Retinal Pigment Epithelium Defects Accelerate Photoreceptor Degeneration in Cell Type–Specific Knockout Mouse Models of Choroideremia

Tanya Tolmachova, Silene T. Wavre-Shapton, Alun R. Barnard, Robert E. MacLaren, Clare E. Futter, and Miguel C. Seabra

PURPOSE. Choroideremia (CHM) is a progressive X-linked degeneration of three ocular layers (photoreceptors, retinal pigment epithelium, and choroid), with a complex and still largely unclear pathogenesis. To investigate the pathophysiology of CHM, the authors engineered mice with a cell type–specific Chm/Rep1 knockout (KO).

METHODS. A mouse line carrying a conditional allele ChmFlox was crossed with the transgenic line IRBP-Cre to achieve Chm KO, specifically in the photoreceptor layer, and Tyr-Cre to produce Chm KO, specifically in the retinal pigment epithelium and other pigmented cells. ChmFlox, Tyr-Cre+ and ChmFlox, IRBP-Cre+ mice were mated to produce mice with Chm KO in both layers. All mouse lines were studied by histology, electron microscopy, electroretinography (ERG), scanning laser ophthalmoscopy (SLO), and biochemical methods.

RESULTS. In ChmFlox, IRBP-Cre+ mice the authors observed the progressive degeneration of photoreceptors in the presence of normal retinal pigment epithelium (RPE). ChmFlox, Tyr-Cre+ mice exhibited coat color dilution and pigment abnormalities of the RPE in the presence of an intact outer nuclear layer. In 6- to 8-month-old ChmFlox, Tyr-Cre+, IRBP-Cre+ mice, the degeneration of photoreceptors was accelerated compared with ChmFlox, IRBP-Cre+ mice but became leveled with age, such that it was comparable at 12 to 14 months. Detailed ERG and SLO analysis supported the histopathologic findings.

CONCLUSIONS. Defects in photoreceptors and RPE can arise because of intrinsic defects caused cell autonomously by the Chm KO. However, when both photoreceptors and RPE are diseased, the dynamics of the degenerative process are altered. Photoreceptor functional deficit and cell death manifest much earlier, suggesting that the diseased RPE accelerates photoreceptor degeneration. (Invest Ophthalmol Vis Sci. 2010;51: 4913–4920) DOI:10.1167/iovs.09-4892

CHM/REP1 is the causative gene in CHM patients and is positioned at the locus Xq21.2. The Chm gene product, also known as Rab Escort Protein 1 (REP1), assists in the lipid modification of Rab GTPases. This modification, known as prenylation, involves the covalent attachment of one or two prenyl groups (geranylgeranyl or farnesyl) to the C terminus of a Rab protein. Catalytic function is performed by the enzyme Rab geranylgeranyl transferase (Rab GGTase) and REP1 assists in the reaction by binding unprenylated Rab substrates to Rab GGTase and then delivering newly prenylated Rab to a specific membrane.

Rabs are small GDP/GTP-binding proteins that are inactive when bound to GDP and that change their conformation when GDP is displaced by GTP. Activation through GTP-binding leads to the recruitment of effectors, which results in downstream biological effects. In humans, the Rab family includes more than 60 known members, whereas other species such as mouse, rat, zebrafish, and yeast also have extensive Rab families. Their main role is to regulate intracellular vesicular transport; prenylation is a prerequisite for their functionality because it provides the hydrophobicity that allows Rabs to bind membranes.

CHM/REP1 loss-of-function mutations in humans lead solely to eye disease. No other tissue or organ has been reported to be affected, presumably because of the functional redundancy provided by the presence of another protein, REP2, also known as CHML (choroideremia-like protein). Both REP1 and REP2 are ubiquitously expressed and have 75% amino acid identity and 90% similarity. Chm knockout (KO) appears to be more severe in animal models. In mice, the Chm KO is embryonically lethal because of abnormalities in extraembryonic mouse tissues such as placenta and yolk sac despite the presence of the Chml gene. Nevertheless carrier females (Chmnull/WT) are viable and exhibit progressive retinal degeneration. In zebrafish, Chml/Rep2 is not present, and Chm KO manifests as an extremely severe systemic illness leading to early lethality at postnatal day 6, which limits its use as a model of CHM.

