cho- roidal and scleral targets. 51 Within the IPS, IRBP constitutes the majority of soluble protein. Whether physiologically relevant or not, growth control signals from the retina must interact with or avoid IRBP if they transit the IPS. For instance, IRBP binds RA at high affinity, 2,52–55 which is known to regulate eye growth 56 and inhibit scleral proteoglycan synthesis. 57 Thus, IRBP may act as a gatekeeper for RA in eye growth. Another possibility is that IRBP may act by regulating cell fate in the inner retina. We found that the INL of the developing IRBP KO mouse retina undergoes reduced stage-specific apoptosis, suggesting that absence of IRBP prevents correct developmental pruning. Lack of IRBP could lead to an aberrant retinal cell population (with a cell body in the INL) that sends improper signals to growth targets. For instance, IRBP could affect the development of GABAergic amacrine or ON-bipolar cells 58 and affect intracellular transduction of regulatory stimu- li.

Spreading and thinning of the retina in young IRBP KO pups were demonstrated by histologic and morphometric ap- proaches. The KO retina elongated on a vertical arc by approx- imately 20% in a burst between P7 and P10 (Fig. 4). Thereafter, the KO retina maintains a 20% longer arc length, as the mouse eye grows. The KO ONL was thinner by mensuration at P12, and at P18, the KO ONL had noticeably fewer nuclei (Fig. 6), both accounted for by spreading, with no evidence of apopto- sis in the KO ONL at these ages (P7–P18). Though artifactual histologic and selective shrinkage of the KO retina is possible, the ONL counts (which are not subject to the same potential problem as thickness measurements) support ac- tual thinning. Also, in tree shrew the retina thins and spreads to cover the enlarged globe in form deprivation myopia, and histologic retinal layer thicknesses are the same as in the living retina measured by spectral domain optical coherence tomography. 59

A critical period in KO and WT eye development occurred at P23 to P25, when the ONL in the KO abruptly began to experience high levels of apoptotic cell death. TUNEL counts indicate that apoptotic cell death is the dominant process controlling the decrease in ONL thickness at P23 to P30 in the KO (Fig. 11B). After P30, a gradual but substantial loss of the ONL occurs over the remaining lifespan of the mouse. This burst of apoptosis at P23 to P25 explains the previously unknown temporal origins and mechanism of photoreceptor cell death in the retinal degeneration in the IRBP KO mouse.

At one month of age and older, one prior study showed a substantially thinner ONL in the KO mouse. 57 This phenotype has remained unchanged over the 10-plus years (and approxi-

**Figure 10.** Cell birth in the IRBP KO and the WT mouse are the same from P2 to P10. Bromodeoxyuridine (BrdU) was used to label cells actively undergoing scheduled DNA synthesis. BrdU-positive cells in the ventricular zone of the retina were counted in sections cut on the vertical meridian. Bins of 100 μm were counted every 250 μm, and averaged across the whole retina. One eye was analyzed from one animal, in each of 4 litters at each age, except at P4: three for WT, and at P10, three for KO. No significant difference was detected between WT and KO at any age. Images indicated active scheduled DNA syn- thesis and cell division at P2 and P4 in both WT and KO mouse retinas; however, there was no difference in the number of labeled nuclei between WT and KO mice. There was approximately 10% as much labeling at P7 than at either P2 or P4, and almost no labeling at P10 suggesting that cell division in the ventricular zone was complete by P10.

