A Population Study of Common Ocular Abnormalities in C57BL/6N rd8 Mice

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PURPOSE. The purpose of this study was to quantify the frequency and severity of ocular abnormalities affecting wild-type C57BL/6N mice, the most common strain used worldwide for the creation of single-gene knockouts.

METHODS. A total of 2773 animals (5546 eyes) were examined at one colony at UC Davis and in three more colonies at the Institut Clinique de la Souris in Strasbourg, France. Mice were examined at 15 to 16 weeks postnatal age by performing anterior segment biomicroscopy, posterior segment examination by indirect ophthalmoscopy, intraocular pressure measurement, and optical coherence tomography of anterior and posterior segment structures.

RESULTS. Common ocular findings in the C57BL/6N strain included corneal deposits (3%), increased optical density of the anterior lens capsule (67%), punctate nuclear cataracts (98%), vitreous crystalline deposits (61%), hyaloid vascular remnant (6%), and retinal dysplasia attributed to the rd8 mutation (58%). Interestingly, retinal dysplasia was more common in male mice in all four breeding colonies evaluated in this study. The thickness of ocular tissues and compartments were measured by spectral-domain optical coherence tomography, including the central cornea, anterior chamber, vitreous, and retinal layers. Intraocular pressure was measured by rebound tonometry.

CONCLUSIONS. Ocular abnormalities are common in anterior and posterior segments of the C57BL/6N mouse, the most common background on which single-gene knockout mice have been made. It is important that vision scientists understand the extent and variability of ocular findings associated with this particular genetic background of mice.

Keywords: knockout animals, mouse models, genetic diseases, morphometry, optical coherence tomography
in the inferior retina. The lesions appear as white to yellow
tistic but variably severe retinal dysplasia that is most prominent

C57BL/6N mice are homozygous for the rd8 mutation, an intimate knowledge of C57BL/6N background
ocular lesions to distinguish them appropriately. Additionally,
C57BL/6N mice are homozygous for the rd8 mutation in
C57BL/6N,4 Crumbs homolog 1 gene, a mouse ortholog of the Drosophila Crumbs gene.5 This mutation leads to a character-

C57BL/6N mice are obtained from various commercial
sources with longstanding established colonies, including the
Jackson Laboratory (Bar Harbor, ME, USA), Charles River
Laboratories (L’Ahresles, France), and Taconic Biosciences
(Silkeborg, Denmark). These founders were used to establish
local breeding colonies at each of the IMPC sites. Mutants and
wild-type littermates were maintained in restricted, specific-
pathogen-free vivaria on a 12:12-hour light-dark cycle. Animal
care and use was conducted under guidelines provided by the
eighth Revision of the Guide for the Care and Use of Laboratory
Animals (Protocol No. 19075), and all procedures were
performed according to the Association for Research in Vision
and Ophthalmology Statement for the Use of Animals
in Ophthalmic (i.e., pupillary light reflexes), adnexal
(i.e., the eyelids, third eyelid, conjunctiva), and anterior segment
structures (i.e., cornea, iris, anterior sclera, and anterior
chamber) were examined by slit lamp biomicroscopy at \( \times 16 \) magnification by using broad-beam illumination at the highest
intensity setting (Kowa SL-15; Kowa, Tokyo, Japan). Intraocu-
lar pressure (IOP) was measured by rebound tonometry
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were pharmacologically dilated with either atropine 1%
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Ocular Abnormalities in C57BL/6N Rd8 Mice