With three affected eye layers that are interdependent (choroid, photoreceptors, and RPE), the pathogenesis of CHM is complex and remains unclear. One possibility is that CHM originates in one layer (primary site of degeneration),
which then leads to the demise of the other two layers. The RPE is the most likely candidate for the primary site of degeneration given its position between the other two degenerating layers. Another hypothesis is that the disease appears in the RPE and photoreceptors simultaneously (cell autonomously). In our previous study, we generated mouse CHM carriers (Chmnull/WT) and two conditional alleles (ChmFlox and Chm3lox) that were crossed with six3-Cre and inducible MerCreMer transgenic lines. We showed that in the RPE and the neuroretina, different subsets of Rabs are autonomously. In our previous study, we generated mouse CHM carriers (Chmnull/WT) and two conditional alleles (ChmFlox and Chm3lox) that were crossed with six3-Cre and inducible MerCreMer transgenic lines. We showed that in the RPE and the neuroretina, different subsets of Rabs are underprenylated, implying that both layers have intrinsic prenylation defects. To investigate the pathogenesis of the disease further, we generated two new mouse models in which Chm KO is achieved specifically in the RPE (and other pigmented cells) or photoreceptors. The aim of our study was to investigate whether the Chm KO in one layer (either the photoreceptors or the RPE) could initiate or affect the degeneration of the other layer.

METHODS

Mice

All mice used in this study were treated humanely and in accordance with the UK Home Office Regulations under project license 70/6176. Care, use, and treatment of the animals were in strict agreement with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. Mice with ChmFlox and Chm3lox alleles and tamoxifen (TM)-inducible MerCreMer transgene have been described previously. The transgenic IRBP-Cre mouse line expresses a Cre-transgene under the control of the human IRBP promoter. The transgenic Tyr-Cre mouse line (Tyr-CreB), with the Cre-recombinase gene under the control of a tyrosinase promoter, was described previously. The tyrosinase promoter is a fusion between 2.5 kb of the region immediately upstream of the first exon. Routine genotyping of mice by PCR was described previously. Primers H7 (5’-agagatctcagcatgctctc) and H9 (5’-ccagagaactagttgagac) allowed the identification of ChmWT, Chmnull, and ChmFlox alleles. Sizes of the resultant PCR products were 780 bp (ChmWT), 860 bp (Chmnull), and 350 bp (ChmFlox). To identify the Tyr-Cre transgene, we used primers LL125 (5’-gtcactccaggggttgctgg) and Cre3 (5’-caattgctaccatgcccc); the size of the product was 150 bp. For the IRBP-Cre transgene, we used Cre3 and IRBP4 (5’-gataccacaggtatatgctcg) and the resultant PCR products were 780 bp (ChmWT), 860 bp (Chmnull), and 350 bp (ChmFlox). To identify IRBP-Cre transgene, we used primers Cre1 (5’-ccccgcagaacctgaagatgttc) and Cre2 (5’-tcccgcagaacctgaagatgttc) and the resultant PCR products were 780 bp (ChmWT), 860 bp (Chmnull), and 350 bp (ChmFlox).

In Vitro Prenylation Assay and Immunoblot

The in vitro prenylation assay was performed on cytosolic fractions of tissue and cell lysates that were collected after ultracentrifugation (100,000 g, 1 hour, 4°C). The assay was performed in a 25-μl reaction.
supplemented with 0.5 μM RabGGTase, 0.5 μM REP1, 1 μM [3H]-GGPP (Perkin Elmer, Wellesley, MA), 50 μM NP-40, 1 mM DTT, 50 mM HEPES, pH 7.2, and 5 mM MgCl₂ for 30 minutes at 37°C. The reaction mixture was separated on a 17.5% SDS-PAGE gel, which was then dried and exposed onto autoradiography film (Hyperfilm; GE Healthcare Biosciences, Piscataway, NJ). The protocol for immunoblotting has been described previously.³⁵

Morphologic Studies

Mouse eyes for histology were fix in 2% paraformaldehyde and 2.5% glutaraldehyde in 0.1 M cacodylate buffer for 1 hour. Samples were embedded in paraffin wax, and sections were cut at 4-μm thickness and stained with hematoxylin and eosin. Mouse eyes for immunohistochemistry were fixed in 4% paraformaldehyde in PBS for 1 hour, cryoprotected in 20% sucrose overnight, and embedded in the OCT compound. Sections were cut at 7-μm thickness and air-dried. Cre-specific antibody used for immunostaining was from Cambridge Bioscience (dilution 1:2500; Cambridge, UK). Iba-1–specific antibody was made to open the eyecup, and the neuroretina was gently peeled off. Electron microscopy was performed as described previously.²⁷

