Gene Therapy for Retinitis Pigmentosa Caused by *MFRP* Mutations: Human Phenotype and Preliminary Proof of Concept

Astra Dinculescu,¹ Jackie Estreicher,² Juan C. Zenteno,³ Tomas S. Aleman,² Sharon B. Schwartz,² Wei Chieh Huang,² Alejandro J. Roman,² Alexander Sumaroka,² QiuHong Li,¹ Wen-Tao Deng,¹ Seok-Hong Min,¹ Vince A. Chiodo,¹ Andy Neeley,¹ Xuan Liu,¹ Xinhua Shu,⁴ Margarita Matias-Florentino,³ Beatriz Buentello-Volante,³ Sanford L. Boye,¹ Artur V. Cideciyan,² William W. Hauswirth,¹ and Samuel G. Jacobson²

Abstract

Autosomal recessive retinitis pigmentosa (RP), a heterogeneous group of degenerations of the retina, can be due to mutations in the *MFRP* (membrane-type frizzled-related protein) gene. A patient with RP with *MFRP* mutations, one of which is novel and the first splice site mutation reported, was characterized by noninvasive retinal and visual studies. The phenotype, albeit complex, suggested that this retinal degeneration may be a candidate for gene-based therapy. Proof-of-concept studies were performed in the *rd6 Mfrp* mutant mouse model. The fast-acting tyrosine-capsid mutant AAV8 (Y733F) vector containing the small chicken β -actin promoter driving the wild-type mouse *Mfrp* gene was used. Subretinal vector delivery on postnatal day 14 prevented retinal degeneration. Treatment rescued rod and cone photoreceptors, as assessed by electroretinography and retinal histology at 2 months of age. This AAV-mediated gene delivery also resulted in robust MFRP expression predominantly in its normal location within the retinal pigment epithelium apical membrane and its microvilli. The clinical features of *MFRP*-RP and our preliminary data indicating a response to gene therapy in the *rd6* mouse suggest that this form of RP is a potential target for gene-based therapy.

Introduction

THE RETINAL PIGMENT EPITHELIUM (RPE) plays a critical role in vision, maintaining the structural integrity, function, and survival of photoreceptor cells (Sparrow *et al.*, 2010). On its apical side, the RPE extends numerous microvilli around the light-sensitive photoreceptor outer segments and into the interphotoreceptor matrix. Microvilli considerably increase the surface area of the RPE apical membrane, and establish a critical interface for many RPE functions including phagocytosis of shed outer segments, visual chromophore transport and regeneration, production of trophic and anti-angiogenic factors, directional transport of oxygen and nutrients from the choroid to sustain the high metabolic rate of photoreceptors, and removal of water and aqueous waste

products from the subretinal space (Strauss, 2005; Bonilha *et al.*, 2006). Mutations in genes expressed in RPE cells can result in retinal degeneration and loss of vision (Sparrow *et al.*, 2010). Some of these include *RPE65* and *LRAT* (Leber congenital amaurosis, LCA), *MERTK* (early-onset retinitis pigmentosa, RP), *BEST1* (Best disease), *TIMP3* (Sorsby fundus dystrophy), *EFEMP1* (malattia leventinese), *RDH5* (fundus albipunctatus), and *RLBP1* (retinitis punctata albescens).

A relative newcomer to this group is autosomal recessively inherited RP caused by mutations in the human *MFRP* (membrane-type frizzled related protein) gene, located on chromosome 11q23 (Ayala-Ramirez *et al.*, 2006; Crespi *et al.*, 2008; Zenteno *et al.*, 2009; Mukhopadhyay *et al.*, 2010). The *MFRP* gene encodes a type II transmembrane protein of 584 amino acid residues, which consists of an N-terminal

¹Department of Ophthalmology, University of Florida, Gainesville, FL 32610.

²Scheie Eye Institute, Department of Ophthalmology, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA 19104.

³Biochemistry Department, Faculty of Medicine, National Autonomous University of Mexico (UNAM); and Department of Genetics-Research Unit, Institute of Ophthalmology Conde de Valenciana, Mexico City, CP 06800, Mexico.

⁴Department of Life Sciences, Glasgow Caledonian University, Glasgow G4 0BA, Scotland.

cytoplasmic region, a transmembrane domain, and an extracellular region with a C-terminal cysteine-rich domain similar to that observed in Wnt-binding frizzled proteins (Katoh, 2001; Kameya *et al.*, 2002). *Mfrp* is expressed as one element of a dicistronic transcript (Kameya *et al.*, 2002; Hayward *et al.*, 2003; Mandal *et al.*, 2006), which also encodes the complement C1q tumor necrosis factor-related protein-5 (C1QTNF5/CTRP5). *CTRP5* also has a human disease association; specifically, a Ser163Arg mutation causes an autosomal dominant late-onset retina-wide degeneration, which can feature neovascular macular degeneration (Milam *et al.*, 2000; Jacobson *et al.*, 2001; Hayward *et al.*, 2003). The functional relationship between the two proteins remains under investigation (Fogerty and Besharse, 2011).

With clinical trials of gene therapy ongoing for another autosomal recessive RPE disease leading to retinal degeneration, *RPE65-LCA* (reviewed in Cideciyan, 2010), we inquired whether *MFRP-RP* was a potential candidate for this form of treatment. A patient with *MFRP-RP* was examined in detail by noninvasive studies and the results were compared with those of patients with other molecularly proven *MFRP-RP* in the literature. Like *RPE65-LCA*, there is an animal model of the *MFRP-RP* disease for proof-of-concept studies. The *rd6* mouse has a naturally occurring autosomal recessive retinal degeneration associated with a 4-bp deletion in a splice donor site in the *Mfrp* gene (Hawes *et al.*, 2000; Kameya *et al.*, 2002). This results in the skipping of exon 4 and deletion of 58 amino acids from the *Mfrp* protein (Kameya *et al.*, 2002). The present study uses subretinal delivery of a self-complementary tyrosine-capsid mutant adeno-associated virus serotype 8 (AAV8) (Y733F) vector carrying the mouse *Mfrp* gene to determine whether photoreceptor degeneration can be prevented in *rd6* mice, thereby paving the way for further proof-of-concept, dosing, and safety studies in gene therapy en route to a clinical trial in patients with *MFRP-RP*.

Materials and Methods

Human subjects

A patient with RP underwent a complete eye examination and detailed studies of retinal phenotype. Genomic DNA from peripheral blood lymphocytes was obtained from the patient and family members and molecular genetic analyses led to the identification of *MFRP* mutations (Ayala-Ramirez *et al.*, 2006; Crespí *et al.*, 2008; Zenteno *et al.*, 2009). Patients with retinal degenerations, including other forms of RP ($n=6$; age, 7–78 years) and choroideremia ($n=3$; age, 13, 34, and 38 years), as well as normal subjects ($n=34$; age, 5–58), were included for comparison of phenotype. Informed consent was obtained for all subjects; procedures were in accordance with the Declaration of Helsinki and were approved by the institutional review board.