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C67-218-40) were approved by the French Local Ethics
Committee No. 17 and the French Ministry of Research (No.
16040511578546) and performed in compliance with the
European Community Regulation for Laboratory Animal Care
and Use (Directive 2010/63/UE). For the purpose of this study,
eye examination data were included only from wild-type
C57BL/6N rd8 control animals from the University of California
davis (UCD) cohort (total 1877 mice; 941 males, 956

females). In addition, 896 mice were imaged in France across
colonies. Advanced ocular imaging using optical coher-
ence tomography (OCT) was performed on 870 of these wild-
type animals (452 males, 418 females) at the Institut Clinique
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For all mice, an initial, general systemic phenotyping schedule
was initiated at \( \frac{4}{2} \) weeks of age. A more in-depth
systemic phenotyping schedule, including ocular examination,
was performed on mice aged 15 through 16 weeks, after which
mice were euthanized and tissues and blood were collected
for anatomic and clinical pathology.7 Rigorous standardized
operating protocols (SOPs) were harmonized across all IMPC
laboratories to ensure reproducible testing and data collect-

Ophthalmic Phenotyping

Ophthalmic phenotyping followed an SOP developed for
complete ophthalmic examinations of both eyes of each
individual mouse at 15 to 16 weeks of age. The eye exams
were performed on mixed, sex-balanced cohorts of knockouts
and wild-type animals, which consisted of seven male and
seven female homozygous knockout mice and two male and
two female wild-type mice as controls. Examiners were
masked to the genetic background of the mouse during
examination. At the University of California-Davis, examina-
tions were performed by veterinarians with advanced training
in ophthalmology (BAM, AC, SGE, BCL, LS) with specific
familiarity and knowledge of anticipated background lesions.

New phenotypes outside of the C57BL/6N genetic back-
ground and mice with ocular abnormalities acquired second-
arily to trauma and/or infection (e.g., corneal scars, periocular
fur or eyelash loss, traumatic cataract) were excluded from the
analysis presented here. All ocular findings were noted for
every animal regardless of genotype, and all findings outside of
an anticipated C57BL/6N background lesion were subse-
quent-
ly reviewed by board-certified veterinary ophthalmologists
(CJM, SMT). The mice were gently restrained and neuro-
ophthalmic (i.e., pupillary light reflexes), adnexal (i.e., the
eyelids, third eyelid, conjunctiva), and anterior segment
structures (i.e., cornea, iris, anterior sclera, and anterior
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Ocular Abnormalities in C57BL/6N rd8 Mice

Broomall, PA, USA) and a 60-diopter double aspheric handheld lens (Volk Optical, Inc., Mentor, OH, USA).

Ocular Imaging

A cocktail of either ketamine/midazolam (50–75/1–2 mg/kg), ketamine/xylazine (100/10 mg/kg), or ketamine/medetomidine (100/0.3 mg/kg) was administered intraperitoneally to induce anesthesia in all mice undergoing ocular imaging. A single drop of both tropicamide 1% and phenylephrine 2.5% (or atropine 1%) was used for dilation, and the ocular surface was lubricated with methylcellulose-containing artificial tears. Anterior segment images were obtained with a BQ900 slit lamp (Haag-Streit, Kôniz, Switzerland), while fundus photographs were acquired with either Micron III (Phoenix Research Laboratories, Pleasanton, CA, USA) or topological endoscopic fundal imaging (TEFI).9 OCT imaging was performed with an Envisu R2200 SD-OCT (spectral-domain OCT; Bioptigen-Leica, Wetzlar, Germany), after dilatation with a single drop of atropine 1% (Virbac, Carros, France). Thickness measurements of the retina (from the nerve fiber layer to the outer boundary of the retinal pigment epithelium [RPE]), its layers, and the vitreous chamber were performed at a distance of 0.5 mm from the optic nerve by using calipers in the InVivoVue 2.4 software (Bioptigen-Leica). Corneal thickness and cornea-lens distance (called hereafter anterior chamber depth) were measured in a similar way, along the eye axis. The presence of rd8 lesions was determined on the OCT en face view obtained from the volume intensity projection of the outer retina. The severity of retinal dysplasia was qualitatively graded as mild (few pinpoint lesions), moderate (denser/larger lesions), or severe (most of the ventral quadrant or greater being affected with lesions).

