Published in final edited form as:

Invest Ophthalmol Vis Sci. 2007 December ; 48(12): 5397–5407.

Late-Onset Cone Photoreceptor Degeneration Induced by R172W Mutation in Rds and Partial Rescue by Gene Supplementation

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Abstract

Purpose—R172W is a common mutation in the human retinal degeneration slow (*RDS*) gene, associated with a late-onset dominant macular dystrophy. In this study, the authors characterized a mouse model that closely mimics the human phenotype and tested the feasibility of gene supplementation as a disease treatment strategy.

Methods—Transgenic mouse lines carrying the R172W mutation were generated. The retinal phenotype associated with this mutation in a low-expresser line (L-R172W) was examined, both structurally (histology with correlative immunohistochemistry) and functionally (electroretinography). By examining animals over time and with various *rds* genetic backgrounds, the authors evaluated the dominance of the defect. To assess the efficacy of gene transfer therapy as a treatment for this defect, a previously characterized transgenic line expressing the normal mouse peripherin/Rds (NMP) was crossed with a higher-expresser Rds line harboring the R172W mutation (H-R172W). Functional, structural, and biochemical analyses were used to assess rescue of the retinal disease phenotype.

Results—In the wild-type (WT) background, L-R172W mice exhibited late-onset (12-month) dominant cone degeneration without any apparent effect on rods. The degeneration was slightly accelerated (9 months) in the *rds*^{+/-} background. L-R172W retinas did not form outer segments in the absence of endogenous Rds. With use of the H-R172W line on an *rds*^{+/-} background for proof-of-principle genetic supplementation studies, the NMP transgene product rescued rod and cone functional defects and supported outer segment integrity up to 3 months of age, but the rescue effect did not persist in older (11-month) animals.

Conclusions—The R172W mutation leads to dominant cone degeneration in the mouse model, regardless of the expression level of the transgene. In contrast, effects of the mutation on rods are dose dependent, underscoring the usefulness of the L-R172W line as a faithful model of the human phenotype. This model may prove helpful in future studies on the mechanisms of cone degeneration and for elucidating the different roles of Rds in rods and cones. This study provides evidence that Rds genetic supplementation can be used to partially rescue visual function. Although this strategy is capable of rescuing haplo-insufficiency, it does not rescue the long-term degeneration associated with a gain-of-function mutation.

A product of the retinal degeneration slow (*RDS*) gene, Rds is a tetraspanning membrane protein expressed in the disc rim of rods and cones.¹ It has been extensively studied for the

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Disclosure: S. Conley, None; M. Nour, None; S.J. Fliesler, None; M.I. Naash, None

past 25 years, and more than 80 disease-causing-mutations in this gene have been identified (<http://www.retina-international.com/sci-news/rdsmut.htm>). One of the most common Rds mutations is a substitution of tryptophan for arginine at position 172 (R172W).^{2–5} This mutation has been described by multiple laboratories and occurs in a large number of families. Affected persons typically have faltering visual acuity in the third to fourth decade of life.² Children and young adults carrying the mutation have been reported as asymptomatic⁵ but may exhibit macular changes. Functionally, patients carrying this mutation have normal rod, but diminished cone, electroretinographic (ERG) amplitudes and elongated implicit times.

Rds was first identified in association with a naturally occurring mouse model called retinal degeneration slow (*rds*^{-/-}), which does not form outer segments and has no detectable rod or cone ERG response. The hemizygous (*rds*^{+/-}) mouse exhibits a haploinsufficiency phenotype associated with decreased rod and cone ERG responses and whorl-like, malformed outer segments.⁶ Rds is a structural protein present as a homodimer or a heterodimer complex, or as both, with its homologue, Rom-1 (rod outer segment membrane protein-1).^{7,8} Results of in vitro studies indicate that the large extracellular D2 loop of Rds (containing more than 70% of disease-causing-mutations) is the site of interactions to form Rds/Rom-1 complexes,⁷ whereas the C terminus of Rds protein is responsible for outer segment targeting of the protein.⁹ Rds is thought to have several hypothesized roles in the outer segment: (1) Rds/Rom-1 complexes function to keep the “hairpin rim” region of the rod outer segment disks intact^{10–12}; (2) Rds/Rom-1 complexes potentially keep the disks connected to the rod outer segment plasma membrane through interactions with GARP (glutamic acid-rich protein) and the cyclic nucleotide-gated ion channels of the plasma membrane¹³; (3) the C terminus of Rds directs complex targeting to the outer segment and helps align outer segment disks⁹; and (4) the C terminus of Rds may mediate disc membrane fusion,^{14–17} possibly in direct cooperation with other binding partners.¹⁸

Recent evidence from our group has suggested that Rds does not function the same way in cone photoreceptors that it does in rod photoreceptors. This observation came from studies of the cone-dominant *Nrl/rds* double knockout mouse,¹⁹ whose phenotype is distinct from that of both the *Nrl*^{-/-} (neural retina leucine zipper)²⁰ and the *rds*^{-/-} retina. In contrast to the *rds*^{-/-} retina, the all-cone *Nrl*^{-/-}/*rds*^{-/-} retina contains cone outer segments that, despite grossly aberrant morphology, are capable of robust phototransduction. Structurally, these outer segments are tubular and do not form lamellae. The reasons for the difference in Rds function in cones and rods are not fully understood, but this observation is supported by the clinical findings that RDS mutations can cause either rod-dominant (retinitis pigmentosa) or cone-dominant (macular dystrophy) retinal degeneration.²¹

Although the R172W mutation occurs within the D2 loop region of Rds, it is not located in the area required for complex assembly.⁷ In fact, it is unclear why the R172W mutation causes this distinct cone-associated phenotype. What is clear is the importance of arginine at position 172 to cone structure. Specifically, each of the three known substitutions at position 172 (R172W, R172G, R172Q) causes a cone- or a fovea-dominant defect in patients.^{4,21,22} In contrast, the N244K Rds mutation causes retinitis pigmentosa, whereas the N244H mutation causes macular dystrophy.^{23,24} To study the mechanism underlying the pathogenesis associated with the R172W mutation, we have generated and characterized multiple transgenic mouse lines carrying this mutation on different *rds* genetic backgrounds. In our previous work,²⁵ we reported the biochemical characterization of the R172W protein in a high-expressing transgenic line (H-R172W), with a level corresponding to approximately 75% of the endogenous Rds. We showed that R172W Rds associates with Rom-1 and exhibits no defects in complex formation.²⁵ These data were supported by findings by Molday et al.⁸ indicating that recombinant R172W protein was glycosylated and could properly form complexes in vitro. However, our in vivo study²⁵ with the higher expresser line demonstrated that the R172W

protein was more susceptible to partial tryptic digestion than wild-type (WT) Rds, suggesting a slight conformational change in complexes containing R172W Rds.

