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The Severity of Retinal Degeneration in *Rp1h* Gene-Targeted Mice Is Dependent on Genetic Background

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Abstract

Purpose—The severity of disease in patients with retinitis pigmentosa (RP) can vary significantly, even among patients with the same primary mutations. It is hypothesized that modifier genes play important roles in determining the severity of RP, including the retinitis pigmentosa 1 (*RPI*) form of disease. To investigate the basis of variation in disease expression for *RPI* disease, the authors generated congenic mice with a gene-targeted retinitis pigmentosa 1 homolog (*Rp1h*) allele (*Rp1h^{tm1Eap}*) on several different genetic backgrounds and analyzed their retinal phenotypes.

Methods—The *Rp1h^{tm1Eap}* allele was placed onto the C57BL/6J, DBA1/J, and A/J backgrounds. Retinal function of the resultant congenic mice was evaluated using electroretino-graphic analyses. Retinal structure and ultrastructure were evaluated using light and electron microscopy. *Rp1h* protein location was determined with immunofluorescence microscopy.

Results—Analysis of the retinal phenotype of incipient congenic (N6) B6.129S-*Rp1h^{+/tm1Eap}*, DBA.129S(B6)-*Rp1h^{+/tm1Eap}*, and A.129S(B6)-*Rp1h^{+/tm1Eap}* mice at 1 year of age showed retinal degeneration only in the A.129S(B6)-*Rp1h^{+/tm1Eap}* mice. Further analyses revealed that the photoreceptors of the fully congenic A.129S(B6)-*Rp1h^{+/tm1Eap}* mice show evidence of degeneration at 6 months of age and are almost completely lost by 18 months of age. In contrast, the photoreceptor cells in the fully congenic B6.129S-*Rp1h^{+/tm1Eap}* mice remain healthy up to 18 months.

Conclusions—The severity of the retinal degeneration caused by the *Rp1h^{tm1Eap}* allele is notably dependent on genetic background. The development and characterization of the B6.129S-*Rp1h^{+/tm1Eap}* and A.129S(B6)-*Rp1h^{+/tm1Eap}* congenic mouse lines will facilitate identification of sequence alterations in genes that modify the severity of *RPI* disease.

Inherited retinal degenerations are important causes of blindness.^{1–3} For the most part, these disorders are thought to be caused by mutations in single genes. Great progress has been made identifying the genes that harbor mutations that cause inherited retinal degenerations; more than 150 disease genes have been identified to date.⁴ Although the identified mutations are clearly pathogenic, a growing body of evidence suggests that mutations or sequence variants in genes other than the primary disease gene make important contributions to disease phenotypes.^{5,6} For example, the severity of retinal disease has been reported to vary significantly in patients who share primary mutations in a number of other retinal degeneration disease genes, including *CRX*, *PRPH2*, *RHO*, and *RPGR*.^{7–13} Variation in disease expression is especially evident in patients with retinitis pigmentosa 1 (*RPI*) disease. Mutations in *RPI* are a common cause of dominant RP.^{14–18} There are also reports of mutations in *RPI* causing recessive and simplex RP.^{19–21} Clinical characterization of patients with *RPI* disease showed

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notable differences in visual field diameters and electroretinographic (ERG) amplitudes in patients of the same age who shared the same primary mutations.^{18,22}

In general, variation in the phenotypes of genetic disorders can be attributed to allelic heterogeneity, environmental factors, and genetic modifiers.^{5,6} For *RPI* disease, genetic modifiers are thought to be especially important because variation in the severity of disease is observed in patients with the same primary mutation and because environmental exposures are likely to be relatively similar within families.^{18,22} Identification of sequence variations in genes that modify disease severity is an important goal because the modifier genes may provide insight into the pathogenesis of disease. Modifier genes may also provide additional targets for therapeutic interventions.^{5,6}