Histology

For selected mice, eyes were either removed from the head or were fixed in situ in preparation for histology. Tissues were immersion fixed in 10% neutral-buffered formalin. If fixed in situ, the formalin-fixed heads were decalcified in 15% formic acid for at least 24 hours, until sufficient decalcification was achieved. Parasagittal sections of eyes or coronal sections through the head including eyes were processed routinely for histopathology, embedded in paraffin, sectioned 4– to 5-μm thick, and stained with hematoxylin-eosin. The histopathology was evaluated by a board-certified veterinary anatomic pathologist (DMI).

Statistics

For comparisons of groups of mice regarding IOP, or presence of retinal dysplasia, a 2-tailed Student’s t-test was performed. A P value < 0.05 was considered statistically significant. The datasets generated and/or analyzed during the current study from UC Davis and France are available as Supplementary Material.

Results

Cornea

Corneal deposits were detected in 2.6% of wild-type C57BL/6N mice during slit lamp biomicroscopy. The deposits ranged in size from pinpoint to occupying up to 60% of the corneal surface (Fig. 1). The deposits were often well demarcated, horizontally ovoid, and formed by multifocal punctate to coalescing stromal crystalline white opacities. When examined by OCT the deposits appeared as a hyperreflective band in the corneal mid-stroma (Fig. 1E). No obvious histopathologic abnormalities were noted that would correlate with the corneal opacities, and no evidence of corneal mineralization was observed; however, possible corneal lipidosis was detected from larger, more rounded stromal clefts than what are normally described in mice.

Lens

Another commonly found lesion was a variable degree of anterior lens capsule translucency (Figs. 2A–C). The anterior lens capsule is normally more lucent than the cornea as viewed by slit-beam biomicroscopy (Fig. 2A). Decreased translucency of the anterior lens capsule ranged from equal optical density of the slit beam on the anterior lens capsule and corneal surface (Fig. 2B) to a markedly denser anterior lens capsule slit beam (Fig. 2C). We found that 33% of mice examined were normal (anterior lens capsule more lucent than cornea), and 67% had decreased anterior lens capsule translucency. Another background lesion found in the lens of C57BL/6N mice consisted of punctate nuclear cataracts (Figs. 2D, 2E), which were the most commonly seen background lesion with 98% of mice being affected. Histopathologically, the altered lens translucency was apparent as pale and flocculent anterior subcapsular lens fibers. Nuclear cataracts were described as disorganized, granular, and whorled nuclear lens fibers.

Vitreous

Vitreous opacities of two kinds are relatively common in C57BL/6N mice. Crystalline-like opacities within the anterior vitreous, sometimes in very close approximation to the posterior lens capsule (Fig. 3), were identified in 61% of eyes. Histologic sampling of these opacities was unable to be obtained. Vitreous pigmentation (Fig. 4) was detected commonly and was noted in two different forms. Axial pigment was noted infrequently (6%), either suspended within the vitreous or in association with the optic nerve or posterior lens capsule, findings characteristic of hyaloid vasculature remnants and/or persistent hyperplastic primary vitreous (Figs. 4A–C). These clinical findings were confirmed histologically. However, vitreous pigment most commonly appeared as small flecks located sporadically throughout the posterior vitreous near the retinal interface (Fig. 4D). These clinical findings were confirmed histologically (e.g., pigment flecks shown in Fig. 4D along with a persistent hyaloid vessel); however, the total number of animals possessing this distinct type of vitreal pigmentation was not documented.