In the present study, we characterized a new R172W model that closely mimics the human retinal phenotype and tested the efficacy of gene transfer therapy as a strategy to combat diseases associated with RDS mutations. As reported previously, the H-R172W line exhibits an early-onset dominant cone-rod dystrophy that is not characteristic of the retinal degeneration observed in human patients.²⁵ Here we present additional data obtained with this model indicating that genetic supplementation with WT Rds provides partial, long-term rescue of rod function but no permanent rescue of cone function. Although functional dominance of the R172W allele is the most likely explanation for the lack of persistent cone rescue, conclusions regarding the potential usefulness of gene transfer as a treatment are limited by the severity of the H-R172W phenotype and its lack of correlation with the human disease phenotype. Therefore, we also have characterized an alternative R172W model (L-R172W), which expresses a lower amount of R172W protein. L-R172W mice exhibit late-onset cone degeneration without manifesting a dominant effect on rods, closely resembling the human clinical phenotype. Thus, the L-R172W line offers a useful model for future studies of the mechanism of late-onset macular dystrophy.

MATERIALS AND METHODS

Generation of the Transgene

R172W transgenic mice were generated as described previously.²⁵ Briefly, full-length mouse RDS cDNA was isolated, and the R172W mutation in the D2 loop and P341Q modification in the C terminus was introduced by site-directed mutagenesis. The cDNA was preceded by a 1.3-kb promoter fragment of the human interphotoreceptor retinoid binding protein (hIRBP) and was followed by a 0.7-kb regulatory fragment of the SV40 polyA. Previous studies show that the P341Q modification exerts no biochemical, structural, or functional effect on Rds^{10, 26} and that it allows specific recognition of the transgenic protein with the monoclonal 3B6 antibody (generated by Robert Molday, University of British Columbia, Canada). Three transgenic lines are used in the present study: H-R172W was characterized previously,²⁵ L-R172W is characterized here, and the normal mouse peripherin/Rds (NMP) line used in our rescue studies was characterized previously.²⁶ All L-R172W mice used in this study were on the C57/BL6 background and were homozygous for the transgene regardless of the *rds* background. Mice were maintained in the breeding colony under cyclic light (14-hour light/10-hour dark) conditions; cage illumination was approximately 7 foot candles during the light cycle. All procedures were approved by the University of Oklahoma Health Science Center Institutional Animal Care and Use Committee (IACUC) and adhered to the ARVO Statement for the Use of Animals in Ophthalmic and Visual Research (<http://www.arvo.org/>).

Expression of the Transgene

Total RNA was extracted from retinas of transgenic mice using reagent (TRIzol; Gibco-BRL, Gaithersburg, MD). Five micrograms retinal RNA was separated by electrophoresis on a 0.7% agarose gel containing 18% formaldehyde. The gel was stained with ethidium bromide to check RNA integrity (judged by the integrity of the 28s and 18s rRNA). RNA was then transferred to nitrocellulose membrane and was hybridized first with α -³²P-labeled SV40 polyA fragment to detect the expression of the transgene. The blot was stripped and rehybridized to the second probe (α -³²P-labeled exon 1 of RDS) to equally detect the endogenous transgene transcripts. Prehybridization and hybridization conditions were the same as described.²⁷

Western Blot Analysis

Rabbit polyclonal antibody against residues 331 to 346 of murine Rds C terminus (1:1000 dilution) was used for the detection of Rds, as described.²⁵ Anti-actin antibody (1:250 dilution; Sigma-Aldrich, St. Louis, MO) was used to control for sample loading. Retinal protein extraction and blot analysis were carried out as previously described.²⁵

Immunohistochemistry

Mice were humanely killed, and whole eyes from transgenic and nontransgenic mice were enucleated and fixed for 2 hours in 0.1 M phosphate buffer (pH 7.4) containing 4% formaldehyde, after which the lens was removed and the remaining tissue was placed in phosphate-buffered paraformaldehyde for an additional 12 hours of fixation at 4°C. Eyes were embedded, sectioned, and stained with appropriate primary and commercially available, fluorescence-conjugated (FITC and Cy3) secondary antibodies (see figure legends), as described.²⁵ The 3B6 anti-Rds antibody (kindly provided by Robert Molday) was used at a 1:5 dilution with phosphate-buffered saline (PBS) containing 0.2% Triton X-100. Anti-human blue opsin antibody (a generous gift from Jeremy Nathans, Johns Hopkins University, Baltimore, MD) was used at a 1:3000 dilution. The density of the blue cones was assessed across a retinal expanse 100- μ m in length at a distance 300 μ m from the edge of the optic nerve head in the inferior hemisphere, corresponding to the retinal region of highest blue cone content.

Light and Electron Microscopy and Quantitative Morphometry

Enucleated eyes were fixed in buffered mixed aldehydes, processed, embedded in plastic resin, and sectioned, as described in detail previously.^{25,28} For light microscopy, sections (0.75- μ m thickness, stained with 1% toluidine blue) were photographed with a photomicroscope (BH-2; Olympus, Tokyo, Japan) in auto-expose mode using 20 $\times$ (air) or 60 $\times$ (oil immersion) DPlanApo objectives. For electron microscopy, ultrathin (silver-gold) sections were poststained with lead citrate and uranyl acetate on-grid and were viewed with an electron microscope (JEM-1200EX; JEOL, Tokyo, Japan) at an accelerating voltage of 80 KeV. Photoreceptor nuclei were counted in a microscopic field that spanned 100 μ m within the central superior and central inferior portions of the retina, approximately 300 μ m away from the edge of the optic nerve head. At least three individual eyes taken from separate litters were used for examination at each time point.