Human phenotype studies

Full-field electroretinograms (ERGs) were performed with bipolar Burian-Allen contact lens electrodes and a standard protocol using an Espion system (Diagnosys, Lowell, MA) with methodology previously described (Jacobson *et al.*, 1989; Aleman *et al.*, 2002). Kinetic visual fields and dark- and light-adapted chromatic static threshold perimetry (200-msec

duration, 650- and 500-nm stimuli, dark-adapted, and 600 nm, light-adapted; 1.7° diameter target) were performed with a modified HFA-750i analyzer (Zeiss-Humphrey, Dublin, CA). Our methods for data collection and analyses have been published (Jacobson *et al.*, 1986, 2010; Roman *et al.*, 2005). Reflectance imaging and reduced-illuminance autofluorescence imaging (RAFI) were performed with near-infrared (NIR) and short-wavelength (SW) lights with a confocal scanning laser ophthalmoscope (SLO) (HRA2; Heidelberg Engineering, Heidelberg, Germany) as described (Cideciyan *et al.*, 2007, 2011; Jacobson *et al.*, 2011). Retinal cross-sectional imaging used a spectral-domain optical coherence tomography (SD-OCT) unit (RTVue-100; Optovue, Fremont, CA) with published recording and analysis techniques (Cideciyan *et al.*, 2011; Jacobson *et al.*, 2011). The three-dimensional SD-OCT raster scans were performed for topographic analysis. Postacquisition processing of OCT data was performed with custom programs (MATLAB 6.5; MathWorks, Natick, MA). Further methodological details are provided in the online supplement (supplementary data are available online at www.liebertonline.com/hum).

Animal studies

rd6 mice (originally provided by B. Chang, Jackson Laboratory, Bar Harbor, ME) were bred and maintained in the University of Florida Health Science Center Animal Care Services Facility (Gainesville, FL) under 12-hr-on/12-hr-off cyclic lighting. Wild-type C57BL/6 mice served as controls and were from the University of Florida or University of Pennsylvania (Philadelphia, PA) Animal Care Services Facility. All experiments were approved by University of Florida and University of Pennsylvania Institutional Animal Care and Use Committees and conducted in accordance with the Association for Research in Vision and Ophthalmology (ARVO) Statement for the Use of Animals in Ophthalmic and Vision Research.

Recombinant AAV preparation and subretinal delivery in mice

A self-complementary AAV8 vector containing a Y733F point mutation in a highly conserved surface-exposed capsid tyrosine residue was used for packaging the wild-type murine *Mfrp* cDNA under the control of the ubiquitous, constitutive smCBA (small chicken β -actin) promoter. AAV8 (Y733F) vector was produced by the two-plasmid cotransfection method in HEK 293 cells and purified according to previously reported methods (Petr-Silva *et al.*, 2009). Viral titer was determined by real-time PCR. Subretinal injections were performed on postnatal day 14 (P14) under anesthesia as described (Pang *et al.*, 2008). In brief, the nasal cornea was penetrated with a 30.5-gauge disposable needle; and a 33-gauge unbeveled, blunt-tip needle on a Hamilton syringe was introduced into the subretinal space. Each eye received 1 μ l of vector at a titer of 1×10^{12} genome copies/ml, leaving the left eye as an untreated control. Preliminary experiments were also performed with buffer-injected *rd6* eyes as possible controls, and it was observed that there was rapid degeneration in these eyes, likely secondary to poor reattachment after retinal detachment. To avoid bias that would lead to an apparent treatment effect due to surgery-related damage to control *rd6* eyes, we chose to use an untreated control eye for

comparison with treatment. Eleven *rd6* mice had subretinal injections of vector in the right eye. At 6 weeks postinjection, mice with no signs of injection-related trauma to the anterior segment were included in further studies.

Electroretinography in mice

Full-field ERGs were elicited in 2-month-old (P14-treated) *rd6* mice ($n=5$) and wild-type C57BL/6 controls ($n=20$; mean age, 3.4 months; age range, 1 to 10 months), using methods previously reported (Roman *et al.*, 2007; Pang *et al.*, 2008; Caruso *et al.*, 2010). A series of stimuli were generated with a UTAS SunBurst system (LKC Technologies, Gaithersburg, MD) or a custom-built ganzfeld stimulator with a computer-based system (EPIC-XL; LKC Technologies). Mice were dark-adapted (>12 hr) and anesthetized by injection of a mixture of ketamine-HCl (72 mg/kg) and xylazine (5 mg/kg). Pupils were dilated with topical agents (phenylephrine hydrochloride, 2.5%; tropicamide, 1%) under dim red light. Dark-adapted ERGs were elicited with 0.02 and 2 scot-cd·sec·m⁻² stimuli. In wild-type mice, the dimmer flash produces a b-wave driven by rod postreceptoral activity whereas the brighter flash produces an a-wave dominated by rod photoreceptor activity and a b-wave that is driven by both rod and cone postreceptoral neurons (Weymouth and Vingrys, 2008). In addition, cone-isolated ERGs were elicited with 25 phot-cd·sec·m⁻² stimuli on a rod-suppressing background light after a preadaptation period. In wild-type mice, this flash produces a b-wave driven by cone postreceptoral neurons (Weymouth and Vingrys, 2008). Efficacy of the unocular treatment in *rd6* eyes was determined by evaluating interocular differences (IODs). IODs for all ERG parameters were expressed as the amplitude difference between the two eyes divided by the mean value. *t* tests were used to determine the statistical significance of differences between IODs of *rd6* mice and wild-type animals.

Retinal histology and immunostaining

For morphological analysis, treated *rd6* mice ($n=4$) had both eyes fixed in 10% formalin solution, processed for paraffin embedding, sectioned at a thickness of 4 μ m, and stained with hematoxylin and eosin. The sections were visualized by light microscopy. Comparisons were made with adult C57BL/6 mice ($n=4$). The outer nuclear layer (ONL) thickness was measured at three locations from within the mid-superior retina of wild-type, treated, and untreated *rd6* eyes (four animals per group). The differences between the ONL thickness of AAV-treated and the uninjected contralateral left eyes were analyzed by Student *t* test for paired samples. A difference was considered significant at $p<0.05$.

For immunostaining, deparaffinized tissue sections were dewaxed in xylene and rehydrated in a graded series of ethanol, and then incubated with 0.5% Triton X-100 for 15 min, followed by blocking with a solution of 2% albumin for 30 min. The sections were incubated with mouse MFRP affinity-purified polyclonal antibody (AF3445, R&D Systems, Minneapolis, MN) or anti-ezrin (Sigma-Aldrich, St. Louis, MO). Secondary antibodies were Alexa-594 or Alexa-488 fluorophore (Molecular Probes/Invitrogen, Eugene, OR) diluted 1:500 in 1× phosphate-buffered saline (PBS). All sections were examined by fluorescence microscopy, using a Leica TCS SP2 laser scanning confocal microscope (Leica,

Heidelberg, Germany). Albino control mouse retinas were used as immunostaining controls to prevent the melanin in the RPE from interfering with the signal.