Electroretinography and Scanning Laser Ophthalmoscopy

Before the ERG procedure, the mice were dark adapted (≥1 hour), and the experimental preparation was performed under dim red illumination. The mice were anesthetized with a single intraperitoneal injection of medetomidine hydrochloride (Dormitor; 1 mg/kg body weight; Pfizer, New York, NY) and ketamine (60 mg/kg body weight; Pfizer, New York, NY) and controlled the light stimulus. Single-flash stimuli were delivered in a Ganzfeld dome with the intensity increasing from −6 to 1 log cd s/m² in 15 approximately half-log unit steps. For dim stimuli (−6 to −5 log cd s/m²), 10 responses were averaged with an interstimulus interval (ISI) of 2 seconds. For midrange stimuli (−4.523 to −2 log cd s/m²), 10 responses were averaged with an ISI of 5 seconds. For the brightest intensities (−1.523 to 1 log cd s/m²), five responses were averaged with an ISI of 10 seconds. A custom-made active electrode (platinum wire loop) was positioned concentrically to the cornea of the left eye using a micromanipulator. Hypromellose eyedrops (0.5% methylcellulose solution) were used to provide good electrical contact and to maintain corneal moisture. A reference electrode (subcutaneous stainless steel needle) was placed in the scruff, and an identical ground electrode was positioned at the base of the tail. Signals were differentially amplified and digitized at a rate of 5 kHz. Amplitudes of the major ERG components (a- and b-wave) were measured (Espion software; Diagnosys LLC) using automated and manual methods. Full datasets were tested for significant differences using two-way ANOVA, with genotype and light intensity as factors. Immediately after ERG recording, imaging of the fundus was performed with a scanning laser ophthalmoscope (HRA2; Heidelberg Engineering, Heidelberg, Germany), as previously described.²⁸

RESULTS

Photoreceptor-Specific Chm KO

To achieve a photoreceptor-specific Chm KO, we crossed Chm<sup>flox</sup> animals with an IRBP-Cre transgenic line, expressing Cre-recombinase under the control of the human IRBP promoter.²⁴ The Chm<sup>flox</sup> allele is a conditional allele that carries Cre-recombinase and an early stop codon appearance (Fig. 1A). To confirm the activity of the IRBP-Cre transgene,
the neuroretina and the RPE were isolated from the eyes of

\[Chm^{\text{Flox}}\], \(IRBP-Cre^{+}\) animals and were analyzed by PCR using pairs of primers that can identify \(Chm^{\text{Flox}}\), \(Chm^{\text{WT}}\), and \(Chm^{\text{null}}\) alleles simultaneously (Fig. 1B). The diagnostic 330-bp band was present in samples of the neuroretina from \(Chm^{\text{Flox}}\), \(IRBP-Cre^{+}\) animals and in control \(Chm^{\text{null}}/\text{WT}\) samples (RPE and neuroretina). To verify the expression of the \(IRBP-Cre\) transgene in the photoreceptors, we prepared frozen sections of the eyes of \(Chm^{\text{WT}}\), \(IRBP-Cre^{+}\) mice at various time points: postnatal day (P) 14, P18, P21, and P70 and stained them with a Cre-specific antibody. We observed staining in the outer nuclear layer, consistent with expression of the transgene in the photoreceptors, that was maximal at P21, with >90% of photoreceptors expressing Cre-recombinase (Figs. 1C, 1D).

Next, we assessed the function of the Rep1 protein using an in vitro prenylation assay that identifies unprenylated Rabs that accumulate only when Rep1 is dysfunctional. Neuroretina and RPE were isolated from the eyes of \(Chm^{\text{Flox}}\), \(IRBP-Cre^{+}\), and control (\(Chm^{\text{Flox}}\) and \(Chm^{\text{WT}}\)) animals. Cytosolic fractions were collected and subjected to an in vitro prenylation reaction using recombinant RabGGTase, REP1, and radiolabeled substrate \([\text{H}]\)-geranylgeranyl pyrophosphate (\([\text{H}]\)GGPP), allowing visualization of the newly prenylated Rabs (Fig. 1E). As expected, there were no Rabs available for prenylation in the RPE of \(Chm^{\text{Flox}}\), \(IRBP-Cre^{+}\), and all control samples. Conversely, we observed radiolabeled bands after in vitro prenylation of Rabs (corresponding to unprenylated Rabs in vivo) in neuroretina samples obtained from \(Chm^{\text{Flox}}\), \(IRBP-Cre^{+}\) eyes. This result confirms that photoreceptors exhibit a functional Rep1 defect.