Electron Microscopic Immunogold Cytochemistry

The procedures used were as described in detail.^{25,28} In brief, ultrathin tissue sections (in LR White plastic, on nickel grids) were briefly rinsed in Tris-buffered saline (TBS; 0.15 M NaCl, 0.05 M Tris) and then were “blocked” for 1 hour at room temperature in blocking buffer (TBS containing 1% [wt/vol] RIA-grade BSA and 5% [vol/vol] normal goat serum [NGS]). The sections were exposed overnight at 4°C to anti-cone opsin mixture antibody (1:2500 in blocking buffer). After brief rinses with TBS, sections were incubated for 2 hours at room temperature with goat anti-rabbit IgG conjugated to 15-nm diameter colloidal gold (Amersham, Arlington Heights, IL), diluted 1:50 in blocking buffer. After brief rinses in TBS and distilled water, sections were treated with 1% aqueous glutaraldehyde (5 minutes) and rinsed with TBS. Sections were then stained with 2% aqueous uranyl acetate (15 minutes) and lead citrate (6 minutes), rinsed again with distilled water, air dried, and exposed to OsO₄ vapors for 30 minutes before viewing with an electron microscope.

Electroretinography

Electroretinograms were recorded from nontransgenic and transgenic mice ($n = 6$ –10 each) at different developmental stages to obtain dark-adapted, light-adapted, and specific cone responses, as described.^{19,29} For spectral electroretinography, a two-flash technique was used

to record the responses of blue and red/green cones. The first flash was used to desensitize the rod response; after a 2-second interval, the second flash was presented through a specific filter (400 nm for blue cone and 500 nm for red/green cone) to the retina. In this study, the flash intensity was held constant at $3.1 \log \text{cd} \cdot \text{s/m}^2$, and the interflash interval was kept at 2 seconds. Mice were not dark adapted before recording. Each response represented the average of 10 stimulating flashes at the same intensity and filter setting. At least two responses were recorded for each filter to make sure the ERG system was stable. Statistical significance was determined by Student's *t*-test (two samples) or ANOVA with Bonferroni post hoc test (more than two samples). ERG amplitudes are presented as averages $\pm$ SEM.

RESULTS

Transgene Generation and Protein Expression

Several transgenic lines were generated and characterized; two have been selected for extensive study. The first was the H-R172W line, which expressed the transgene at approximately 75% of the WT level and showed a severe, early-onset, dominant cone-rod dystrophy.²⁵ Here we describe the phenotype in an additional line (L-R172W) that expressed the transgene at approximately 40% of the WT level.

The first step in characterizing the L-R172W line was to confirm the expression of the transgenic protein. Retinas from L-R172W mice were taken at postnatal day (P) 30 and used for Northern and Western blot analyses to document the presence of the R172W transcript and protein, respectively. As shown in Figure 1, the R172W transcript (Fig. 1A) and protein (Fig. 1B) were present in retinas from transgenic mice of multiple genetic backgrounds. Based on Western blot analysis of nonreducing gels, R172W protein in L-R172W animals was capable of forming dimers and higher-order complexes, even in the absence of native Rds. Although Rom-1 was nearly undetectable in the *rds*^{-/-} retina, the presence of R172W protein led to a significant increase in the level of Rom-1 (Fig. 1B, right). Based on previously published quantitative analysis,²⁵ animals homozygous for the L-R172W transgene on the *rds* null background expressed the R172W Rds protein at approximately 40% of the endogenous Rds protein level. To confirm that the IRBP promoter was capable of driving transgene expression in rods and cones, immunohistochemical labeling of R172W protein from L-R172W mice was undertaken. As shown in Figure 1C, R172W protein was found in rods and cones (labeled for S-opsin).

L-R172W Mice Exhibit a Dominant, Late-Onset Cone Dystrophy in the Wild-Type Background

Because the human R172W phenotype was dominant, our next step was to examine whether the L-R172W model was associated with a dominant effect on retinal structure or function. L-R172W eyes at 12 months of age were examined histologically and morphometrically. As shown in Figure 2A, at 12 months of age, the R172W protein had no apparent structural effect on the retina; this was confirmed by morphometric analysis. Next, electroretinograms from dark-adapted animals were used to examine rod function. Consistent with the lack of a structural effect on the rods, ERG analysis (Fig. 2B) indicated that, at 6 months of age, L-R172W mice exhibited scotopic a-wave amplitudes well within the normal range for rod function. Although at 12 months of age the values decreased, they did not diverge statistically from the WT range.

To test the possibility that cone structure and function were adversely affected in L-R172W animals, we used immunohistochemistry to quantify cone nuclei at 12 months and light-adapted spectral electroretinography to examine function at multiple time points. There was a statistically significant (25%) decrease in the number of blue (S) cones found in the retinas of 12-month-old L-R172W animals (Fig. 2C). A similar decrease was observed for red/green (L/

M) cones (not shown). This deficit corresponded with a significant decrease in the function of blue cones (approximately 25%) and red/green cones (approximately 20%), as measured by spectral electroretinograms (Fig. 2D), even though these mice have two native Rds alleles. This functional alteration was only observed in 12-month-old animals (the latest age tested).

Cone Degeneration in L-R172W Is Accelerated in the *rds*^{+/-} Background

Because humans affected by the R172W mutation have one WT and one mutant allele, we studied retinal structure and function of L-R172W animals in the *rds* hemizygous background. As shown in Figure 3A, in some L-R172W/*rds*^{+/-} mice, retinal structure at 12 months of age was slightly improved when compared with *rds*^{+/-} (6–7 vs. 3–4 rows of outer nuclear layer [ONL] nuclei, respectively, in the example shown). However, detailed morphometric analysis indicated no generalized improvement—that is, L-R172W/*rds*^{+/-} mice, as a group, did not have more photoreceptor nuclei (per 100-μm area) than nontransgenic littermates at 12 months of age (L-R172W/*rds*^{+/-} 124 ± 12 nuclei/100 μm vs. *rds*^{+/-} 129 ± 13 nuclei/100 μm; *n* = 6). To test the effect of the R172W protein on rod function in the *rds*^{+/-} mice, we examined scotopic ERG amplitudes. At early time points (2 months), the presence of the L-R172W protein seemed beneficial to rod function: L-R172W/*rds*^{+/-} mice had significantly larger scotopic a-wave amplitudes than age-matched *rds*^{+/-} controls (221.4 ± 47.4 μV vs. 149.0 ± 17.0 μV, respectively; *P* < 0.05). Consistent with observations of human populations, L-R172W mice in the hemizygous background did not exhibit rod-dominant degeneration. Long-term rod degeneration in L-R172W/*rds*^{+/-} mice was consistent with the standard haploinsufficiency phenotype seen in *rds*^{+/-} mouse retinas.