Although they are important factors in determining disease severity, few human modifier genes have been identified. One of the best examples comes from Bardet Biedl syndrome (BBS), a pleiotropic cilia disorder that includes retinal degeneration.^{23,24} BBS is an autosomal recessive disorder, but much of the observed phenotypic variability in patients with BBS cannot be explained by mutations at a single locus. It has been observed that some BBS patients have mutations at two BBS loci, indicating that inheritance of this disorder can be oligogenic.^{25–27} Further, a hypomorphic sequence variant in the *CCDC28B* gene has been observed to modify the severity of BBS caused by primary mutations in other BBS genes.²⁸ Given that mutations in *CCDC28B* itself were not found to cause BBS, this is a BBS modifier gene.²⁸ Similarly, cases of digenic RP caused by mutations in the *PRPH2* and *ROM1* genes have been reported.²⁹ Sequence variants of the *ROM1* gene serve as the modifiers. Several modifier loci for inherited retinal degenerations in mice have been identified. This includes quantitative trait loci for the *Rd3*, *Nr2e3*, and *Rs1* genes and for age-related retinal degeneration.^{30–33}

To investigate the basis of the variation in disease expression for *RPI* disease, we generated congenic mice with the retinitis pigmentosa 1 homolog (*Rp1h*)-myc allele on several different genetic backgrounds.³⁴ The official designation for this allele is *Rp1h^{tm1Eap}* (<http://www.informatics.jax.org/>). *Rp1h^{tm1Eap}* is a gene-targeted allele in which the mouse *Rp1h* gene is truncated to mimic most human *RPI* mutations, which are either nonsense or frameshift mutations predicted to result in truncation of the RP1 protein after the N-terminal one-third.^{18,34} Homozygous *Rp1h^{tm1Eap/tm1Eap}* mice on a mixed 129/B6 background experience a relatively rapid photoreceptor degeneration, characterized by loss of organization of outer segment discs. In contrast, heterozygous *Rp1h^{+/tm1Eap}* mice on the mixed 129/B6 background do not show signs of retinal degeneration up to 1 year of age.³⁴ We generated several strains of *Rp1h^{+/tm1Eap}* congenic mice and analyzed their retinal phenotypes. Results show that A.129S(B6)-*Rp1h^{+/tm1Eap}* congenic mice, with the heterozygous mutant allele transferred to the A/J background, experienced photoreceptor degeneration whereas pigmented B6.129S-*Rp1h^{+/tm1Eap}* congenic mice did not.

Materials and Methods

Animals

This research was conducted in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research and the University of Pennsylvania Guidelines for Animal Care and Use. *Rp1h^{tm1Eap}* mice were generated by gene targeting, as described.³⁴ Wild-type C57BL/6J, DBA1/J, and A/J mice were purchased from the Jackson Laboratories (Bar Harbor, ME). Heterozygous *Rp1h^{+/tm1Eap}* mice on a mixed 129-B6 background were crossed with C57BL/6J, DBA1/J, and A/J mice to initiate the production of congenic *Rp1h^{tm1Eap}* strains. N1 heterozygous *Rp1h^{+/tm1Eap}* offspring from each of these outcrosses were repeatedly backcrossed with wild-type C57BL/6J, DBA1/J, and A/J mice to generate the B6.129S-*Rp1h^{tm1Eap}*, DBA.129S(B6)-*Rp1h^{tm1Eap}*, and A.129S(B6)-*Rp1h^{tm1Eap}* lines, respectively. The

notation used for describing these congenic mice follows the Rules and Guidelines for Nomenclature of Mouse and Rat Strains, which are available at the Mouse Genome Informatics Web site (<http://www.informatics.jax.org/mgihome/nomen/strains.shtml>). Lighting in the animal facility was maintained on a 12-hour light/12-hour dark cycle, with 5 to 10 lux of dim white light at the cage surface. Mice were genotyped by PCR amplification of a 612-bp product specific for the *Rp1h^{tm1Eap}* allele using primers 5'-GTGCCACAACAATGCTTTACCGT-CAAC-3' and 5'-GGACTGAGGGGCCTGAAATGA-3'.³⁴ To identify the *Rpe65₄₅₀* allele, total RNA was reverse transcribed, and a 400-bp fragment containing codon 450 was amplified using primers 5'-GTCA-CACTGCCCCATACAAC-3' and 5'-CAGCCCTGGCAATTTCACTCAA-3'. PCR products were purified (QIAquick kit; Qiagen, Valencia, CA) and sequenced.³⁵

Immunofluorescence Microscopy

Eyes from wild-type or heterozygous mice from the B6.129S-*Rp1h^{tm1Eap}* and A.129S(B6)-*Rp1h^{tm1Eap}* congenic lines were processed for immunostaining experiments, as described previously.³⁴ Briefly, the eyes were enucleated after cardiac perfusion and fixed in 4% paraformaldehyde for 3 hours, then embedded in OCT freezing media and cryosectioned at 10 μ m. Frozen sections were immunostained with anti-Rp1h and anti-myc antibodies, followed by Alexa 488- and Alexa 555-conjugated secondary antibodies (Invitrogen, Carlsbad, CA). Stained sections were viewed with a confocal microscope (LSM 510 Meta; Zeiss, Thornwood, NY), and the images were processed with Zeiss software (Meta 510).