Retina

C57BL/6N mice are nearly universally homozygous for the rd8 mutation in the Crb1 gene, which causes a variable amount of retinal dysplasia. These mice develop small multifocal to coalescing yellow flecks of varying degrees (e.g., mild [Figs. 5A, 5A’], moderate [Figs. 5B, 5B’], or severe [Figs. 5C, 5C’]) that are predominantly located in the inferior nasal fundus. Histologically, folds and rosettes were present, primarily affecting the photoreceptor layer (Fig. 5D), which are not seen in areas lacking dysplastic flecks (Fig. 5D’). Systematic analysis of the en face view of OCT data from 1792 eyes of C57BL/6N mice (summed from Taconic, Charles River-A, and Charles River-B colonies in France) revealed that 75.9% had no rd8 lesions in the area centered on the optic nerve. Among the 24.1% of animals that did have an rd8 phenotype on photos, these were divided into 21.0% mild, 1.3% moderate, and 1.7% severe rd8 phenotype as shown in Figure 5.
Interestingly, we observed a higher percentage of rd8 lesions in male animals (29.6%) than in female animals (17.8%) when combining all animals from all three colonies (Taconic, Charles River-A, and Charles River-B) in France. This pattern of male predominance was consistently observed across all three C57BL/6N colonies in France, though to varying degrees (Table). Interestingly, there was a significant difference between mice obtained from two different providers: only 15.0% of eyes of those obtained from Charles River were affected (157 of 1046), compared to 26.7% of eyes of those obtained from Taconic (274 of 746). The cause and mechanism of the sexual dimorphism seen in these animals are not known.

In contrast to the more limited view obtained during fundus photography and/or OCT, indirect ophthalmoscopy enables the examiner to evaluate the entire retinal landscape. When indirect ophthalmoscopy was used on 1877 mice (3754 eyes) from a fourth independent colony of C57BL/6N mice at UC Davis to determine the presence of rd8 retinal lesions, we found that 1850 eyes (49%) had some evidence of retinal dysplasia. Since some animals only had one affected eye at the time of ocular examination, the rate of affected animals was higher than the rate of affected eyes: overall, 1087 of 1877 animals (58%) had at least one eye with evidence of retinal dysplasia by indirect ophthalmoscopy. The degree of retinal dysplasia seen on indirect ophthalmoscopy was not graded in this fourth cohort. The increased rate of retinal dysplasia detected by indirect ophthalmoscopy, compared to the rate seen on fundus photos/OCT analysis, may be due to the more limited field of view obtained during imaging, which does not capture the peripheral retina. The pattern of increased penetrance of the retinal phenotype in males compared to females was confirmed in the UC Davis colony (Table). The prevalence of dysplasia in the UC Davis colony was 1097 of 1882 (58%) in male eyes, while 822 of 1872 (44%) female eyes were affected. In male mice, 597 of 941 animals (63%) had at least one affected eye, compared to 490 of 936 (52%) in female animals.

Several infrequent ocular anomalies were also observed. Notably, microphthalmia and dyscoria/corectopia were described in approximately 1% to 2% of mice. Other rare anomalies included severely atrophic retina. These rare anomalies described here are likely sporadic and not inherent background lesions of this mouse strain.

Optical Coherence Tomography Measurements

SD-OCT measurements are represented in Figure 6, including retinal thickness and manual segmentation of various retinal layers, vitreous thickness, anterior chamber depth, and corneal thickness. The average measurements of these ocular compartments and tissues were based on 1466–1736 eyes, and the results of these measurements are summarized in Figure 6. Central corneal thickness was 88 ± 11 μm, and anterior chamber depth was 411 ± 17 μm, vitreous depth was 530 ± 18 μm, and retinal thickness was 223 ± 5 μm. OCT is capable of producing nearly histologic-quality thickness measurements of the retina (223 ± 5 μm) and its various constitutive layers: inner plexiform layer (IPL: 61 ± 4 μm), inner nuclear layer (INL: 29 ± 3 μm), outer nuclear layer (ONL: 58 ± 3 μm), and photoreceptor inner/outer segments (40.0 ± 3 μm), all measured at 0.5 mm from the optic nerve head.
Intraocular Pressure

IOP in C57BL/6N mice was measured and found to be 10.9 mm Hg ± 1.9 SD with no significant differences between males and females or between the right and left eyes (Fig. 7).