*Figure 11.** (A) Normal pruning in the INL of the IRBP KO mouse is diminished at P7 to P12. Programmed cell death in the WT and KO mouse retina was measured. TUNEL-positive cells were counted in the whole retina at the indicated postnatal ages. No significant differences were detected at P2 and P4. There was a trend of more TUNEL-positive cells at P7 and P10 in the WT, but the differences were not significant. There were more TUNEL-positive cells in WT at P12 than in the KO mouse (P 0.05). One eye was analyzed from each animal. Per group, the sample size was four mice at P2, P10, and P12. At P4 and P7, the sample size was five mice per group. The errors bars designate SD. A single asterisk denotes a significant difference at P 0.05. At P7 and P10, TUNEL-positive nuclei were located in the developing INL, in both WT and KO retinas, suggestive of normal pruning in the INL in WT. This normal pruning was reduced in the KO mouse. (B) Summary of TUNEL-positive cell counts in the ONL of P12 to P30 mice. There was a burst of apoptosis in the KO mouse retinas starting at P23 and continuing through P30. The peak was at P25, with roughly five times as many TUNEL-positive cells in the KO mouse retina as in the WT mouse ONL. The difference was significant at P 0.00001. Afterward, there continued to be more apoptotic cell death in the KO than the WT mouse ONL. n = 4 mice from independent litters at P12–P18 for both WT and KO. At P20, n = 3 for WT, and n = 4 for KO. At P22 and P23, n = 4 for WT and n = 5 for KO. At P25, n = 5 for both WT and KO. At P27, n = 4 for WT and n = 5 for KO. At P30, n = 6 for both WT and KO. *P 0.05, **P < 0.01.
mately 30 generations of breeding) since this mouse was first described. We observed a 38% reduction in ONL counts \((P < 0.01)\) at P30 (Fig. 6). Also, the INL, RIS, and ROS were thinner in the KO compared with WT, and the ROS were more sparse and disorganized (Figs. 3 and 9). The retinal degeneration continued to progress by slow loss of the ONL (Fig. 7) as is characteristic of many retinal degenerations and forms of RP.

Cone accounts for approximately 3% of the photoreceptors in the WT mouse.\(^{60,61}\) Cone counts in Figure 8 indicate a 30% decrease in cone density in vertical cross-sections of the KO mouse at P30, which is slightly less loss than in the ONL at the same age. Parker et al.\(^{23}\) find that cone cell densities in flat mounts of isolated retinas are the same in KO and C57BL/6 from one to nine months of age. This seems inconsistent with the present measurements of cone densities on a vertical meridian. It may be accounted for by known differences in preparation, fixation, and by use of antibodies against different cone-specific antigens. Also, the C57BL/6 mice at two different institutions (Emory versus Medical University of South Carolina) appear to originate from two different sources (c.f., Jackman C57BL/6) versus Harlan C57BL/6) with potential genetic drift. These differences remain to be resolved.

Many retinal cells pass through S-phase\(^ {33,34}\) at P2 and P4, and progressively fewer at P7 and P10. There was no difference in BrdU-labeled cell counts between age-matched KO and WT (Fig. 10), suggesting that differential cell division rates did not account for the reduced ONL thickness in the KO mouse. This suggests that the critical difference is the reduced amount of apoptotic pruning of the INL cells at P7 to P10 caused by the deficiency of IRBP in the KO (Fig. 10). Likely, this early apoptosis in WT represents normal retina remodeling in the INL that fails when IRBP is absent. This failure is associated with the large myopic shift and the increased globe size in the KO mouse.

**CONCLUSIONS AND FUTURE DIRECTIONS**

We ruled out the possibility that the ONL was simply born thin in the KO mouse: the ONL is born normal in thickness. Our data revealed two distinct disease processes in the IRBP KO mouse eye. First, starting between P7 and P10, a lengthening of the posterior segment concomitant with insufficient INL pruning results in substantial enlargement of the eye developmentally. This occurs before the eyelids open, suggesting a light-independent mechanism. Second, much later (P23–P25), sudden apoptotic cell death, peaking at P25, in the ONL of the KO mouse results in retinal degeneration, which continues to progress as the KO mouse ages. The temporal separation between the early aberrant posterior segment growth and later photoreceptor degeneration raises the possibility that these two disease processes are largely independent of each other, or require two different functions of IRBP. Alternatively, the later retinal degeneration may be a direct consequence of globe elongation. To test these hypotheses, conditional knockouts of IRBP, knockdowns, and other experiments are needed. These future studies will advance our understanding of the types and etiology of human eye disease caused by IRBP mutations.

Finally, knowledge of how a deficiency in IRBP causes the myopic shift would help in understanding the mechanisms and steps involved in normal eye growth. It is possible that IRBP is an essential intracellular gatekeeper in mechanisms controlling eye growth and emmetropia.

**References**