Light and Electron Microscopy

Eyecups from the desired age and genetic background of heterozygous *Rp1h^{+/tm1Eap}* mice and wild-type littermate controls were fixed in 2% paraformaldehyde + 2% glutaraldehyde in 0.2 M sodium cacodylate buffer (pH 7.4) for 4 hours. Eyecups were trimmed and postfixed in 1% osmium tetroxide. Tissues were then dehydrated in a graded ethanol series, infiltrated, and embedded in Epon resin (EMbed812; Electron Microscopy Sciences, Hatfield, PA) according to the manufacturer's instructions. Sections of 1- μ m thickness were cut and stained with alkaline toluidine blue for light microscopy. Ultrathin sections of 60- to 80-nm thickness were cut, stained with lead citrate and uranyl acetate, and examined using a transmission electron microscope.^{34,36}

Measurement of Outer Nuclear Layer Thickness

The thickness of the outer nuclear layer in 1- μ m thickness of toluidine blue-stained retinal sections from B6.129S-*Rp1h^{tm1Eap}* and A.129S(B6)-*Rp1h^{tm1Eap}* congenic mice was measured using image processing software (Image-pro Plus 6.0; Media Cybernetics, Bethesda, MD) essentially as described.³⁷ ONL thickness measurements from the *Rp1h^{+/tm1Eap}* mice were compared with those observed in littermate control mice using Student's *t*-test.

Electroretinography

Electroretinography was performed as previously described.^{34,38} Briefly, mice were dark adapted for a minimum of 12 hours before the ERG experiments. Preparations of the animals for recordings were made under dim red light. Pupils were dilated with 1% tropicamide. Full-field electroretinograms were recorded in a Ganzfeld dome on dark-adapted, anesthetized mice, with care taken to maintain 37°C body temperature at all times. Retinal responses were detected with platinum electrodes embedded in contact lenses on the cornea and recorded using custom software. Rod a-wave, rod b-wave, and cone b-wave amplitudes measured in the *Rp1h^{+/tm1Eap}* mice were compared with those observed in the littermate control mice using Student's *t*-test.

Results

We followed the recommendations of the Banbury Conference on phenotypic analyses of gene-targeted mouse strains to analyze the effect of genetic background on the phenotype of the *Rp1h^{tm1Eap}* allele.³⁹ The phenotypes of all three lines of *Rp1h^{+/tm1Eap}* mice were analyzed at the incipient congenic (N6) stage. Backcrosses of the B6.129S-*Rp1h^{tm1Eap}* and A.129S(B6)-*Rp1h^{tm1Eap}* lines were continued to generate fully congenic (N10) lines.

Retinal Function in the *Rp1h^{tm1Eap}* Congenic Mice

To examine the retinal function of mice with the *Rp1h^{tm1Eap}* allele on different genetic backgrounds, we measured full-field ERG responses of 12-month-old *Rp1h^{+/tm1Eap}* mice and litter-mate controls from all three lines at the incipient congenic (N6) stage. The retinal function of heterozygous B6.129S-*Rp1h^{+/tm1Eap}* and DBA.129S(B6)-*Rp1h^{+/tm1Eap}* mice was the same as that observed in wild-type littermate controls (Fig. 1). In contrast, rod and cone function was decreased in the A.129S(B6)-*Rp1h^{+/tm1Eap}* mice compared with controls (Fig. 1). Specifically, the saturating amplitude of the rod a-wave ($a_{\max}$) of A.129S(B6)-*Rp1h^{+/tm1Eap}* mice was reduced by approximately 36% at 12 months of age ($P < 0.01$). Rod b-wave amplitudes were significantly reduced in the A.129S(B6)-*Rp1h^{+/tm1Eap}* mice compared with wild-type littermate controls. Cone b-wave amplitudes were also reduced by approximately 28%, though this difference was not statistically significant (Fig. 1). Of note, the amplitudes of the rod and cone responses in the control albino mice were less than those observed in the control pigmented mice of the same age, consistent with the age-related retinal degeneration described in these mice.³¹