On examination of cone function, it was determined that the late-onset defect we observed in the WT background (Fig. 2D) was accelerated in L-R172W animals hemizygous for *rds* (Fig. 3B). These animals exhibited approximately 20% decreases in blue and red/green cone ERG function at 9 months compared with *rds*^{+/-} mice. Compared with WT mice, the decrease in cone ERG amplitudes was even more pronounced; blue cone function was decreased by approximately 45% compared with age-matched WT animals, even though the quantity of Rds protein was approximately equivalent between the two groups (two L-R172W alleles plus one native Rds allele approximately equivalent two native Rds alleles).

R172W Rds Does Not Promote Outer Segment Formation

Given our observations that L-R172W can improve rod structure in the hemizygous background (albeit without permanent functional rescue) and that L-R172W stabilizes Rom-1 expression in the absence of native Rds, we proceeded to test the hypothesis that the L-R172W protein could support the formation of photoreceptor outer segments in the *rds*^{-/-} (null) background. A structural and functional developmental study was undertaken; eyes were collected from L-R172W/*rds*^{-/-} and *rds*^{-/-} mice at P10, P20, and P30. The eyes were examined histologically (Fig. 4), but, in spite of its biochemical similarity to native Rds in terms of complex formation with itself and Rom-1 (as shown in Fig. 1 and Ding et al.²⁵), the amount of R172W protein expressed in L-R172W animals, even dysmorphic ones, was not capable of supporting the formation of outer segments during ocular development. Even though they have similar quantities of Rds protein, the phenotype in L-R172W/*rds*^{-/-} mice (no outer segments) was significantly worse than in *rds*^{+/-} mice (retention of dysmorphic outer segments even at 1 year of age; see Fig. 3A). Finally, ERG analysis indicated that L-R172W/*rds*^{-/-} animals did not have more function than *rds*^{-/-} mice (not shown).

Genetic Supplementation of Wild-Type Rds to Rescue the Phenotype in the R172W Retina

Our next step was to test the hypothesis that gene transfer would be a suitable strategy for treating the R172W disease phenotype. For these proof-of-principle experiments, we chose to use the higher-expresser line, H-R172W, because the severe phenotype can be detected at

earlier time points than the more clinically relevant phenotype in the lower-expresser line (L-R172W). Genetic supplementation was our chosen gene transfer method; we used the Rds overexpressing transgenic mouse line known as NMP (normal mouse peripherin/Rds). This mouse line has been fully characterized elsewhere.²⁶ Briefly, the NMP transgene is expressed in rods and cones, is not toxic to the photoreceptor, and has been used effectively to rescue the *rds*^{+/-} haploinsufficiency phenotype and the loss-of-function model expressing the C214S mutation in Rds. Mice homozygous for the transgene express NMP protein at approximately 60% of WT levels.²⁶ Heterozygotes of single (H-R172W^{+/-}/*rds*^{+/-}) and double (H-R172W^{+/-}/NMP^{+/-}/*rds*^{+/-}) transgenic mice were generated on an *rds*^{+/-} genetic background, and structural and functional rescue of the H-R172W disease phenotype by NMP were assessed at multiple time points.

Light microscopy (Fig. 5A) indicated that, when the animals were 3 months of age, supplementation with NMP led to grossly normal retinal morphology and rescue of ONL thickness (in the H-R172W/*rds*^{+/-} background). Correlative electron microscopy indicated that supplementation with NMP could restore normal rod outer segment (ROS) structures. However, this structural rescue was not sustained when the animals were 11 months of age (data not shown). As previously reported, the NMP^{+/-}/*rds*^{+/-} retinas (shown with WT as controls in Fig. 5B) exhibited infrequent abnormalities in ROS ultrastructure as a result of relative Rds underexpression (approximately 80% of WT) but showed normal ONL thickness.²⁶

To examine the ability of NMP to rescue the structural defect seen in cones, retinas from 3-month-old mice were immunogold labeled with antibodies to S-cone opsin. Figure 6 shows representative images of immunogold-positive labeling in cone, but not rod, photoreceptor outer segments. As shown in Figure 6A, supplementation with NMP led to a remarkable rescue of cone outer segment (COS) structural defects associated with the dominant effect of the R172W mutation. Double-transgenic (R172W^{+/-}/NMP^{+/-}/*rds*^{+/-}) retinas generated compact, well-aligned, left-eye disks, and, though not fully returned to the morphology seen in WT retinas (Fig. 6B), they were significantly improved when compared with the retinal morphology observed in single-transgenic mice (R172W^{+/-}/*rds*^{+/-}). In marked contrast to the relatively normal H-R172W ROS structure (Fig. 5A), the devastating effect of R172W on cones is evident from the severely degenerated COS structure (Fig. 6A) in these animals.

To determine the functional relevance of NMP-mediated structural rescue of H-R172W^{+/-}/*rds*^{+/-} mice, ERG studies were undertaken. Figure 7A shows representative scotopic and photopic ERG wave forms from single- and double-transgenic mice taken at 1 month of age. As reported previously, the introduction of the H-R172W transgene onto an *rds*^{+/-} background (H-R172W^{+/-}/*rds*^{+/-}) alleviates rod functional defects associated with the haploinsufficiency phenotype as early as 1 month of age,²⁵ whereas expression of the R172W mutant protein causes a significant ($P < 0.05$) reduction in cone function (Fig. 7B). This early-onset phenotype makes H-R172W a perfect model for our rescue studies. In H-R172W mice, the enhancement in rod function persisted over time, whereas cone function continued to decline in an age-dependent manner. As shown in Figure 7B, the addition of one NMP allele significantly improved rod and cone ERG amplitudes; at 1 month of age, NMP/H-R172W double-transgenic mice had scotopic and photopic amplitudes (90%–91% of WT), significantly higher than H-R172W single-transgenic mice (71% and 50% of WT, respectively). Rescue continued in the short term; at 3 months of age, H-R172W animals carrying an NMP allele had scotopic and photopic ERG amplitudes 82% and 73% of WT levels, respectively, whereas H-R172W animals without NMP continued to degenerate (56% vs. 34% of WT responses, respectively). At later time points (11 months), we found double-transgenic (H-R172W^{+/-}/NMP^{+/-}/*rds*^{+/-}) animals continued to have significantly ($P < 0.05$) better rod function than single-transgenic (R172W^{+/-}/*rds*^{+/-}) mice (Fig. 7C, left panel). Although Rds supplementation showed a

statistically significant ($P < 0.001$) amelioration of the cone defect in H-R172W^{+/-}/*rds*^{+/-} mice at 1 and 3 months of age (Fig. 7B), this rescue did not persist over the long-term, and cone ERG amplitudes in the H-R172W^{+/-}/*NMP*^{+/-}/*rds*^{+/-} were not different from H-R172W^{+/-}/*rds*^{+/-} by the age of 11 months (Fig. 7C, right panel).