Western blot analysis

AAV8 (Y733F)-smCBA-MFRP-treated and untreated *rd6* eyes were carefully dissected, and the MFRP protein was detected by Western blotting. The eyecups were homogenized by sonication in 1× PBS containing complete protease inhibitor cocktail (Roche Diagnostics, Mannheim, Germany). After centrifugation, each pellet was resuspended in a buffer containing 50 mM Tris-HCl (pH 7.4), 1% Triton X-100, 2% sodium dodecyl sulfate (SDS), and 10% glycerol, and used for Western blot analysis. Aliquot extracts containing equal amounts of protein were subjected to SDS-polyacrylamide gel electrophoresis (PAGE), using a 4–12% gradient gel, and transferred to polyvinylidene difluoride (PVDF) membranes (Millipore, Bedford, MA). After incubation for 1 hr in Odyssey blocking buffer (Li-Cor, Lincoln, NE), the membranes were probed either with a primary antibody against MFRP (mouse MFRP AF3445; R&D Systems) or an antibody against α -tubulin (rabbit polyclonal ab4074; Abcam, Cambridge, MA) as an internal control. The signals were detected with an infrared IRDye 800 dye-conjugated secondary antibody (Rockland Immunochemicals, Gilbertsville, PA). Visualization of specific bands was performed with the Odyssey infrared fluorescence imaging system (Odyssey; Li-Cor).

Results

MFRP-RP human phenotype

A 19-year-old female patient had spectacle correction in childhood. There were some complaints of night and peripheral vision disturbances as well as difficulty with reading; these visual symptoms were noted mainly from the second decade of life. Ancestry was German/English/French with no known parental consanguinity; parents and siblings had no visual complaints. Visual acuity was 20/30 with a refractive error of +11.00 sphere in each eye. Corneal curvatures, ultrasound A-scan, and anterior chamber depth were 52.00D spherical equivalent (normal mean $\pm$ SD, 43.95 $\pm$ 1.470) (AlMahmoud *et al.*, 2011), 16.4 mm (normal, 23.67 $\pm$ 0.9) (Oliveira *et al.*, 2007), and 2.2 mm (normal, 2.9 $\pm$ 0.3) (Fontana and Brubaker, 1980), respectively. Corneal diameters and intraocular pressures were normal. Near-infrared (NIR) reflectance images of the fundus showed irregular margins of the optic nerve head, and peripheral retinal regions with chorioretinal atrophy and bone spicule-like pigment (Fig. 1A). Autofluorescence imaging with SW and NIR excitation were consistent with a $\sim$ 20° diameter central region of retained RPE with normal or nearly normal signals originating from lipofuscin and melanin fluorophores. Surrounding the central region was an annulus of hyperautofluorescence apparent in both SW- and NIR-RAFI. This annulus could originate from unmasking of existing fluorophores, accumulation of additional fluorophores, or chemical changes affecting their fluorescence efficiency. Surrounding the hyperautofluorescent ring was an intermediate level of autofluorescence like that previously described in other forms of RP (Cideciyan *et al.*, 2011). Optic disc

FIG. 1. Human *MFRP-RP* phenotype. **(A)** *En face* imaging. Digitally stitched wide-field, near-infrared reflectance (NIR-REF) imaging of the patient (*top*). Arrows point to bone spicule-like pigment. Melanin abnormalities are visible on reduced-illumination autofluorescence imaging (RAFI) with NIR light (*bottom left*) and lipofuscin abnormalities are demonstrated on RAFI with short-wavelength (SW) light (*bottom right*). *Insets*: Representative normal images for each modality. Images are individually contrast-stretched for visibility of features. **(B)** Electrophysiology. Standard full-field electroretinograms (ERGs) from a normal subject and a patient with *MFRP-RP*. Rod b-waves were reduced to 4% of mean normal; mixed ERGs had reduced a-waves (11% of normal) and b-waves (7% of normal); and cone ERGs (1 and 30 Hz) were reduced to 35% and 10% of normal, respectively. Stimulus onset was at the start of traces. Calibrations are shown to the right and below the waveforms. **(C)** Psychophysics. Dark- and light-adapted static threshold perimetry results are displayed as grayscale maps of rod and cone sensitivity loss. The physiological blind spot is shown as a black square at 12° in the temporal field. N, T, I, and S, nasal, temporal, inferior, and superior visual field, respectively. Kinetic perimetry results (*inset, top right*) illustrate some visual field constriction (nasally) for the larger target (V-4e) with 70% of normal extent, and a central island with 10% normal extent, using the small target (I-4e). **(D and E)** Thickness topography of total retina, inner retina, and outer nuclear layer mapped in the central retina for normal subjects (**D**, $n=6$; age, 21–41 years) and the patient with *MFRP-RP* (**E**). *Insets* in the lower right-hand corner of patient data indicate whether the thickness measurements are within normal limits (white), abnormally thin (blue, less than 2 SD), or abnormally thickened (pink, greater than 2 SD). **(F–H)** Retinal laminar architecture. Cross-sectional optical coherence tomography (OCT) images along the horizontal meridian through the fovea in a normal subject (**F**) are compared with those of a patient with *MFRP-RP* (**G**). Examples of OCT cross-sections in other retinopathies with abnormal foveal shapes are shown along with measurements of the hyperreflectivity at the vitreoretinal interface in normal subjects; patients with RP with cystoid macular edema (CME) and choroideremia (CHM); and the patient with *MFRP-RP* (**H**). Error bars, 1 SD. *MFRP-RP*, *MFRP* (membrane-type frizzled-related protein) gene mutation-associated retinitis pigmentosa.

drusen were detectable as small hyperautofluorescent dots on SW-RAFI.

The high hyperopia with pigmentary retinopathy prompted questions concerning whether the patient had *MFRP*-RP (Ayala-Ramirez *et al.*, 2006; Crespi *et al.*, 2008; Zenteno *et al.*, 2009; Mukhopadhyay *et al.*, 2010). Partial nucleotide sequencing of *MFRP* exon 5 from the proband showed a heterozygous 1-bp deletion (Supplementary Fig. S1A; c.498delC, arrow) (supplementary data are available online at www.liebertonline.com/hum), which predicts a prematurely truncated protein (p.Asn167ThrfsX25). The normal exon 5 sequence is shown (Supplementary Fig. S1B). In the second allele a novel heterozygous G-to-T mutation (Supplementary Fig. S1C, arrow) at the conserved 5' donor splice site was demonstrated at exon/intron 9. The normal exon/intron 9 sequence is also illustrated (Supplementary Fig. S1D). Parental DNA analysis showed that the father carried one allele with the one base deletion and the mother carried one allele with the splice site mutation.