We also assessed the retinal function of fully congenic (N10) B6.129S-*Rp1h^{tm1Eap}* and A.129S(B6)-*Rp1h^{tm1Eap}* mice. As with the N6 mice, the retinal function of the N10 B6.129S-*Rp1h^{+/tm1Eap}* mice was the same as that observed in wild-type littermate controls at 6 and 12 months of age (Fig. 2). Rod a-wave amplitudes of the 6-month-old N10 A.129S(B6)-*Rp1h^{+/tm1Eap}* mice were significantly decreased, in this case by 23% ($P < 0.05$). By 12 months of age, the retinal function of the A.129S(B6)-*Rp1h^{+/tm1Eap}* congenic mice was decreased further, similar to the results obtained from the N6 mice (Fig. 2).

Retinal Morphology of *Rp1h^{tm1Eap}* Congenic Mice

To evaluate the extent of photoreceptor degeneration in the A.129S(B6)-*Rp1h^{+/tm1Eap}* congenic mice, we examined the thickness of the outer nuclear layer (ONL) in semithin retinal sections from heterozygous mice and wild-type littermate controls at 6, 12, and 18 months of age. Eyes from three to four *Rp1h^{+/tm1Eap}* and *Rp1h^{+/+}* mice were evaluated at each time point. At the light microscopic level, the A.129S(B6)-*Rp1h^{+/tm1Eap}* mice demonstrated progressive degeneration of photoreceptor cells (Fig. 3). At 6 months of age, the ONL of retinas from the A.129S(B6)-*Rp1h^{+/tm1Eap}* mice was one to two rows of nuclei thinner than controls. By 12 months of age, the ONL of the A.129S(B6)-*Rp1h^{+/tm1Eap}* mice decreased to five to six rows of nuclei, which was two to three rows thinner than the ONL of wild-type littermate controls. This difference in ONL thickness was present throughout the retina (Fig. 4) and was statistically significant ($P < 0.0001$). Retinal degeneration was more severe in 18-month-old A.129S(B6)-*Rp1h^{+/tm1Eap}* mice, with only two to three rows of photoreceptor nuclei remaining. Although some age-related degeneration was observed in the control albino mice, five to six rows of photoreceptor nuclei were still present in the control retinas at 18 months of age (Fig. 3). In contrast, the retinal morphology in 6-, 12-, and 18-month-old heterozygous B6.129S-*Rp1h^{+/tm1Eap}* mice was similar to that of age-matched control animals, without evidence of photoreceptor cell loss (Fig. 3).

Defective Outer Segments in *Rp1h^{tm1Eap}* Congenic Mice on the A/J Genetic Background

The ultrastructure of photoreceptors in *Rp1h^{tm1Eap}* congenic mice was evaluated by electron microscopy. On the A/J genetic background, photoreceptor outer segment length and organization decreased progressively in the heterozygous *Rp1h^{+/tm1Eap}* mice. At 12 months of age, the photoreceptor outer segments of A.129S(B6)-*Rp1h^{+/tm1Eap}* mice were shorter and demonstrated early signs of disorganization (Figs. 5I, 5J). By 18 months of age, the outer segments of the A.129S(B6)-*Rp1h^{+/tm1Eap}* mice were notably shorter and more disorganized than those in controls. Small packets of enlarged and incorrectly oriented discs were seen in place of intact outer segments (Figs. 5K, 5L). Ultrastructural analyses of retinas from the B6.129S-*Rp1h^{+/tm1Eap}* mice demonstrated normal photoreceptor outer segment structure up to 18 months of age (Figs. 5C, 5D).