DISCUSSION

We characterized the background ocular lesions present in C57BL/6N mice along with their relative frequency by examining 5546 wild-type eyes from 2773 mice (roughly equal numbers of females and males) at 15 to 16 weeks of age. The value of the findings presented here will only increase as the IMPC continues to grow as a major resource for knockout mice that have already been created and phenotyped. With the increased utilization of C57BL/6N genetic knockouts from IMPC centers for more detailed ophthalmic analysis and characterization, the background abnormalities common in the C57BL/6N strain must be well understood, as the presence of background lesions can confound phenotypic screening of genetic knockouts. Results from this large population screen provide a valuable reference standard for vision scientists using C57BL/6N mice.

Corneal opacities were noted infrequently as a background lesion in C57BL/6N mice (Fig. 1). For most strains of mice, reports of congenital corneal defects typically involve a relationship with the lens (e.g., delayed or abnormal keratolenticular cleavage/separation from the lens vesicle) or in association with more global ocular abnormalities such as microphthalmia. However, we identified discrete, anterior stromal deposits that ranged in size from pinpoint to affecting up to 60% of the corneal area. The C57BL strain of mice has been reported to have a background corneal lesion that consists of a stromal defect with disorganized overlying epithelium. The corneal lesions documented here appear to be an anterior to mid-stromal deposit without epithelial...
Ocular Abnormalities in C57BL/6N rd8 Mice

Disorganization as based on OCT. The limited histologic findings were suggestive of lipid deposits.

Lens abnormalities were extremely common in C57BL/6N mice. More than half of the eyes examined (67%) had a decreased translucency of the anterior lens capsule relative to the cornea (Figs. 2A–C). These altered translucencies are not true cataracts, as light is able to pass through them; however, the altered translucencies when severe would subtly impede light, and with time could progress toward capsular cataract formation. Histologically, severe altered translucencies were described as having subtly pale and flocculent anterior capsular cataracts, but no histopathologic findings could be detected in mild or moderate forms. It has been reported that decreased anterior lens translucency and even cataract formation can occur after anesthesia in mice, which has been demonstrated to be a result of tear film evaporation and a subsequent rise in aqueous humor osmolarity.11 Our assessment is that lens changes were primary in nature rather than a consequence to drugs and/or dessication. All mice were examined without sedation or anesthesia and mice retained the ability to blink throughout examinations, and it is unlikely that topical administration of a mydriatic agent would result in an increased aqueous osmolarity significant enough to cause lens opacification. Furthermore, decreased lens translucency was detected before pharmacologic pupil dilation. Lens opacities are an extremely common background lesion in mice that have rapidly increased in reported frequency in the past 30 years owing to extensive manipulation of the mouse genome.12 The lens nucleus of mice is less easily distinguished than that of humans in nonpathologic conditions. By far the most common lens abnormality in C57BL/6N mice is nuclear cataracts, which were found in nearly every mouse examined (98%; Figs. 2D, 2E).

The vitreous was affected with a high frequency of abnormalities in the form of both crystalline deposits and pigment. Crystalline deposits were common (61%) in the vitreous chamber of C57BL/6N mice. Several mutations are known to be associated with lenticular crystalline opacities within the vitreous chamber due to posterior lens capsule rupture,13–16 which often results in rapid increase in cataract density but not marked intraocular inflammation like that observed in humans.11 Although the crystalline opacities described here typically occurred in the anterior vitreous, often in close proximity to the posterior lens (Fig. 3), they occurred without evidence of any posterior lens capsule rupture. These structures were not detected via light microscopy. Pigmentation in the vitreous chamber was present in two forms: small scattered pigment and pigment associated with incomplete regression of the hyaloid vasculature or persistent hyperplastic primary vitreous. The most common form of pigment detected was found scattered throughout the vitreous chamber in small or large aggregates (Fig. 4). Pigmentation overlying the optic nerve, often attached via a stalk, or adhered to the posterior pole of the lens (sometimes with associated posterior capsular cataract), likely represents a remnant of the hyaloid vasculature or persistent primary hyperplastic vitreous (Fig. 4C).