DISCUSSION

Here we present the phenotype of a transgenic line (L-R172W) that expresses the R172W mutation in Rds at approximately 40% of the WT level. We first confirmed the presence of the R172W transcript and protein level in this line. Functionally, L-R172W mice in the WT background did not exhibit rod-dominant degeneration at either 6 or 12 months compared with WT controls, an observation supported by a lack of morphometric change in the ONL. Conversely, cone function and numbers were significantly reduced in mice carrying the R172W allele. In the *rds*^{+/-} background, L-R172W cone degeneration was accelerated (compared with the WT background), but again no dominant effect was observed in rods. Furthermore, the presence of the R172W protein could not rescue the *rds* null phenotype during development and failed to support the formation of photoreceptor outer segments. The L-R172W mouse exhibited a phenotype remarkably similar to that seen in patients carrying the R172W mutation. In the second part of our study, we used the H-R172W line to determine whether genetic supplementation of WT Rds (NMP) would lead to functional or structural rescue of the early-onset dominant phenotype. We observed significant structural and functional rescue of rods and cones at early time points (1–3 months), but at a later time point (11 months), cone rescue was not sustained and rod rescue was only partially sustained.

Our early observation of the R172W protein as incapable of providing significant structural rescue to the *rds*^{-/-} retina merits further discussion. In our previous work,²⁵ we reported that at early time points, the R172W protein from the H-R172W model was capable of mediating significant structural rescue of ROS in the *rds*^{-/-} background. H-R172W^{+/-}/*rds*^{-/-} retinas have outer segments that look similar to those in the *rds*^{+/-} mice. This partial rescue could be interpreted as a positive function of the R172W allele in rods; however, because so much R172W protein is present in that mouse line, the adverse effects of the R172W mutation were seen much earlier, and the cone degeneration was correspondingly more severe. Thus, we may say that the effects of R172W are clearly dose dependent: at low doses (L-R172W), the dominant cone defect appears later in life (9–12 months) and is moderate (approximately 20% reduction in cone function); however, at high doses (H-R172W), the dominant defect appears very early (1 month) and is devastating (approximately 75% reduction in cone function²⁵). These trends persist regardless of the *rds* background and clearly highlight the importance of a physiologically relevant model for studying disease pathogenesis. The H-R172W line was able to provide us with invaluable biochemical information, but the clinically more relevant phenotype of the lower-expresser line (L-R172W) leads to more accurate interpretations of disease progression and mechanism.

In this study, the functional phenotype of the L-R172W mouse model closely resembles that seen in human patients harboring this same RDS mutation. The R172W mutation in humans causes a late-onset disease characterized by visual deficits and a decrease in cone function. Although humans affected by this mutation typically do not exhibit any statistically significant decrease in rod function, there is evidence that their rod function tends to be toward the lower limits of the normal range (Fishman G, personal communication, 1999), similar to that observed in our mouse model. Our morphometric observations indicating a decrease in the number of cone nuclei is a first step toward understanding the mechanism of disease progression in patients with the R172W mutation. The fact that the observation is limited to cones further supports the hypothesis that Rds functions differently in rods versus cones.

Here we observed that cone degeneration occurred in older animals in the WT background but was accelerated in the *rds* hemizygous background. This is probably a result of the dominant effect of the R172W protein combined with the moderate effect of the Rds haploinsufficiency phenotype. Although we observed no dominant effect of the L-R172W on rod function, L-R172W/*rds*^{+/-} animals did exhibit the standard *rds*^{+/-} haploinsufficiency phenotype. In this one aspect, the L-R172W/*rds*^{+/-} diverged from the human phenotype. R172W patients did not exhibit clinical signs of Rds haploinsufficiency; rather, they had pure cone degeneration. This disparity might have occurred because our L-R172W mouse model had slightly less R172W protein than what would be afforded by one WT allele (approximately 40% of total Rds in the mouse vs. 50% in the human). However, this disparity likely resulted from species-specific differences between mice and humans; mice exhibit a decidedly lower tolerance for Rds deficiency than do humans. For example, when examined on an *rds*^{+/-} background, a typical Rds loss-of-function mutation, such as C214S, leads to an early-onset haploinsufficiency phenotype indistinguishable from that seen in *rds*^{+/-} mice.³⁰ Conversely, patients with the C214S mutation exhibit rod defects (e.g., night blindness), but not until later in life (fourth to fifth decade).³¹ Far from being a detriment to our models, the differential requirements of human rods versus mouse rods for Rds is further proof of the underlying differences in the role of Rds in rods and cones. In other words, human rods need less Rds than do mouse rods, whereas human cones and mouse cones need similar quantities of Rds (as evidenced by the similarity in cone-dominant disease patterns in humans and mouse models).

The second portion of our study dealt with characterizing the ability of wild-type Rds (NMP) to rescue the severe cone-rod degeneration seen in the H-R172W model and provided us with valuable information regarding the usefulness of gene transfer therapy to rescue dominant disease phenotypes. In the simplest case, one might hypothesize that genetic supplementation would have no effect on a dominant mutation. However, our data do not support this theory. At early time points with H-R172W, rod degeneration is not dominant. In other words, in terms of rod function, having one allele of H-R172W is better than not having it at all. This phenotype is best explained by hypothesizing that at early time points, R172W, if enough is present, it can support rod function, though not as well as native Rds. Thus, the observation that NMP can rescue rod function at early time points is not unexpected. In fact, the H-R172W^{+/-}/NMP^{+/-}/*rds*^{+/-} rod function looks strikingly similar to the homozygous L-R172W/*rds*^{+/-} phenotype: rod function at the lower end of the normal WT range. What is surprising is that the severe, early-onset, dominant-cone degeneration observed in the H-R172W^{+/-}/*rds*^{+/-} can be rescued almost completely. For example, at 1 month of age, the H-R172W cone degeneration is a completely dominant effect (not associated with haploinsufficiency), yet the presence of one allele of NMP provides considerable rescue of cone function (approximately 90% of WT). This observation is exciting from a mechanistic point of view and begs the question; how does NMP overcome the dominant structural and functional effect of H-R172W in cones?