Expression of Truncated Rp1h-myc Protein in Congenic Mice

Locations of Rp1h proteins in the A/J and C57BL/6J congenic strains were evaluated using immunofluorescence analyses. The truncated Rp1h-myc protein produced from the *Rp1h^{tm1Eap}* allele was detected using antibodies to the myc tag at the C-terminal end of this protein, and the full-length Rp1h protein was detected using antibodies to the C-terminal portion of the protein, which is not present in the truncated Rp1h-myc protein.^{34,40} Immunofluorescence analyses using frozen retinal sections from B6.129S-*Rp1h^{+/tm1Eap}* and A.129S(B6)-*Rp1h^{+/tm1Eap}* mice showed that the Rp1h-myc protein was correctly located in the axoneme of photoreceptor outer segments, as reported previously (Fig. 6).³⁴ The Rp1h-myc protein colocalized with the full-length Rp1h protein in both *Rp1h^{+/tm1Eap}* congenic lines (Fig. 6). Signal intensities of the wild-type Rp1h and mutant Rp1h-myc proteins were similar in the B6.129S-*Rp1h^{+/tm1Eap}* and A.129S(B6)-*Rp1h^{+/tm1Eap}* mice.

Modifiers of Rp1 Phenotype in A/J Congenic Mice

Previous studies have shown that a sequence variation in the *Rpe65* gene can act as a genetic modifier of inherited retinal degeneration in mice. C57BL/6J mice have a variant in their *Rpe65* gene (*Rpe65_{450Met}*) that is protective against light damage and retinal degeneration caused by the VPP rhodopsin transgene.^{41–43} The *Rpe65_{450Met}* variant leads to decreased levels of Rpe65 protein, a decreased rate of rhodopsin regeneration, and protection against photoreceptor damage.^{35,41,42} As for most other mouse strains, A/J mice have Leu at position 450 of their Rpe65 protein. Although no mechanistic connection is evident between the RP1 and RPE65 proteins, we investigated the potential contribution of the *Rpe65*-Leu450Met variation on the phenotype of the A.129S(B6)-*Rp1h^{+/tm1Eap}* mice by evaluating the retinal function of albino mice generated in the N2 cross during development of the A.129S(B6)-*Rp1h^{tm1Eap}* congenic line. At 1 year of age, rod and cone ERG amplitudes were not significantly different between the *Rpe65^{450Leu/Leu}* mice ($n = 13$) and the *Rpe65_{450Met/Leu}* mice heterozygous for the *Rp1h^{tm1Eap}* allele ($n = 7$).

Discussion

The results described show that the severity of the retinal degeneration caused by the *Rp1h^{tm1Eap}* allele is notably dependent on genetic background. Photoreceptors of the A.129S(B6)-*Rp1h^{+/tm1Eap}* congenic mice show evidence of degeneration as early as 6 months of age and are almost completely lost by 18 months of age. In contrast, photoreceptor cells in the B6.129S-*Rp1h^{+/tm1Eap}* congenic mice remain healthy up to 18 months of age. Incipient congenic N6 DBA.129S(B6)-*Rp1h^{+/tm1Eap}* mice also retain normal retinal function. These results establish the basis for using the A.129S(B6)-*Rp1h^{+/tm1Eap}* and B6.129S-*Rp1h^{+/tm1Eap}* congenic mice to map and identify sequence alterations in genes that modify the severity of *RP1* disease.^{18,22} As described, identification of a modifier gene or genes for *RP1* would be valuable for several reasons, including improved understanding of disease pathogenesis and

insight into potential therapies.⁶ Genes that harbor sequence alterations that modify the severity of *RP1* disease may also be disease genes in their own right. In addition, genetic modifiers of *RP1* disease severity may affect other inherited retinal degenerations; thus, their identification will help improve our understanding of the genetic variations that cause and modulate inherited retinal diseases.

Identification of modifier loci in patient populations can be difficult because of the large number of patients required.^{5,44} Mouse models of genetic disorders can facilitate modifier screens by providing genetically homogeneous populations and controlled environments. Further, inbred mouse strains replicate much of the genetic diversity of the human population, and several modifiers identified in mice have been found to be relevant to human disease. For example, polymorphisms in the *SORCS1* (sortilin-related VPS10 domain containing receptor 1) gene have recently been linked to susceptibility to diabetes in patients; this modifier gene was originally identified in mice.⁴⁵

The finding that truncation of the RP1 protein can cause dominant disease in mice is also important because this is the primary mode of inheritance of *RP1* disease in humans.^{14–17, 19–21} This finding confirms the value of gene-targeted mice as models of *RP1* disease. It also confirms that loss of the C-terminal two-thirds of the RP1 protein leads to loss or alteration of protein function.^{14,34} Based on the dominant mode of inheritance in humans, it has been suggested that the truncated RP1 protein has a toxic or dominant-negative effect on photoreceptor cells.¹⁴ The observation that dominant disease also occurs in mice is consistent with this hypothesis, but additional studies are needed to determine whether *RP1* disease is caused by loss or gain of function.