IOP was remarkably uniform in C57BL/6N mice. Most studies of IOP require sedation/anesthesia, but with proper restraint and use of a rebound tonometer we were able to assess IOP reliably. The rebound tonometry measurements were found to be consistent with previous reports of IOP in wild-type mice, using similar methods.17

The OCT measurements of the thicknesses of ocular tissues and spaces represent a significant contribution to the vision science community, especially regarding C57BL/6N mice, and probably to the murine eye in general. Other studies have reported retinal thickness measurements on various strains of mice,18–25 but differences in OCT devices, authors’ preferenc-
es regarding segmentation and groupings of the various retinal layers, and the way the authors chose to present the quantitative OCT data are all variables that make direct comparisons between studies difficult. One study using mice designated simply as C57BL/6 reports retinal measurements using the same instrument and in a similar fashion as those presented here, and is perhaps the most appropriate reference for comparison.26 They report a total retinal thickness (retina $204.4 \pm 18.2 \mu m$) of $222.6 \mu m$ (compared to our $223.1 \mu m$), IPL thickness of $59.6 \mu m$ (compared to our $60.8 \mu m$), INL thickness of $27.8 \mu m$ (compared to our $28.6 \mu m$), and ONL thickness of $62.8 \mu m$ (compared to our $58.2 \mu m$). While the measurements between these two studies are very similar, differences may be due to their study having been done using just 30 male mice purchased from the Jackson Laboratory (Bar Harbor, ME, USA), which may have been 6J and may not have had retinal abnormalities.

C57BL/6N mice are homozygous for the rd8 mutation in $\text{Crb1}$ (Crumbs homolog 1) gene. The rd8 mutation is a single-nucleotide deletion causing a frame shift, leading to an early stop codon and nonfunctional gene product.27 This gene is a cell surface membrane-bound protein required for the correct determination of cell polarity in several tissues, most notably in photoreceptors of the mammalian retina. Crb1 localizes primarily to the subapical region (SAR) of Müller glial cells, and, to a lesser extent, to the SAR of the photoreceptors. It is known to interact directly with the protein associated with Lin seven 1 ($\text{Pals1}$), and through it, with the Pals1-associated tight junction protein ($\text{Patj}$), and the multi-PDZ domain protein 1 ($\text{Mupp1}$), to form tight junctions at the SAR and to maintain the integrity of the external limiting membrane.28,29 Retinal dysplasia of C57BL/6N mice is attributed to the rd8 mutation in $\text{Crb1}$, leading to a variable degree of rosette formation due to photoreceptor polarity defects. The dysplastic changes are consistently most prominent in the inferior retina.

Despite the presence of retinal dysplasia, even with marked histologic changes, affected rd8 mice have relatively normal full-field electroretinograms.18 It is reported that the severity of the phenotype in rd8 mice varies from retina to retina, and the degeneration of B6N rd8 mice is less severe than in the Crb1−/− retinas.30 However, humans with $\text{CRB1}$ mutations develop autosomal recessive retinitis pigmentosa, or are born blind owing to Leber’s congenital amaurosis (LCA8), both severe photoreceptor diseases. $\text{CRB1}$ mutations typically

table sexual dimorphism in penetrance of rd8 phenotype in C57BL/6N Mice

<table>
<thead>
<tr>
<th></th>
<th>Taconic</th>
<th>Charles River-A</th>
<th>Charles River-B</th>
<th>UC Davis</th>
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<tbody>
<tr>
<td>Males rd8 affected</td>
<td>183</td>
<td>52</td>
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<td>Males total</td>
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<td>374</td>
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<td>Females rd8 affected, %</td>
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</table>