Histologic analysis of the paraffin eye sections prepared from \(Chm^{\text{Flox}}\), \(IRBP-Cre^{+}\) mice revealed slow progressive degeneration of photoreceptors (Figs. 1F-I), with a nearly normal presence of healthy RPE, confirming our previous finding that \(Chm^{\text{null}}/\text{WT}\) animals exhibited degeneration of photoreceptors in the presence of healthy RPE, confirming our previous finding that photoreceptors are a primary site of the disease.

**RPE-Specific \(Chm\) KO**

To achieve \(Chm\) KO specifically in the RPE, we used the transgenic line \(Tyr-Cre\) expressing Cre-recombinase under the control of an artificial tyrosinase promoter.\(^{25}\) This promoter is active in pigmented cells, including RPE and skin melanocytes. \(Chm^{\text{Flox}}\), \(Tyr-Cre^{+}\) animals have a lighter (chocolate) coat color than the \(Chm^{\text{WT}}\) animals on the same C57BL/6 genetic background (Fig. 2A). This is presumably a result of the \(Chm\) deletion in melanocytes, which affects melanosomal biogenesis and transport.\(^{29}\) To determine whether the transgene was expressed in the RPE, we performed immunoblot analysis on isolated RPE and neuroretina from \(Chm^{\text{Flox}}\), \(Tyr-Cre^{+}\) animals and showed that total amounts of Rep protein in the RPE of \(Chm^{\text{Flox}}\), \(Tyr-Cre^{+}\) animals were markedly reduced compared with \(Chm^{\text{Flox}}\) animals (Fig. 2B). A small amount of Rep1 protein was still detectable, which could be derived from the outer segment discs shed from the overlying photoreceptors in which Rep1 was present. There was no difference between the expression level of Rep1 protein in \(Chm^{\text{Flox}}\), \(Tyr-Cre^{+}\) and \(Chm^{\text{Flox}}\) animals in the respective neuroretina samples.

Next, we isolated cytosolic fractions from RPE cells of \(Chm^{\text{Flox}}\), \(Tyr-Cre^{+}\) mice and performed in vitro prenylation (Fig. 2C). As positive controls, we used \(Chm^{\text{null}}/\text{WT}\) and TM-induced \(Chm^{\text{Flox}}\), \(MerCreMer^{+}\) animals with significant RPE defects, as described in our previous study.\(^{19}\) We observed bands corresponding to unprenylated Rabs in \(Chm^{\text{Flox}}\), \(Tyr-Cre^{+}\) animals that were similar to \(Chm^{\text{null}}/\text{WT}\) and TM-induced \(Chm^{\text{Flox}}\), \(MerCreMer^{+}\) animals,\(^{19}\) confirming a prenylation defect in the RPE of the \(Chm^{\text{Flox}}\), \(Tyr-Cre^{+}\) mice. To further demonstrate the \(Chm\) KO in the RPE, we observed positive staining in this layer after immunohistochemistry on frozen eye sections of the \(Chm^{\text{WT}}\), \(Tyr-Cre^{+}\) embryos at embryonic day (E) 12.5 using Cre-specific antibody, confirming that the expression of the transgene in RPE occurred relatively early in eye development (Figs. 2D-G).

Retinal morphology of \(Chm^{\text{Flox}}\), \(Tyr-Cre^{+}\) animals was analyzed at 6, 12, and 19 months. There was no obvious pathology, including no apparent reduction in the number of photoreceptors as determined by counting the rows of nuclei in the ONL (Figs. 2H-K). Electron microscopy was used to analyze the morphology of the RPE in more detail (Fig. 3). In the samples from \(Chm^{\text{Flox}}\), \(Tyr-Cre^{+}\) animals we observed pigment irregularities (Fig. 3F). More specifically, melanosomal distribution was not homogeneous throughout the RPE, and in some areas melanosomes remained in the main body of the cell and did not enter apical processes as seen in wild-type mice. This is a

![Figure 3](https://example.com/fig3.png)
hallmark of Rab27a dysfunction, also observed in Rab27a mutant (ashen) mice. Another striking feature was disorganization of basal infoldings. In contrast to control mice (Figs. 3A, 3B), basal infoldings in all RPE-KO mice lost their regular thickness and organization (Chmnull/WT, Fig. 3D; TM-induced ChmFlox, MerCreMer+, Fig. 3E; ChmFlox, Tyr-Cre+, Fig. 3F). Overall, the data confirm our previous conclusion that the RPE and photoreceptors degenerate because of intrinsic defects in each layer that do not lead to a significant secondary degeneration of the other layer. Hence, both layers could be considered the primary pathogenic sites in CHM.