Full-field ERGs were abnormal (Fig. 1B). Rod b-waves were barely detectable (11 μ V; normal, 299 ± 52 μ V); a mixed cone-rod ERG had reduced a- and b-wave amplitudes (a-wave, 33 μ V [normal, 297 ± 65 μ V]; b-wave, 35 μ V [normal, 497 ± 111 μ V]); and cone ERGs were reduced in amplitude (for single flash, 61 μ V [normal, 173 ± 32 μ V]; and for flicker, 17 μ V [normal, 172 ± 35 μ V]). A rod sensitivity loss map by psychophysics showed no detectable rod function in the far peripheral field, but there was some retained rod sensitivity (between ~ 1.5 and 2.5 log₁₀ units reduced) in a wide region of the central field (Fig. 1C, left). Cone sensitivity loss was most evident in the nasal field, but there was detectable cone function elsewhere in the field. At fixation, cone sensitivity was within normal limits but was reduced by 0.5 – 1.5 log₁₀ units with increasing eccentricity into the temporal peripheral field (Fig. 1C, right). A kinetic visual field showed slight generalized constriction (more evident in the nasal field) to the V-4e target (70% of normal extent; 90% is 2 SD less than the normal mean) (Jacobson *et al.*, 1989); the I-4e target was detected only centrally (10% of normal extent; 90% is 2 SD less than the normal mean) (Fig. 1C, inset).

Topographical maps using OCT of retinal thickness and maps of inner retina and photoreceptor outer nuclear layer (ONL) are shown for normal subjects compared with the patient with *MFRP*-RP (Fig. 1D and E). Whereas retinal thickness was within normal limits across most of the retina except in the very central macula, the inner retina was significantly thicker than normal across the retinal area studied. The ONL, on the other hand, was thicker than normal at the fovea, within normal limits in the parafoveal region, but thinner than normal across the remainder of the retina sampled (Fig. 1D and E, insets, are comparison maps to normal limits). Cross-sectional images revealed abnormal retinal laminar architecture in the patient with *MFRP*-RP. A normal fovea has a structural pit due to the concentric displacement of an inner nuclear layer (INL) and limited hyperreflective tissue vitreal to the cone outer nuclear layer (Fig. 1F). In contrast, the *MFRP*-RP fovea was devoid of a normal central depression and had substantial inner retinal lamination with a discernable INL, microcystic changes anterior to the ONL, and considerable tissue vitreal to the INL (Fig. 1G). The deep hyporeflexive layer had no cystic structures and measured 182.8 μ m (normal foveal ONL in the

central 0.6 mm, 90.1 ± 10.5 μ m; $n=16$; age, 11–28 years) (Jacobson *et al.*, 2007).

We then asked whether the inner and outer segment (IS/OS) layer thickness was also different from normal in the patient with *MFRP*-RP and found the thickness across the central 4 mm to be within normal limits (data not shown). This would suggest that this hyperthick layer may be complicated by Muller cell swelling, such as we suggested may be the cause of a similar-appearing effect in patients with choroideremia (CHM) (Jacobson *et al.*, 2006). Thickness at the fovea with concomitant loss of the foveal pit at certain stages of CHM could reach 150 – 190 μ m, similar to that seen in this patient with *MFRP*-RP. A more common cause of thickened central structure in RP is cystoid macular edema (CME), but no cysts were visible within the ONL layer scans of the patient with *MFRP*-RP. The thick hyperreflective inner retinal layer across the foveal region in this patient measured 160 μ m, which is in dramatic contrast to the relatively thin foveal hyperreflectivity at the vitreoretinal interface in normal subjects (9.8 ± 1.5 μ m; $n=7$; age, 9–45 years). Patients with CHM with hyperthick foveal hyporeflexivity and no cystic changes ($n=3$; age, 13, 34, and 38 years) and patients with RP with CME ($n=6$; age, 7–78 years) showed thicker than normal hyperreflectivity at the vitreoretinal interface, but the values were small (average, RP with CME, 34.8 μ m; CHM, 30.7 μ m) by comparison with that in the patient with *MFRP*-RP (Fig. 1H).

Retinal function and morphology in AAV vector-treated *rd6* mice

The natural history of retinal function in the *rd6* mouse has been reported to show a decrease in ERG amplitude detectable by P25 followed by a progressive decline (Hawes *et al.*, 2000; Won *et al.*, 2008). Dark-adapted ERGs, dominated by rod function, show greater reduction than light-adapted ERGs, mediated by cone function, at early disease stages (Won *et al.*, 2008). Representative ERG waveforms driven by the activity of rods, both rods and cones (mixed rod-cone), and cones are shown for a wild-type mouse and for the untreated and treated eyes of a 2-month-old *rd6* mouse (Fig. 2A). At the ages studied, for rod b-wave amplitudes, the mean of untreated *rd6* eyes was 58% of mean wild-type, whereas, for cone-isolated b-waves, the mean of untreated *rd6* eyes was 89% of mean wild-type. Despite the tendency for lower values compared with wild-type, all *rd6* eyes were within normal limits for these parameters. For mixed rod-cone ERGs, the majority (60%) of the untreated *rd6* eyes had abnormally reduced amplitudes with mean values being reduced to 45 and 56% of mean wild-type for a- and b-waves, respectively.

ERGs performed 6 weeks after the uniocular subretinal injection of 1 μ l (10^9 total vector genomes) of AAV8 (Y733F) vector carrying wild-type mouse *Mfrp* cDNA showed there were higher amplitudes in treated versus untreated *rd6* eyes. ERGs for all treated *rd6* eyes were within normal limits. For the representative *rd6* mouse (Fig. 2A), the treated eye had a rod-isolated b-wave amplitude of 462 μ V as compared with 225 μ V in the untreated eye (interocular difference, 69%). Across all *rd6* animals, treated eyes had larger amplitudes (range, 316 – 462 μ V) compared with untreated eyes (160 – 238 μ V) with interocular differences (treated minus untreated)

FIG. 2. Functional and structural consequences of gene therapy in *rd6* mice. **(A)** Representative ERG traces from a wild-type (WT) mouse eye compared with those from the two eyes of a 2-month-old *rd6* mouse that was treated unilaterally with vector-gene 6 weeks previously. Rod-isolated responses were elicited under dark-adapted conditions with dim ($0.02 \text{ scot-cd} \cdot \text{s} \cdot \text{m}^{-2}$) flashes; mixed rod- and cone-driven responses were elicited with brighter ($2 \text{ scot-cd} \cdot \text{s} \cdot \text{m}^{-2}$) flashes. Cone-isolated responses were evoked with 25 phot-cd $\cdot \text{s} \cdot \text{m}^{-2}$ stimuli on a rod-desensitizing background. **(B)** Interocular difference of four ERG parameters plotted for individual *rd6* animals (red circles) and wild-type mice (black squares). $*p < 0.01$ for *t* tests comparing the means of two groups of animals. L-R, left – right; T-U, treated – untreated. **(C)** Light microscopy of a 2-month-old wild-type retina (left) and untreated (middle) and treated *rd6* retinas (right). Note the shorter outer segments (OS), reduced outer nuclear layer (ONL) thickness, and the presence of phagocytic-like cells (arrows) in the untreated *rd6* eye. RPE, retinal pigment epithelium; IS, inner segments; INL, inner nuclear layer; GCL, ganglion cell layer. Scale bar, 20 μ m.