From a gene therapy perspective, however, our gene transfer strategy is less promising. For a gene therapy to be clinically viable, rescue must be long term. Our data clearly show that over the long term, the presence of WT Rds does not provide rescue to cone function. That rescue persists to a certain level in rods is of little clinical usefulness because rod degeneration is not associated with the R172W phenotype in humans. Even with the persistent partial rescue in rods combined with observations at later time points, H-R172W/*rds*^{+/-} has equal or better rod function than *rds*^{+/-} (see Fig. 7 and our previous work²⁵), which supports our contention that the R172W mutation does not have a dominant effect on rods in the traditional sense.

Interestingly, however, evidence suggests that the R172W allele may have a small, virtually undetectable dominant effect on the rod. First, the long-term rod ERG in L-R172W animals and R172W patients is at the far lower end of the normal range, though the divergence from normal is not statistically significant. Second, though NMP-mediated rescue of rods in the H-

R172W line is certainly a more efficient rescue than of cones over time, this rescue also declines (Figs. 7C-D), in marked contrast to the rescuing ability of NMP in both the *rds*^{+/-} and in the C214S/*rds*^{+/-} loss-of-function mutation in which rescue persists at similar levels throughout the life of the animal.³² All these observations suggest that, though gene transfer therapy of the WT Rds may not be a suitable strategy for R172W patients, understanding the reasons for the differential requirement of rods and cones for Rds and the subtle differences in the effect of the R172W mutation in rods and cones is critical for the design of any form of effective treatment. Future rescue studies of the R172W model will likely make use of gene replacement with concurrent gene knockdown approaches. Studies on other dominant retinal degenerations (e.g., the P23H rhodopsin mutation) have used ribozyme^{33,34} or small interfering or small hairpin RNA (siRNA/shRNA) approaches as a way to decrease expression of a dominant mutant protein.³⁵⁻³⁷ Similar approaches could be used to overcome gain-of-function mutations in Rds. Because the dose of normal and mutant Rds is critical to disease phenotype and rescue, treatments will have to be fine-tuned to deliver the appropriate amount of therapeutic vector.

Here we have characterized a mouse model of cone dystrophy associated with the R172W mutation (L-R172W), which closely mimics the late-onset cone dystrophy seen in human patients harboring this mutation. Although gene replacement therapy alone is not likely to be an effective treatment strategy for R172W-associated macular degeneration, the temporary cone rescue we observed after supplementation with NMP raises exciting questions about the mechanism of the R172W-mediated degeneration. Future studies with this model in native and cone-dominant (*Nrl*^{-/-}) retinas may enable us to gain greater understanding of the pathogenesis and intricacies of dominant cone dystrophies and may help us to elucidate some of the underlying reasons for differences in rod/cone biogenesis and degeneration.

Acknowledgements

The authors thank Chibo Li for generating the transgenic mice used in this study, Huijun Yang and Barbara Nagel for their exceptional technical assistance with histology, and Mark Ballard for his assistance with the manuscript.

References

1. Arikawa K, Molday LL, Molday RS, Williams DS. Localization of peripherin/rds in the disk membranes of cone and rod photoreceptors: relationship to disk membrane morphogenesis and retinal degeneration. *J Cell Biol* 1992;116:659–667. [PubMed: 1730772]
2. Downes SM, Fitzke FW, Holder GE, et al. Clinical features of codon 172 RDS macular dystrophy: similar phenotype in 12 families. *Arch Ophthalmol* 1999;117:1373–1383. [PubMed: 10532447]
3. Payne AM, Downes SM, Bessant DA, Bird AC, Bhattacharya SS. Founder effect, seen in the British population, of the 172 peripherin/RDS mutation-and further refinement of genetic positioning of the peripherin/RDS gene. *Am J Hum Genet* 1998;62:192–195. [PubMed: 9443872]
4. Piguet B, Héon E, Munier FL, et al. Full characterization of the maculopathy associated with an Arg-172-Trp mutation in the RDS/peripherin gene. *Ophthalmic Genet* 1996;17:175–186. [PubMed: 9010868]
5. Wroblewski JJ, Wells JA 3rd, Eckstein A, et al. Macular dystrophy associated with mutations at codon 172 in the human retinal degeneration slow gene. *Ophthalmology* 1994;101:12–22. [PubMed: 8302543]
6. Cheng T, Peachey NS, Li S, Goto Y, Cao Y, Naash MI. The effect of peripherin/rds haploinsufficiency on rod and cone photoreceptors. *J Neurosci* 1997;17:8118–8128. [PubMed: 9334387]
7. Ding XQ, Stricker HM, Naash MI. Role of the second intradiscal loop of peripherin/rds in homo and hetero associations. *Biochemistry* 2005;44:4897–4904. [PubMed: 15779916]
8. Goldberg AF, Molday RS. Expression and characterization of peripherin/rds-rom-1 complexes and mutants implicated in retinal degenerative diseases. *Methods Enzymol* 2000;316:671–687. [PubMed: 10800708]