**Double-Layer Chm KO**

To test whether one layer could influence the adjacent layer, we produced mice with the Chm KO in both photoreceptors and RPE cells (ChmFlox, IRBP-Cre+, Tyr-Cre+). Histopathologic studies were performed in paraffin wax-embedded eyes (Fig. 4). In 6- to 8-month-old ChmFlox, IRBP-Cre+, Tyr-Cre+ mice, we observed a sharp decline in the number of photoreceptors (Fig. 4D) compared with control and single-layer KO mice, we observed a sharp decline in the number of photoreceptors was accelerated in the 

**Figure 4.** Histologic analysis of the retina of the 6-month old Chmnull (A), ChmFlox, Tyr-Cre+ (B), ChmFlox, IRBP-Cre+ (C), ChmFlox, IRBP-Cre+, Tyr-Cre+ (D) and 12-month old ChmFlox (E), IRBP-Cre+, Tyr-Cre+ (F), (G) Morphometric analysis of the number of photoreceptor rows in the central retina in the Chmnull, IRBP-Cre+ (purple bars) and ChmFlox, IRBP-Cre+, Tyr-Cre+ (blue bars). Numbers of animals analyzed were: ChmFlox, IRBP-Cre+: 6 to 8 months (n = 15), 12 to 14 months (n = 19); Chmnull, IRBP-Cre+, Tyr-Cre+: 6 to 8 months (n = 7), 12 to 14 months (n = 11).
Scanning Laser Ophthalmoscopy Analysis of Chm KO mice

In the same cohort of animals, a scanning laser ophthalmoscope (SLO) was used with autofluorescent (AF) visualization to investigate the pathologic changes of the fundus in vivo (Fig. 6). For ChmFlox, Tyr-Cre+ mice, we observed abnormal autofluorescent mottling of the retina that was equally obvious at 8 and 14 months (Figs. 6B, 6F, 6J) and was consistent with pigment irregularities found in these animals by electron microscopy (Fig. 3E). ChmFlox, IRBP-Cre+ mice exhibited a dramatic change from the ostensibly normal appearance of the fundus in the 8-month-old animals (Fig. 6C) to the multiple dot-shape autofluorescent spots of the retina in the older animals (14 months) (Figs. 6G, 6K). Abnormal autofluorescence of both types (mottling and dot-like specks) was present in 8- and 14-month-old ChmFlox, IRBP-Cre+, Tyr-Cre+ mice in abundance.

To establish the origin of these autofluorescent dot-like structures, we prepared whole mounts and stained them with antibody specific for ionized calcium adapter molecule (Iba-1), a marker for cells of microglia origin (Fig. 7). We observed punctate staining that resembled the SLO images, suggesting that at least some of the autofluorescent dots are of microglia origin (Figs. 7A, 7B). Quantification of the Iba-1-positive structures showed a significant increase in the number of these cells in ChmFlox, IRBP-Cre+, Tyr-Cre+ animals compared with ChmFlox, IRBP-Cre+ and ChmFlox, Tyr-Cre+ strains (Fig. 7C). Overall these data further suggest that the dynamics of retinal degeneration are altered when both photoreceptors and RPE contain the Chm mutation.

**FIGURE 5.** Quantification of the amplitude of the major components (a- and b-wave) of the dark-adapted ERG across a range of stimulus intensity. Two groups of animals (8-month- and 14-month-old) were studied. Numbers of 8-month-old animals were: ChmFlox, Tyr-Cre+ (n = 5); ChmFlox, IRBP-Cre+ (n = 3); ChmFlox, Tyr-Cre+, IRBP-Cre+ (n = 5); and ChmFlox (n = 4). Numbers of 14-month-old animals were: ChmFlox, Tyr-Cre+ (n = 6); ChmFlox, IRBP-Cre+ (n = 5); ChmFlox, Tyr-Cre+, IRBP-Cre+ (n = 6), and ChmFlox (n = 6). All genotypes are shown on each graph: ChmFlox, Tyr-Cre+ (red line), ChmFlox, IRBP-Cre+ (blue line), ChmFlox, IRBP-Cre+, Tyr-Cre+ (green line). Mean amplitudes ± SEM are plotted. Values for control animals (ChmFlox, mean ± SEM) are indicated by gray shading.