FIG. 3. Immunolocalization of MFRP and Western blot analysis in *rd6* mice with and without gene therapy. **(A)** Detection of MFRP expression in wild-type albino control retinas (scale bar, 20 μ m). Note the increased distance between the outer nuclear layer (ONL) and retinal pigment epithelium (RPE) [compared with **(B)** and **(C)**] caused by artifactual postmortem retinal detachment. *Inset*: Image of the entire retina at lower magnification (scale bar, 0.5 mm). **(B)** Untreated *rd6* retina showing no detectable MFRP expression. **(C)** Vector-treated *rd6* retina section showing robust MFRP immunostaining predominantly in the RPE apical membrane. *Inset*: Full retinal section at low magnification, showing the widespread area of transduction (scale bar, 0.5 mm). DAPI (blue) staining of nuclei. **(D)** and **(E)** Higher magnification of the RPE layer from **(A)** and **(C)**, respectively, depicting strong MFRP expression in the apical RPE membrane and its microvilli (arrowhead) (scale bars, 10 μ m). **(F)** Western blot analysis of whole eyecup protein extracts from a 2-month-old untreated (U) *rd6* eye and the partner vector-treated (T) eye. Note the presence of an immunoreactive MFRP band in the treated eye only.

averaging 54% (range, 34–69%) as compared with 3% (range, –21 to +31%) in wild-type mice (Fig. 2B). With the mixed rod–cone ERGs, the treated *rd6* eyes had larger amplitudes (a-wave range, 132–265 μ V; b-wave range, 326–604 μ V) as compared with untreated eyes (a-wave range, 47–147 μ V; b-wave range, 153–369 μ V) with interocular differences averaging 83% (range, 35–99%) for a-waves and 57% (range, 40–72%) for b-waves. Wild-type mice for the mixed rod–cone ERGs had a mean interocular difference near zero (range, –24 to +32%) (Fig. 2B). For the cone-isolated ERG, the treated *rd6* eyes also had larger b-wave amplitudes (range, 129–175 μ V) as compared with untreated eyes (range, 84–128 μ V), with interocular differences averaging 35% (range, 22–50%). Interocular differences in wild-type mice for the cone-isolated ERG had a mean near zero (range, –35 to +38%) (Fig. 2B). In summary, interocular differences for all four ERG parameters in *rd6* mice were significantly larger than in wild-type mice. Taken together with the fact that untreated eyes at this age had reduced retinal function compared with wild-type, the results suggest that the vector–gene injection rescued rod- and cone-mediated ERG function in the *rd6* mice.

Light microscopy was used to evaluate the effect of the gene therapy on *rd6* mice (Fig. 2C). Treated eyes had greater numbers of photoreceptor nuclei compared with the contralateral untreated eyes. The ONL of untreated *rd6* eyes was about 5–7 nuclei thick (Fig. 2C, middle), whereas the vector-injected retina had 9 or 10 nuclei (Fig. 2C, right), which is similar to that in the wild-type eyes (Fig. 2C, left). ONL thickness (mid-superior retina) was $47.8 \pm 4.9 \mu\text{m}$ in treated retinas compared with $29.9 \pm 1.7 \mu\text{m}$ in untreated *rd6* retinas ($p < 0.05$) (Supplementary Fig. S2A). Treated *rd6* retinas also had well-organized, normal length outer segments, in contrast to the untreated eye, in which outer segments were both shortened and disorganized. Abnormal cells, likely of phagocytic nature (Hawes *et al.*, 2000), were visible in the subretinal space of untreated *rd6* mice (Fig. 2C, arrows, middle) but absent in treated eyes.

MFRP expression after gene therapy in *rd6* mice

MFRP expression was evaluated by immunohistochemistry in retinal sections (Fig. 3). Previous studies have shown that *MFRP* in wild-type mice is predominantly expressed in the RPE and ciliary epithelium whereas *MFRP* protein is not detected in *rd6* eyes (Kameya *et al.*, 2002; Mandal *et al.*, 2006a; Won *et al.*, 2008). This is confirmed in the present study (Fig. 3A and B). After gene therapy, intense labeling was present in the RPE (Fig. 3C), and it was similar to the pattern of *MFRP* in wild-type retina (Fig. 3A). When viewed at low magnification, the treated *rd6* eye displayed widespread and nearly continuous expression of *MFRP* in the RPE (Fig. 3C, inset). This indicates that a wide expanse of retina was included in the subretinal injection. In addition, *MFRP* expression, as driven by the ubiquitous smCBA promoter, was detectable in photoreceptor inner segments and ONL when the images were purposely overexposed to enhance the low-level non-RPE transgene expression pattern throughout the retina (Supplementary Fig. S2B).

Western blot analysis confirmed the presence of an immunoreactive *MFRP* band in treated eyes only (Fig. 3F). The *MFRP* immunoreactive band migrated at about 120 kDa, a much larger size than the predicted molecular mass of

65 kDa of wild-type protein, but consistent with results of previous studies (Won *et al.*, 2008; Fogerty and Besharse, 2011). This high molecular weight band suggests that *MFRP* may exist as a dimer in RPE cells. Previous studies have shown that some of the frizzled proteins are capable of forming homodimers, and a region containing the cysteine-rich domain is implicated in this process (Carron *et al.*, 2003). Cysteine-rich domains have also been shown to form a conserved dimer interface within crystal structures, suggesting that dimerization may have a biological function in frizzled receptor signaling (Dann *et al.*, 2001).

In earlier studies *MFRP* expression was found to be restricted to the base of the RPE apical membrane (Mandal *et al.*, 2006a), but we observed intense labeling of RPE microvilli in both wild-type and treated *rd6* mice (Fig. 3D and E). We further confirmed this finding by staining with an anti-ezrin antibody, a marker of RPE apical processes (Supplementary Fig. S3).

Discussion

MFRP-RP: A complex phenotype showing developmental and degenerative abnormalities

Before relatively routine molecular analysis, clinical reports noted the rare association of high hyperopia, microphthalmos, and retinal degeneration with or without other ocular abnormalities (e.g., Buys and Pavlin, 1999; Ghose *et al.*, 1985; MacKay *et al.*, 1987). The *MFRP* gene, first identified more than a decade ago (Katoh, 2001), was initially associated only with humans having high hyperopia, reduced axial length, but no retinal degeneration (Sundin *et al.*, 2005). Screening for *MFRP* mutations in a wide spectrum of retinal degenerations was negative (Pauer *et al.*, 2005) and species differences were used to explain the lack of retinal degeneration in humans (Sundin, 2005). More recently, however, autosomal recessive families with *MFRP* mutations were identified with RP, posterior microphthalmos, foveal abnormalities, and optic disc drusen (Ayala-Ramirez *et al.*, 2006; Crespi *et al.*, 2008; Zenteno *et al.*, 2009; Mukhopadhyay *et al.*, 2010). A list of clinical and molecular features of the 16 published patients (age range, 16–60 years), representing 7 families, is provided (Supplementary Table S1). Visual acuities varied from 20/25 to no light perception (NLP). All had high hyperopia (range, +13.50 to +29.00) and abnormal ERGs. Corneal diameters were normal. Data from the *MFRP*-RP patient in the present study are consistent with the clinical phenotype in these other reports (Supplementary Table S1).