9. Tam BM, Moritz OL, Papermaster DS. The C terminus of peripherin/rds participates in rod outer segment targeting and alignment of disk incisures. *Mol Biol Cell* 2004;15:2027–2037. [PubMed: 14767063]
10. Molday RS, Hicks D, Molday L. Peripherin: a rim-specific membrane protein of rod outer segment discs. *Invest Ophthalmol Vis Sci* 1987;28:50–61. [PubMed: 2433249]
11. Travis GH, Groshan KR, Lloyd M, Bok D. Complete rescue of photoreceptor dysplasia and degeneration in transgenic retinal degeneration slow (rds) mice. *Neuron* 1992;9:113–119. [PubMed: 1385966]
12. Travis GH, Sutcliffe JG, Bok D. The retinal degeneration slow (rds) gene product is a photoreceptor disc membrane-associated glycoprotein. *Neuron* 1991;6:61–70. [PubMed: 1986774]
13. Poetsch A, Molday LL, Molday RS. The cGMP-gated channel and related glutamic acid-rich proteins interact with peripherin-2 at the rim region of rod photoreceptor disc membranes. *J Biol Chem* 2001;276:48009–48016. [PubMed: 11641407]
14. Boesze-Battaglia K. Fusion between retinal rod outer segment membranes and model membranes: functional assays and role for peripherin/rds. *Methods Enzymol* 2000;316:65–86. [PubMed: 10800669]
15. Boesze-Battaglia K, Goldbereg AF, Dispoto J, Katragadda M, Cesarone G, Albert AD. A soluble peripherin/Rds C-terminal polypeptide promotes membrane fusion and changes conformation upon membrane association. *Exp Eye Res* 2003;77:505–514. [PubMed: 12957149]
16. Boesze-Battaglia K, Lambda OP, Napoli AA Jr, Sinha S, Guo Y. Fusion between retinal rod outer segment membranes and model membranes: a role for photoreceptor peripherin/rds. *Biochemistry* 1998;37:9477–9487. [PubMed: 9649331]
17. Boesze-Battaglia K, Stefano FP. Peripherin/rds fusogenic function correlates with subunit assembly. *Exp Eye Res* 2002;75:227–231. [PubMed: 12137768]
18. Boesze-Battaglia K, Song H, Sokolov M, et al. The tetraspanin protein peripherin-2 forms a complex with melanoregulin, a putative membrane fusion regulator. *Biochemistry* 2007;46:1256–1272. [PubMed: 17260955]
19. Farjo R, Skaggs JS, Nagel BA, et al. Retention of function without normal disc morphogenesis occurs in cone but not rod photoreceptors. *J Cell Biol* 2006;173:59–68. [PubMed: 16585269]
20. Daniele LL, Lillo C, Lyubarsky AL, et al. Cone-like morphological, molecular, and electrophysiological features of the photoreceptors of the Nrl knockout mouse. *Invest Ophthalmol Vis Sci* 2005;46:2156–2167. [PubMed: 15914637]
21. Wells J, Wroblewski J, Keen J, et al. Mutations in the human retinal degeneration slow (RDS) gene can cause either retinitis pigmentosa or macular dystrophy. *Nat Genet* 1993;3:213–218. [PubMed: 8485576]
22. Nakazawa M, Wada Y, Tamai M. Macular dystrophy associated with monogenic Arg172Trp mutation of the peripherin/RDS gene in a Japanese family. *Retina* 1995;15:518–523. [PubMed: 8747448]
23. Kikawa E, Nakazawa M, Chida Y, Shiono T, Tamai M. A novel mutation (Asn244Lys) in the peripherin/RDS gene causing autosomal dominant retinitis pigmentosa associated with bull's-eye maculopathy detected by nonradioisotopic SSCP. *Genomics* 1994;20:137–139. [PubMed: 8020945]
24. Nakazawa M, Kikawa E, Chida Y, Tamai M. Asn244His mutation of the peripherin/RDS gene causing autosomal dominant cone-rod degeneration. *Hum Mol Genet* 1994;3:1195–1196. [PubMed: 7981698]
25. Ding XQ, Nour M, Ritter LM, Goldberg AF, Fliesler SJ, Naash MI. The R172W mutation in peripherin/rds causes a cone-rod dystrophy in transgenic mice. *Hum Mol Genet* 2004;13:2075–2087. [PubMed: 15254014]
26. Nour M, Ding XQ, Stricker H, Fliesler SJ, Naash MI. Modulating expression of peripherin/rds in transgenic mice: critical levels and the effect of overexpression. *Invest Ophthalmol Vis Sci* 2004;45:2514–2521. [PubMed: 15277471]
27. Li C, Ding XQ, O'Brien J, Al-Ubaidi MR, Naash MI. Molecular characterization of the skate peripherin/rds gene: relationship to its orthologues and paralogues. *Invest Ophthalmol Vis Sci* 2003;44:2433–2441. [PubMed: 12766040]
28. Tan E, Wang Q, Quiambao AB, et al. The relationship between opsin overexpression and photoreceptor degeneration. *Invest Ophthalmol Vis Sci* 2001;42:589–600. [PubMed: 11222515]

29. Nour M, Quiambao AB, Peterson WM, Al-Ubaidi MR, Naash MI. P2Y(2) receptor agonist INS37217 enhances functional recovery after detachment caused by subretinal injection in normal and rds mice. *Invest Ophthalmol Vis Sci* 2003;44:4505–4514. [PubMed: 14507899]
30. Stricker HM, Ding XQ, Quiambao A, Fliesler SJ, Naash MI. The Cys214 → Ser mutation in peripherin/rds causes a loss-of-function phenotype in transgenic mice. *Biochem J* 2005;388:605–613. [PubMed: 15656787]
31. Saga M, Mashima Y, Akeo K, Oguchi Y, Kudoh J, Shimizu N. A novel Cys-214-Ser mutation in the peripherin/RDS gene in a Japanese family with autosomal dominant retinitis pigmentosa. *Hum Genet* 1993;92:519–521. [PubMed: 8244346]
32. Nour, M.; Fliesler, S.J.; Naash, M.I. Genetic supplementation of Rds alleviates a loss-of-function phenotype in the C214S model of retinitis pigmentosa. In: Anderson, R.E.; LaVail, M.M.; Hollyfield, J.G., editors. *Recent Advances in Retinal Degeneration Research*. New York: Springer; In press
33. LaVail MM, Yasumura D, Matthes MT, et al. Ribozyme rescue of photoreceptor cells in P23H transgenic rats: long-term survival and late-stage therapy. *Proc Natl Acad Sci USA* 2000;97:11488–11493. [PubMed: 11005848]
34. Lewin AS, Drenser KA, Hauswirth WW, et al. Ribozyme rescue of photoreceptor cells in a transgenic rat model of autosomal dominant retinitis pigmentosa. *Nat Med* 1998;4:967–971. [PubMed: 9701253]
35. O'Reilly M, Palfi A, Chadderton N, et al. RNA interference-mediated suppression and replacement of human rhodopsin in vivo. *Am J Hum Genet* 2007;81:127–135. [PubMed: 17564969]
36. Tessitore A, Parisi F, Denti MA, et al. Preferential silencing of a common dominant rhodopsin mutation does not inhibit retinal degeneration in a transgenic model. *Mol Ther* 2006;14:692–699. [PubMed: 16979943]
37. Gorbatyuk M, Justilien V, Liu J, Hauswirth WW, Lewin AS. Suppression of mouse rhodopsin expression in vivo by AAV mediated siRNA delivery. *Vision Res* 2007;47:1202–1208. [PubMed: 17292939]

Figure 1.