The observation that the retinal degeneration caused by the *Rp1h^{tm1Eap}* allele is more severe in albino mice raises the possibility that the lack of pigment in these mice or their increased susceptibility to light-induced retinal degeneration contributes to the *Rp1h^{tm1Eap}* phenotype.⁴⁶ A/J mice are albino because of a mutation (C103S) in the tyrosinase (*Tyr*) gene.⁴⁷ In humans, defects in tyrosinase result in oculocutaneous albinism type 1 (OCA1). Patients with OCA1 have several ocular abnormalities, including iris transillumination, foveal hypoplasia, and misrouting of ganglion cell axons in the optic chiasm.⁴⁸ It is not known why diminished melanin pigment formation leads to defects in axon routing or foveal development. Albino mice share many of these defects and have been reported to have approximately 25% fewer rod photoreceptors than C57BL/6 mice.⁴⁹ A/J mice have also been reported to experience faster age-related decline in photoreceptor cell numbers than C57BL/6J mice.³¹ It is therefore possible that the more severe *Rp1* phenotype observed in the A/J congenics is related to lack of pigmentation. What is not known, however, is how the amount of melanin in RPE cells could influence the RP1 protein in the axoneme of photoreceptor cells.^{34,36} Further, the difference in the rates of age-related retinal degeneration between the A/J and C57BL/6J mice is not the result of pigmentation because C57BL/6J-c2J mice, which are albino but otherwise identical to C57BL/6J mice, have the same relatively slow rate of age-dependent retinal degeneration as C57BL/6 mice.³¹

Lack of pigmentation in albino mice contributes to their increased susceptibility to light-induced retinal degeneration compared with pigmented mice, in part because of lack of pigment in the irides of albino mice, which permits increased transmission of light to the retina.⁴⁶ It is not likely that light-induced damage played a role in the degeneration observed in the A.129S (B6)-*Rp1h^{+/tm1Eap}* congenic mice because all mice used in these studies were raised in dim light, with approximately 5 to 10 lux of light at the cage surface. This level of light is too low to cause light-induced degeneration.⁵⁰

An additional issue related to the more severe retinal degeneration observed in the A.129S(B6)-*Rp1h*^{+/tm1Eap} congenic mice compared with the pigmented B6.129S-*Rp1h*^{+/tm1Eap} congenic mice relates to the *Rpe65* gene. C57BL/6J mice have a variant in the *Rpe65* gene (*Rpe65*_{450Met}) that is protective against light damage. The *Rpe65*_{450Met} variant leads to decreased levels of Rpe65 protein, decreased rhodopsin regeneration, and consequent protection against light-induced damage.^{35,41,42} The *Rpe65*_{450Met} variant is also protective against retinal degeneration caused by the VPP rhodopsin transgene and thus can function as a modifier allele for retinal degenerations caused by mutations in genes associated with photo-transduction.⁴³ Like most other strains of mice, the A/J strain has the nonprotective *Rpe65*_{450Leu} variant of the *Rpe65* gene, raising the possibility that this could contribute the increased severity of the *Rp1* phenotype in the A/J congenic mice. Several results from the studies described indicate that this is not the case. First, no degeneration was observed in the DBA incipient congenic mice, which have the *Rpe65*_{450Leu} allele. Second, we compared the retinal function of albino mice with *Rpe65*_{450Met/Met} and *Rpe65*_{450Met/Leu} alleles generated in the N2 cross during development of the A.129S(B6)-*Rp1h*^{tm1Eap} congenic line and did not observe any difference in retinal function in *Rp1h*^{+/tm1Eap} mice that were *Rpe65*_{450Met/Met} compared with *Rpe65*_{450Met/Leu}.

Mice heterozygous at position 450 of *Rpe65*, with one Met and one Leu allele, have an intermediate sensitivity to light damage because of intermediate levels of Rpe65 and a twofold reduction in the rate of rhodopsin regeneration compared with *Rpe65*_{450Leu/Leu} mice.^{35,42} These results suggest that the retinal degeneration observed in the A.129S(B6)-*Rp1h*^{tm1Eap} congenic mice is not caused by the loss of the protective *Rpe65* allele in the albino mice.