The convexity forming a domelike configuration in the central retina with persistent inner retinal structure across the presumptive fovea in human *MFRP* disease suggests a disturbance in the complex early cell migrations that lead to normal foveal development. Nine of the previously published patients with *MFRP*-RP had OCT scans and all showed the lack of a foveal pit and thickening of inner retinal tissue across the foveal region (Ayala-Ramirez *et al.*, 2006; Crespi *et al.*, 2008; Zenteno *et al.*, 2009; Mukhopadhyay *et al.*, 2010). We postulate that this is the result of foveal maldevelopment (Sundin *et al.*, 2008), likely complicated by effects of degenerative retinal disease. Formation of the normal foveal pit occurs during the second half of gestation and is due to progressive thinning of ganglion cell and inner

nuclear cell layers that overlie the foveal cone cells due to their centrifugal displacement (reviewed in Provis *et al.*, 1998). The process is complete at about 1 year of life. Another process, but a centripetal one, leads to increasing cone cell density in the foveal pit and this continues for years after birth. Taken together, the good visual acuity in many patients with *MFRP*-RP (Supplementary Table S1), normal inner and outer segment thickness measurements in our patient, and thicker than normal foveal ONL suggest that the cone cell centripetal movement has occurred. It is also conceivable that Muller cell swelling secondary to noncystic macular edema is the basis of the thickened hyporeflective layer (Jacobson *et al.*, 2006). The excessively thickened superficial hyperreflective layer above the cone cells suggests persistence of tissue that should have moved centrifugally if there was normal development. A comparison with different retinopathies indicated that even the thickest of other pre-cone cell layers we examined are far thinner than the *MFRP*-RP layer. The OCT appearance without a foveal depression has some similarities but also differences from other maldevelopment retinopathies such as albinism (Marmor *et al.*, 2008; McAllister *et al.*, 2010) and nanophthalmic eyes without retinal degeneration (Bijlsma *et al.*, 2008). The findings in the extracentral retina of photoreceptor degeneration and inner retinal thickening point to retinal remodeling in *MFRP*-RP, as we have noted in many retinal degenerative diseases (e.g., Jacobson *et al.*, 2006; Aleman *et al.*, 2009; Cideciyan *et al.*, 2011).

Gene therapy in the *rd6* mouse model of *MFRP* disease

The AAV8 (Y733F) tyrosine capsid mutant has emerged as a promising vector for the treatment of rapidly degenerating animal models of RP caused by mutations in photoreceptor cells (Pang *et al.*, 2011). Here, we demonstrate the ability of a subretinally delivered AAV8 (Y733F) vector containing a smCBA promoter driving expression of the murine *Mfrp* gene to rescue retinal function and prevent photoreceptor cell death in the *rd6* mouse, the early-onset RPE-based model of *MFRP*-RP. To minimize the surgical trauma associated with subretinal injections and to still be able to provide rapid and widespread transgene expression before the initiation of cell death, treatment was provided on P14 in *rd6* mice, coincident with eye opening when the photoreceptor cell layer was still fully intact. AAV8 (Y733F)-smCBA-*MFRP* vector delivery at this age led to significantly higher ERG amplitudes relative to untreated eyes when assessed 6 weeks later. Histology of treated retinas revealed preserved photoreceptor ONL with better organized inner and outer segments than control untreated *rd6* retinas. Moreover, accumulation of abnormal, phagocytic-like cells in the subretinal space was not observed in vector-treated eyes. Treatment also led to intense and widespread expression of the *MFRP* transgene, predominantly localized to the apical RPE membrane and its microvilli, where the wild-type protein normally resides in unaffected controls.

The function of the *MFRP* protein is not clear at this time, and the mechanism of photoreceptor degeneration in *rd6* mice could be multifactorial. The presence of *MFRP* throughout the apical membrane and its actin-rich microvilli, both in wild-type and treated *rd6* mice, suggests that it

may play a structural role by maintaining the normal morphology of the RPE apical processes. *MFRP* expression normally increases markedly after birth, coincident with the development of microvilli and outer segments in mice (Won *et al.*, 2008). Thus, *rd6* retinal degeneration may be caused by compromised RPE microvilli leading to adhesion defects between RPE and photoreceptor outer segments. In addition, experiments suggest that *MFRP* and *CTRP5* physically interact, and could therefore be both functionally and transcriptionally dicistronic (Mandal *et al.*, 2006a; Shu *et al.*, 2006). *CTRP5* contains a short-chain collagen sequence and a C-terminal globular complement 1q (C1q) domain that appears to bind the extracellular *MFRP* region containing tandem repeats of two cubilin (CUB) domains and a low-density lipoprotein receptor-related sequence (Shu *et al.*, 2006). C1q is a key component of the classical pathway of complement activation, and is known to be involved in many critical processes including innate and adaptive immunity, inflammation, apoptosis, cell adhesion, and monocyte chemotaxis (Kishore *et al.*, 2004; Lu *et al.*, 2008). Interestingly, a study describing a murine *Mfrp* null mutant has shown that *CTRP5* is upregulated in both *rd6* and *Mfrp*^{174delG} (Fogerty and Besharse, 2011). Thus, the lack of *MFRP* protein might lead to uncontrolled signaling by *CTRP5*, triggering the migration of phagocytic cells from the choroid into the subretinal space, as seen in *rd6* mice. Morphological characterization of an *Mfrp/CTrp5* double-knockout mouse could prove useful for testing this hypothesis in future studies.

Realistic potential for gene-based therapy in *MFRP*-RP

Is *MFRP*-RP a target for future gene therapy trials? Although a limited number of patients have been reported to date, there is a recognizable human phenotype with a dramatic refractive error that should make clinical and genetic screening more productive in the future than previously (Pauer *et al.*, 2005). Evidence of central retinal maldevelopment and early peripheral retinal degeneration with loss of normal retinal architecture indicating remodeling makes a complex target for gene therapy as it is currently performed. Despite reports of safety and efficacy of subretinal gene therapy as currently performed in the *RPE65* form of LCA (reviewed in Cideciyan, 2010), a surgically induced subfoveal retinal detachment in a well-functioning but maldeveloped central retina may present more risk than benefit. The patient with *MFRP*-RP whom we examined, however, had preserved although abnormal peripheral retinal function (at the end of the second decade of life) by ERG and psychophysics, both for rod and cone photoreceptor systems. This suggests the non-foveal retina is a potential therapeutic target. In terms of relevant *rd6* experiments to perform, our relatively short-term and preliminary studies with one vector serotype demand to be confirmed and extended to longer term efficacy and safety studies, dose-response experiments, and future work to address the potentially safer approach of intravitreal delivery of *Mfrp* cDNA driven by RPE cell-specific promoters (e.g., tyrosine capsid mutant AAV vectors; Petrs-Silva *et al.*, 2011).

Acknowledgments

Supported in part by NIH grants R01EY11123 and P30EY021721, and by grants from the Macula Vision

Research Foundation, Foundation Fighting Blindness, and Research to Prevent Blindness. A.V.C. is an RPB Senior Scientific Investigator.