Penetrance of the rd8 phenotype was increased in males in all four colonies used in this study, though the Charles River-A colony did not meet statistical significance, perhaps owing to the relatively low overall rate of affected animals. The number listed in each group is the number of eyes. Comparisons between male and female eyes within each colony were made with a Student’s $t$-test. $P$ value < 0.05 was considered statistically significant. Taconic and Charles River Laboratories–based colonies (A and B) are in France. The UC Davis colony is in the United States (California).
exhibit a more severe retinal phenotype than CRB2 in humans, though a minority of patients with CRB2-related syndrome have congenital retinal and/or optic nerve disease. In mice, the Crb1 deficiency is less severe than in humans, because photoreceptor expression of Crb2 compensates for the absence of Crb1 in this species. Consistent with this explanation, mice lacking both Crb1 and Crb2 have severe photoreceptor degeneration more similar to the human CRB1 phenotype of LCA. CRB2 is not expressed in human photoreceptors, but rather only in Müller glial cells. For this reason, humans with CRB1 mutations typically develop more severe degeneration than that seen in mice.

We have also observed that while all C57BL/6N mice are homozygous for the rd8 mutation, the phenotype is variably penetrant at the time of ocular examination (4 months postnatally). Furthermore, the rd8 background does not always worsen the retinal degeneration phenotype of all knockout mouse models of retinal disease. The variable penetrance of the retinal dysplasia phenotype is also correlated with the location from where the mice were obtained. For example, the mice derived from Taconic had a more severe and more frequent dysplasia phenotype than animals from Charles River Laboratories. It has been shown previously that the rd8 phenotype is modified by genetic and epigenetic factors. We attribute the variability in the retinal phenotype to genetic drift and mutation of unknown presumed modifying genes and epigenetic factors, which either amplify or dampen the photoreceptor disease.

Retinal dysplasia in the C57BL/6N mice is rightfully attributed to the rd8 mutation, but the other ocular phenotypes described here may also be related to Crb1, or may be due to other genetic factors on this inbred strain. Since advanced anterior segment phenotyping occurred in the UC Davis cohort, and detailed fundus grading occurred on mice in
Intraocular Pressure

![Graph showing intraocular pressure measurements for male and female eyes of C57BL/6N rd8 mice.](Image)

**Figure 7.** Intraocular pressure measurements. The mean intraocular pressure across all measured eyes was 10.9 mm Hg ± 1.9 SD (n = 1082). Male eye IOP (mean, 10.9; n = 436) and female eye IOP (mean, 10.8; n = 646) were indistinguishable. The mean IOP of left and right eyes of each sex is shown on the graph. Error bars represent standard deviation. N represents the number of eyes.

France, it is not possible to draw statistically meaningful conclusions regarding possible correlations between the frequency and severity of anterior segment abnormalities and posterior segment abnormalities in the same eyes. However, OCT of mice with severe retinal dysplasia did not seem to correlate with the presence of vitreous opacities (data not shown). Determining if the rd8 mutation is responsible for not only the retinal dysplasia in C57BL/6N mice, but also the other common ocular abnormalities described in this report, is a matter of future investigation. Future studies could also evaluate the heterogeneity and frequency of background eye lesions in other strains, in order to determine which strains have the least variation and are therefore more ideal choices for modeling the genetics of ocular disease.

The value of this report lies in the systematic documentation of the spectrum and frequency of ocular findings. We acknowledge that both a strength and limitation of the study was the specific age range (15–16 weeks), and it is possible that progression of lesions identified and appearance of new lesions could occur with advancing age. As OCT becomes more and more prevalent, there is a need for established normative data of ocular structures in the C57BL/6N strain of mice. We hope that the large numbers of animals involved in this study, the systematic nature in which the examinations were performed, the proficiency of the examiners, and the precision of the advanced imaging measurements will combine to make these data an important benchmark for our colleagues in the vision science community.

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**References**