Based on the data presented here, we conclude that sequence variations in one or more modifier genes in the B6 or A/J strains are responsible for the observed difference in phenotypes between the A.129S(B6)-*Rp1h*^{+/tm1Eap} and the B6.129S-*Rp1h*^{+/tm1Eap} congenic mice. An analysis of 8.27 million SNPs in 16 inbred strains of mice showed that C57BL/6J and A/J were discordant at 14% (approximately 1 million) of the 7.47 million SNPs successfully tested in the two strains.⁵¹ We hypothesize that this genetic variation underlies the phenotypic variation between the albino and black congenic *Rp1h*^{tm1Eap} mice. Among the SNPs that were discordant between the B6 and A/J strains were 28 SNPs that resulted in premature stop codons, 9 SNPs that altered stop codons, 16 SNPs that altered start codons, and 42 SNPs that altered splice sites in the A/J strain.⁵¹ One of the genes that harbors a premature stop codon in the A/J strain is *Gpr98*. Mutations in human *GPR98* cause Usher syndrome 2C, in addition to familial febrile seizures.^{52,53} The A/J allele of *Gpr98* results in an Arg4296Ter mutation (GenBank NM_054053.4; NP_473394.3). Because this mutation is in exon 64 of 90, the mutant allele would be expected to be null secondary to non-sense-mediated decay.⁵⁴ The Gpr98 protein has been localized to the transition zones (connecting cilia) of photoreceptor cells, which is adjacent to the location of the Rp1h protein in the axoneme of photoreceptor cells.^{36,40,55} These proteins are thus part of the photoreceptor sensory cilium, which encompasses the outer segments, transition zone, and basal bodies of photoreceptor cells.⁵⁶ The mutant allele of *Gpr98* in the A/J mice raises the possibility that the Gpr98 and Rp1 proteins interact or participate in the same cell biological pathway. The A.129S(B6)-*Rp1h*^{+/tm1Eap} and the B6.129S-*Rp1h*^{+/tm1Eap} congenic mouse lines provide the opportunity to identify sequence differences between the B6 and A/J strains that modify the *Rp1* phenotype in mice.

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Figure 1.

Amplitudes of components of electroretinograms from 12-month-old N6 *Rp1h*^{+/tm1Eap} mice. Rod and cone ERG measurements obtained from 12-month-old N6 incipient congenic (A) B6.129S-*Rp1h*^{tm1Eap}, (B) DBA.129S(B6)-*Rp1h*^{tm1Eap}, and (C) A.129S(B6)-*Rp1h*^{tm1Eap} mouse lines. Decreased rod photoreceptor function is evident in the A.129S(B6)-*Rp1h*^{tm1Eap} incipient congenics. Rod a-wave- and b-wave-saturated amplitudes were decreased by approximately 36% and 34%, respectively. *Decreases were statistically significant. wt, wild-type littermate control mice; het, heterozygous *Rp1h*^{+/tm1Eap} mice.

Figure 2.

Retinal function of fully congenic (N10) B6.129S-*Rp1h*^{tm1Eap} and A.129S(B6)-*Rp1h*^{tm1Eap} mice. Rod and cone ERG measurements obtained from fully congenic (A, B) B6.129S-*Rp1h*^{tm1Eap} and (C, D) A.129S(B6)-*Rp1h*^{tm1Eap} mice at 6 and 12 months of age, as indicated. Decreased rod photoreceptor function is evident in the A.129S(B6)-*Rp1h*^{tm1Eap} congenics by 6 months of age, with a 23% decrease in rod a-wave amplitudes. Rod a-wave and b-wave amplitudes were decreased by 31% and 33% at 12 months of age in the A.129S(B6)-*Rp1h*^{tm1Eap} congenics. *Decreases were statistically significant. No changes in ERG amplitudes were detected in the B6.129S-*Rp1h*^{tm1Eap} congenics. Wild-type littermate controls used in these experiments had the same coat color as the *Rp1h*^{+/tm1Eap} mice. wt, wild-type littermate control mice; het, heterozygous *Rp1h*^{+/tm1Eap} mice.

Figure 3.