Author Disclosure Statement

W.W.H. and the University of Florida have a financial interest in the use of AAV therapies, and own equity in a company (AGTC Inc.) that might, in the future, commercialize some aspects of this work.

References

- Aleman, T.S., Cideciyan, A.V., Volpe, N.J., *et al.* (2002). Spinocerebellar ataxia type 7 (SCA7) shows a cone-rod dystrophy phenotype. *Exp. Eye Res.* 74, 737–745.
- Aleman, T.S., Soumitra, N., Cideciyan, A.V., *et al.* (2009). CERKL mutations cause an autosomal recessive cone-rod dystrophy with inner retinopathy. *Invest. Ophthalmol. Vis. Sci.* 50, 5944–5954.
- AlMahmoud, T., Priest, D., Munger, R., and Jackson, W.B. (2011). Correlation between refractive error, corneal power, and thickness in a large population with a wide range of ametropia. *Invest. Ophthalmol. Vis. Sci.* 52, 1235–1242.
- Ayala-Ramirez, R., Graue-Wiechers, F., Robredo, V., *et al.* (2006). A new autosomal recessive syndrome consisting of posterior microphthalmos, retinitis pigmentosa, foveoschisis, and optic disc drusen is caused by a *MFRP* gene mutation. *Mol. Vis.* 12, 1483–1489.
- Bijlsma, W.R., van Schooneveld, M.J., and Van der Lelij, A. (2008). Optical coherence tomography findings for nanophthalmic eyes. *Retina* 28, 1002–1007.
- Bonilha, V.L., Rayborn, M.E., Bhattacharya, S.K., *et al.* (2006). The retinal pigment epithelium apical microvilli and retinal function. *Adv. Exp. Med. Biol.* 572, 519–524.
- Buys, Y.M., and Pavlin, C.J. (1999). Retinitis pigmentosa, nanophthalmos, and optic disc drusen: A case report. *Ophthalmology* 106, 619–622.
- Carron, C., Pascal, A., Djiane, A., *et al.* (2003). Frizzled receptor dimerization is sufficient to activate the Wnt/ β -catenin pathway. *J. Cell Sci.* 116, 2541–2550.
- Caruso, R.C., Aleman, T.S., Cideciyan, A.V., *et al.* (2010). Retinal disease in Rpe65-deficient mice: Comparison to human Leber congenital amaurosis due to RPE65 mutations. *Invest. Ophthalmol. Vis. Sci.* 51, 5304–5313.
- Cideciyan, A.V. (2010). Leber congenital amaurosis due to RPE65 mutations and its treatment with gene therapy. *Prog. Retin. Eye Res.* 29, 398–427.
- Cideciyan, A.V., Swider, M., Aleman, T.S., *et al.* (2007). Reduced-illumination autofluorescence imaging in *ABCA4*-associated retinal degenerations. *J. Opt. Soc. Am. A Opt. Image Sci. Vis.* 24, 1457–1467.
- Cideciyan, A.V., Rachel, R.A., Aleman, T.S., *et al.* (2011). Cone photoreceptors are the main targets for gene therapy of NPHP5 (IQCB1) or NPHP6 (CEP290) blindness: Generation of an all-cone Nphp6 hypomorph mouse that mimics the human retinal ciliopathy. *Hum. Mol. Genet.* 20, 1411–1423.
- Crespi, J., Buil, J.A., Bassaganyas, F., *et al.* (2008). A novel mutation confirms *MFRP* as the gene causing the syndrome of nanophthalmos–retinitis pigmentosa–foveoschisis–optic disc drusen. *Am. J. Ophthalmol.* 146, 323–328.
- Dann, C.E., Hsieh, J.C., Rattner, A., *et al.* (2001) Insights into Wnt binding and signalling from the structures of two Frizzled cysteine-rich domains. *Nature* 412, 86–90.
- Fogerty, J., and Besharse, J.C. (2011). 174delG mutation in mouse *MFRP* causes photoreceptor degeneration and RPE atrophy. *Invest. Ophthalmol. Vis. Sci.* 52, 7256–7266.
- Fontana, S.T., and Brubaker, R.F. (1980). Volume and depth of the anterior chamber in the normal aging human eye. *Arch. Ophthalmol.* 98, 1803–1808.
- Ghose, S., Sachdev, M.S., and Kumar, H. (1985). Bilateral nanophthalmos, pigmentary retinal dystrophy, and angle closure glaucoma—a new syndrome? *Br. J. Ophthalmol.* 69, 624–628.
- Hawes, N.L., Chang, B., Hageman, G.S., *et al.* (2000). Retinal degeneration 6 (*rd6*): A new mouse model for human retinitis punctata albescens. *Invest. Ophthalmol. Vis. Sci.* 41, 3149–3157.
- Hayward, C., Shu, X., Cideciyan, A.V., *et al.* (2003). Mutation in a short-chain collagen gene, *CTRP5*, results in extracellular deposit formation in late-onset retinal degeneration: A genetic model for age-related macular degeneration. *Hum. Mol. Genet.* 12, 2657–2667.
- Jacobson, S.G., Voigt, W.J., Parel, J.M., *et al.* (1986). Automated light- and dark-adapted perimetry for evaluating retinitis pigmentosa. *Ophthalmology* 93, 1604–1611.
- Jacobson, S.G., Yagasaki, K., Feuer, W.J., and Roman, A.J. (1989). Interocular asymmetry of visual function in heterozygotes of X-linked retinitis pigmentosa. *Exp. Eye Res.* 48, 679–691.
- Jacobson, S.G., Cideciyan, A.V., Wright, E., and Wright, A.F. (2001). Phenotypic marker for early disease detection in dominant late-onset retinal degeneration. *Invest. Ophthalmol. Vis. Sci.* 42, 1882–1890.
- Jacobson, S.G., Cideciyan, A.V., Sumaroka, A., *et al.* (2006). Remodeling of the human retina in choroideremia: Rab escort protein 1 (REP-1) mutations. *Invest. Ophthalmol. Vis. Sci.* 47, 4113–4120.
- Jacobson, S.G., Aleman, T.S., Cideciyan, A.V., *et al.* (2007). Human cone photoreceptor dependence on RPE65 isomerase. *Proc. Natl. Acad. Sci. U.S.A.* 104, 15123–15128.
- Jacobson, S.G., Roman, A.J., Aleman, T.S., *et al.* (2010). Normal central retinal function and structure preserved in retinitis pigmentosa. *Invest. Ophthalmol. Vis. Sci.* 51, 1079–1085.
- Jacobson, S.G., Cideciyan, A.V., Aleman, T.S., *et al.* (2011). Human retinal disease from *AIP1* gene mutations: Foveal cone loss with minimal macular photoreceptors and rod function remaining. *Invest. Ophthalmol. Vis. Sci.* 52, 70–79.
- Kameya, S., Hawes, N.L., Chang, B., *et al.* (2002). *Mfrp*, a gene encoding a frizzled related protein, is mutated in the mouse retinal degeneration 6. *Hum. Mol. Genet.* 11, 1879–1886.
- Katoh, M. (2001). Molecular cloning and characterization of *MFRP*, a novel gene encoding a membrane-type Frizzled-related protein. *Biochem. Biophys. Res. Commun.* 282, 116–123.
- Kishore, U., Gaboriaud, C., Waters, P., *et al.* (2004). C1q and tumor necrosis factor superfamily: Modularity and versatility. *Trends Immunol.* 25, 551–561.
- Leonard, K.C., Petrin, D., Coupland, S.G., *et al.* (2007). XIAP protection of photoreceptors in animal models of retinitis pigmentosa. *PLoS One* 2, e314.
- Lu, J.H., The, B.K., Wang, L., *et al.* (2008). The classical and regulatory functions of C1q in immunity and autoimmunity. *Cell. Mol. Immunol.* 5, 9–21.
- MacKay, C.J., Shek, M.S., Carr, R.E., *et al.* (1987). Retinal degeneration with nanophthalmos, cystic macular degeneration, and angle closure glaucoma: A new recessive syndrome. *Arch. Ophthalmol.* 105, 366–371.
- Mandal, M.N., Vasireddy, V., Jablonski, M.M., *et al.* (2006). Spatial and temporal expression of *MFRP* and its interaction with *CTRP5*. *Invest. Ophthalmol. Vis. Sci.* 47, 5514–5521.