Expression profile of L-R172W. Eyes from L-R172W animals were examined at P30 for transgene expression. (A) Northern blot showing that L-R172W mRNA is expressed in transgenic animals with multiple backgrounds, as indicated by RDS-specific probes (*left*) and transgene-specific (SV40) probes (*right*). 28s RNA (*bottom*) is shown as a loading control. Two standard RDS transcripts are detected in all samples, whereas *rds^{+/-}* animals also have the larger RDS null transcript. Only transgenic animals are positive for SV40 transcripts. (B) Reducing and nonreducing Western blot analysis probed with antibodies against the C-terminal regions of Rds and Rom-1. Immunoreactive bands at M_r 34 kDa represent Rds/Rom-1 monomers, whereas higher bands represent dimers and oligomers. L-R172W is expressed on the M_r protein level and is capable of stabilizing Rom-1 levels in the absence of native Rds. (C) Immunohisto-chemistry (confocal image of left-eye region) of a L-R172W adult (P30) retina was stained with antibody to S-cone opsin and Cy3-conjugated secondary antibody (to label cones *red*) and with monoclonal antibody 3B6 and FITC-conjugated secondary antibody (to label R172W transgenic protein *green*). In L-R172W mice, the R172W protein is expressed in cones and rods.

Figure 2.

Evaluation of L-R172W retinal structure and function in the WT background. **(A)** Histologic and morphometric analyses indicate that, at 12 months of age, L-R172W does not affect rod structure. Scale bar, 20 μm . **(B)** Serial scotopic ERG analyses at 6 months (6M) and 12 months (12M) show that rod function and sensitivity are not significantly altered by the presence of L-R172W. Dashed lines ($+/+$) indicate the upper and lower limits of the normal range (based on SD) of WT responses. **(C)** Immunohistochemical analysis (using S-cone opsin antibody) indicates that presence of L-R172W leads to a statistically significant decrease in the number of blue cones. **(D)** Spectral ERG with 400-nm light (S-cones) and 500-nm light (L/M cones) was conducted on L-R172W and age-matched WT animals. L-R172W animals exhibited a late-onset cone defect (* $P < 0.05$). Shaded column: WT. Open columns: L-R172W. $n = 5$ to 7 animals per time point and genotype.

Figure 3.

Evaluation of L-R172W retinal structure and function in the *rds*^{+/-} background. Because the L-R172W/*rds*^{+/-} is the model most representative of the human condition in terms of protein levels, we analyzed retinal structure and function in this model. **(A)** Histologic analysis of 12-month-old R172W/*rds*^{+/-} retinas shows moderate structural rescue of the rods compared with *rds*^{+/-}. Scale bar, 20 μm. **(B)** ERG amplitudes recorded with 400-nm light (S-cones) and 500-nm light (L/M-cones) indicate that L-R172W/*rds*^{+/-} is associated with statistically significant, dominant, late-onset cone degeneration (both S and L/M-cones). This degeneration occurs sooner in the hemizygous background than in the WT (see Fig. 2). *Shaded columns*: hemizygous. *Open columns*: L-R172W. **P* < 0.05 for differences between *rds*^{+/-} and L-R172W/*rds*^{+/-}.

Figure 4.

Evaluation of L-R172W retinal structure in the absence of endogenous Rds. Retinal structure of L-R172W/*rds*^{-/-} and age-matched *rds*^{-/-} retinas was examined by light microscopy during development. *Rds*^{-/-} mice did not make outer segments; note that the presence of L-R172W did not improve retinal development or enable the formation of outer segments. Scale bar, 20 μ m.

Figure 5.

Evaluation of structural rescue by NMP in R172W transgenic mice at 3 months of age. Supplementation of Rds to H-R172W transgenic mice restored left-eye histologic appearance at both the light microscopic (*upper panels*) and the electron microscopic (*lower panels*) levels. (A) Ultrastructural analysis reveals an improvement in OS alignment and integrity in double-transgenic retinas (H-R172W^{+/-}/NMP^{+/-}/rds^{+/-}) when compared with single-transgenic retinas (H-R172W^{+/-}/rds^{+/-}). (B) WT, rds^{+/-} and NMP^{+/-}/rds^{+/-} images are shown as controls. All tissue sections were taken from 3-month-old animals and evaluated at the superior central region of the retina. Scale bars: light microscopic, 20 μ m; electron microscopic, 4 μ m.

Figure 6. Ultrastructural and immunogold analysis of rod and cone outer segment structure at 3 months of age. Electron microscopic immunogold analysis using anti-cone opsin antibody. Retinas from all genotypes show labeling of cone, but not rod, outer segments. (A) Cone outer segments (*asterisks*) are grossly malformed in H-R172W^{+/}/rdst^{+/} transgenic retinas. However, expression of Rds in H-R172W^{+/}/NMP^{+/}/rdst^{+/} double-transgenic mice dramatically restored alignment and organization of cone outer segment structure. (B) WT, rdst^{+/} and NMP^{+/}/rdst^{+/} images are shown as controls. Scale bar, 1 μ m.

Figure 7.

Rescue of rod and cone ERG function in H-R172W transgenic mice. (A) Representative rod and cone ERG wave forms at 1 month of age illustrate the improvement in rod and rescue in cone function ($P < 0.001$) in double-transgenic (H-R172W^{+/-}/NMP^{+/-}/rds^{+/-}) compared with single-transgenic (H-R172W^{+/-}/rds^{+/-}) mice. (B) Rod a-wave and cone b-wave averages show a significant enhancement in rod function ($P < 0.05$) and rescue in cone function ($P < 0.001$) in double-transgenic (H-R172W^{+/-}/NMP^{+/-}/rds^{+/-}) compared with single-transgenic (H-R172W^{+/-}/rds^{+/-}) mice at 1 month of age. At 3 months of age, enhancement in rod function and significant ($P < 0.001$) improvement in cone function resulting from the supplementation of WT Rds in H-R172W transgenic mice (H-R172W^{+/-}/NMP^{+/-}/rds^{+/-}) persisted. (C) Rod a-

wave and cone b-wave averages at 11 months of age in H-R172W mice demonstrate a significant ($P < 0.001$) rescue in rod function in double-transgenic (H-R172W^{+/-}/NMP^{+/-}/*rds*^{+/-}) compared with single-transgenic (H-R172W^{+/-}/*rds*^{+/-}) mice. However, at this age, double-transgenic mice lost their rescue of cone function provided by NMP supplementation. ERG wave amplitudes for **(B)** represent an average of 12 to 16 eyes for each genotype, and ERG amplitudes for **(D)** represent 8 eyes for each genotype.