Retinal histology in congenic *Rp1h^{+/tm1Eap}* mice. Histology of retinas from B6.129S-*Rp1h^{tm1Eap}* (**A–C**) and A.129S(B6)-*Rp1h^{tm1Eap}* (**D–F**) congenic mice at ages indicated. In each pair of images, #1 is from a wild-type *Rp1h^{+/+}* littermate control and #2 is from a heterozygous *Rp1h^{+/tm1Eap}* animal. No photoreceptor degeneration was detected in the retinas of B6.129S-*Rp1h^{+/tm1Eap}* mice at ages up to 18 months (**A–C**), though some age-related thinning of the retina is evident. In contrast, photoreceptor degeneration was observed in the A.129S(B6)-*Rp1h^{+/tm1Eap}* congenic mice beginning at 6 months of age (**D–F**). By 18 months of age, the retinas of the A.129S(B6)-*Rp1h^{+/tm1Eap}* congenic mice have only two to three rows of photoreceptor nuclei remaining (**F1, F2**). As for the C57BL/6J mice, age-related degeneration of the retina is evident in the A/J congenics. GC, ganglion cell; IPL, inner plexiform layer; INL, inner nuclear layer; IS, inner segment; OS, outer segment; RPE, retinal pigment epithelium.

Figure 4.

Measurements of ONL thickness in congenic *Rp1h^{+/tm1Eap}* mice. The thickness of the ONL was measured in retinas from 12-month-old (A) B6.129S-*Rp1h^{tm1Eap}* (C57BL/6J) and (B) A.129S(B6)-*Rp1h^{tm1Eap}* (A/J) congenic mice. Each graph represents data from 1-μm retinal sections taken along the vertical meridian in the superior retinas of three mice. On each section, 12 data points with 200-μm intervals starting from the optic nerve head were measured, and each data point was the mean of nine measurements, with three measurements taken in each section. There was no difference in ONL thickness between heterozygous B6.129S-*Rp1h^{+/tm1Eap}* mice and littermate controls. The ONLs of the A.129S(B6)-*Rp1h^{tm1Eap}* congenic mice were 8 to 10 μm thinner throughout the retina. Similar results were obtained from sections in the inferior retina. ONH, optic nerve head; wt, wild-type littermate control mice; het, heterozygous *Rp1h^{+/tm1Eap}* mice. Error bars indicate SD. *Significant differences between *Rp1h^{+/tm1Eap}* mice and littermate controls.

Figure 5.

Ultrastructure of photoreceptors in *Rp1h^{+/tm1Eap}* mice. Electron micrographs of photoreceptor cells from B6.129S-*Rp1h^{tm1Eap}* (A–D) and A.129S(B6)-*Rp1h^{tm1Eap}* (E–L) congenic and littermate control mice at 12 and 18 months of age. (A–D) Photoreceptor inner and outer segments in the B6.129S-*Rp1h^{+/tm1Eap}* congenic mice retain normal organization at 12 and 18 months of age. In contrast, the photoreceptor outer segments of the A.129S(B6)-*Rp1h^{+/tm1Eap}* congenic mice (E, F) were shorter and less organized than those in littermate controls (I, J). By 18 months of age, only short disordered outer segments remained in the A.129S(B6)-*Rp1h^{+/tm1Eap}* congenic mice (H, L). This is distinct from the 18-month-old A/J littermate controls in which normal outer segment structure was retained despite shortening of the outer segments associated with age. Scale bars: (A–H) 10 μ m; (I, J) 5 μ m; (K, L) 1 μ m. IS, inner segment; OS, outer segment; RPE, retinal pigment epithelium.

Figure 6.

Immunofluorescence analysis of Rp1 and Rp1-myc protein localization in *Rp1h^{+/tm1Eap}* mice. Frozen sections of retinas from 1-month-old B6.129S-*Rp1h^{+/tm1Eap}* (C57BL/6J) and A.129S (B6)-*Rp1h^{+/tm1Eap}* (A/J) congenic mice were probed with antibodies to both the C terminus of Rp1 (Rp1, *red*) and the myc tag (myc, *green*). Both the full-length Rp1 protein (*red*) and the truncated Rp1-myc protein (*green*) localize correctly to the axoneme of photoreceptor outer segments, as indicated by the overlap of the *red* and *green* signals in the merged images. Locations and levels of the Rp1 and Rp1-myc proteins are similar in the C57BL/6J and A/J congenic mice. Scale bars, 10 μ m. AX, axoneme; IS, inner segment; OS, outer segment.