- Marmor, M.F., Choi, S.S., Zawadzki, R.J., and Werner, J.S. (2008). Visual insignificance of the foveal pit: Reassessment of foveal hypoplasia as fovea plana. *Arch. Ophthalmol.* 126, 907–913.
- McAllister, J.T., Dubis, A.M., Tait, D.M., *et al.* (2010). Arrested development: High-resolution imaging of foveal morphology in albinism. *Vision Res.* 50, 810–817.
- Milam, A.H., Curcio, C.A., Cideciyan, A.V., *et al.* (2000). Dominant late-onset retinal degeneration with regional variation of sub-retinal pigment epithelium deposits, retinal function, and photoreceptor degeneration. *Ophthalmology* 107, 2256–2266.
- Mukhopadhyay, R., Sergouniotis, P.I., Mackay, D.S., *et al.* (2010). A detailed phenotypic assessment of individuals affected by MFRP-related oculopathy. *Mol. Vis.* 16, 540–548.
- Oliveira, C., Harizman, N., Girkin, C.A., *et al.* (2007). Axial length and optic disc size in normal eyes. *Br. J. Ophthalmol.* 91, 37–39.
- Pang, J.J., Boye, S.L., Kumar, A., *et al.* (2008). AAV-mediated gene therapy for retinal degeneration in the *rd10* mouse containing a recessive PDE β mutation. *Invest. Ophthalmol. Vis. Sci.* 49, 4278–4283.
- Pang, J.J., Dai, X., Boye, S.E., *et al.* (2011). Long-term retinal function and structure rescue using capsid mutant AAV8 vector in the *rd10* mouse, a model of recessive retinitis pigmentosa. *Mol. Ther.* 19, 234–242.
- Pauer, G.J., Xi, Q., Zhang, K., *et al.* (2005). Mutation screen of the membrane-type frizzled-related protein (MFRP) gene in patients with inherited retinal degenerations. *Ophthalmic Genet.* 26, 157–161.
- Petrs-Silva, H., Dinculescu, A., Li, Q., *et al.* (2009). High-efficiency transduction of the mouse retina by tyrosine-mutant AAV serotype vectors. *Mol. Ther.* 17, 463–471.
- Petrs-Silva, H., Dinculescu, A., Li, Q., *et al.* (2011). Novel properties of tyrosine-mutant AAV2 vectors in the mouse retina. *Mol. Ther.* 19, 293–301.
- Provis, J.M., Diaz, C.M., and Dreher, B. (1998). Ontogeny of the primate fovea: A central issue in retinal development. *Prog. Neurobiol.* 54, 549–580.
- Roman, A.J., Schwartz, S.B., Aleman, T.S., *et al.* (2005). Quantifying rod photoreceptor-mediated vision in retinal degenerations: Dark-adapted thresholds as outcome measures. *Exp. Eye Res.* 80, 259–272.
- Roman, A.J., Boye, S.L., Aleman, T.S., *et al.* (2007). Electroretinographic analyses of Rpe65-mutant *rd12* mice: Developing an *in vivo* bioassay for human gene therapy trials of Leber congenital amaurosis. *Mol. Vis.* 13, 1701–10.
- Rüfer, F., Schröder, A., Arvani, M.K., and Erb, C. (2005). [Central and peripheral corneal pachymetry—standard evaluation with the Pentacam system] [in German]. *Klin. Monbl. Augenheilkd.* 222, 117–122.
- Shu, X., Tulloch, B., Lennon, A., *et al.* (2006). Disease mechanisms in late-onset retinal macular degeneration associated with mutation in *C1QTNF5*. *Hum. Mol. Genet.* 15, 1680–1689.
- Sparrow, J.R., Hicks, D., and Hamel, C.P. (2010). The retinal pigment epithelium in health and disease. *Curr. Mol. Med.* 10, 802–823.
- Strauss, O. (2005). The retinal pigment epithelium in visual function. *Physiol. Rev.* 85, 845–881.
- Sundin, O.H. (2005). The mouse's eye and *Mfrp*: Not quite human. *Ophthalmic Genet.* 26, 153–155.
- Sundin, O.H., Leppert, G.S., Silva, E.D., *et al.* (2005). Extreme hyperopia is the result of null mutations in *MFRP*, which encodes a Frizzled-related protein. *Proc. Natl. Acad. Sci. U.S.A.* 102, 9553–9558.
- Sundin, O.H., Dharmaraj, S., Bhutto, I.A., *et al.* (2008). Developmental basis of nanophthalmos: MFRP is required for both prenatal ocular growth and postnatal emmetropization. *Ophthalmic Genet.* 29, 1–9.
- Weymouth, A.E., and Vingrys, A.J. (2008). Rodent electroretinography: Methods for extraction and interpretation of rod and cone responses. *Prog. Retin. Eye Res.* 27, 1–44.
- Won, J., Smith, R.S., Peachey, N.S., *et al.* (2008). Membrane frizzled-related protein is necessary for the normal development and maintenance of photoreceptor outer segments. *Vis. Neurosci.* 25, 563–574.
- Zenteno, J.C., Buentello-Volante, B., Quiroz-Gonzalez, M.A., and Quiroz-Reyes, M.A. (2009). Compound heterozygosity for a novel and a recurrent *MFRP* gene mutation in a family with the nanophthalmos–retinitis pigmentosa complex. *Mol. Vis.* 15, 1794–1798.

Address correspondence to:

Dr. Samuel G. Jacobson

Scheie Eye Institute, Department of Ophthalmology

Perelman School of Medicine

University of Pennsylvania

Philadelphia, PA 19104

E-mail: jacobson@mail.med.upenn.edu

Dr. William W. Hauswirth

Department of Ophthalmology

University of Florida

Gainesville, FL 32610

E-mail: hauswirth@ufl.edu

Received for publication September 9, 2011;
accepted after revision December 4, 2011.

Published online: December 5, 2011.