**DISCUSSION**

The aim of this study was to dissect the complex pathologic changes involving photoreceptors and RPE that occur during the CHM retinal degenerative process. For this, we created two new cell layer-specific Chm KO models: ChmFlox, IRBP-Cre+ (photoreceptor-specific) and ChmFlox, Tyr-Cre+ (RPE-specific) and produced a double transgenic line (ChmFlox, IRBP-Cre+, Tyr-Cre+). The main novel finding we report here is that the combination of RPE and photoreceptor disease is not equivalent to the sum of the effects observed in each cell type. We observed enhanced functional defects and photoreceptor cell loss in younger double transgenic mice, implying that the presence of a diseased RPE impacts the rate of photoreceptor degeneration. Therefore, this study highlighted that the adjacent cell layers in the eye influence each other and that these interactions are important factors that determine the CHM retinal degenerative process. Despite these significant novel findings, the results of this study are consistent with our previous results because they further support the idea that both layers are primary sites of CHM disease.19 We observed slow, progressive degeneration of photoreceptors but normal RPE in the photoreceptor-specific KO and RPE abnormalities but normal photoreceptors in the RPE-specific line.

The combined histologic and functional data reported here suggest a complex relationship between the survival and function of photoreceptors, RPE status, and aging. First, there was a mild but consistent decrease in a-wave amplitude in younger (8-month) ChmFlox, Tyr-Cre+ animals in comparison with ChmFlox control mice. ChmFlox, Tyr-Cre+ animals retain a normal ONL thickness into advanced age (>1.5 years) and it seems likely, therefore, that the a-wave deficit was caused by an indirect impairment of photoreceptors because of changes in the function of the choroid-eremic RPE. Importantly, the a-wave deficit receded in older
ChmFlox, Tyr-Cre+ animals, suggesting that Chm KO in the RPE somehow functionally mimics the normal aging processes. This possibility is exciting and certainly warrants further investigation. Second, we found a considerable increase in the rate of photoreceptor degeneration in combined photoreceptor and RPE Chm KO (ChmFlox, IRBP-Cre+, Tyr-Cre+) in comparison with photoreceptor Chm KO (ChmFlox, IRBP-Cre+). Therefore, choroideremic changes in photoreceptors and RPE do not progress independently. Interestingly however, the photoreceptor Chm KO alone eventually exhibited levels of photoreceptor degeneration similar to those of the double Chm KO, suggesting that the diseased RPE does not have an additive detrimental effect on ultimate Chm KO photoreceptor survival. These observations further suggest that the Chm RPE may behave as a prematurely aged RPE. Third, we observed a significantly increased amount of microglia cells in the double Chm KO, which are scavenging cells presumably recruited to clear the debris produced by dying photoreceptors, in agreement with the increased rate of photoreceptor degeneration in these mice. Similar observations were reported in other recently published studies.28,31,32 Interestingly, retinal thickening was observed in early-stage CHM patients and was proposed to be a surrogate marker for retinal remodeling through Müller (microglia) cell hypertrophy.33 This study is consistent with that hypothesis. Fourth, we report the surprising observation that the double Chm KO had less ERG suppression at 14 months than the photoreceptor Chm KO. This might have been an artifact caused by a nonretinal factor relevant to these mice because it is known that tyrosinase is expressed in some neuronal cells, where it is proposed to contribute to the formation of neuromelanin.34 Alternatively, it could be that the absence of Rep1 in the RPE somehow partially compensates for the reduced amplitude normally present in the photoreceptor-specific KO, possibly by electrical changes such as improved conductance occurring as a result of impaired vesicle transport.

We observed mild functional impairment of photoreceptors that were morphologically normal in the RPE Chm KO. Hence, our model supports the notion that CHM is a retinal disease that initially causes sight impairment by functional defects and that cell death occurs later. This would be highly relevant for potential gene therapy because any functional deficit might be reversed by expression of the transgenic REP1 in photoreceptor cells. Because both layers could be considered primary sites of the disease, it is logical to propose that future gene therapy approaches for CHM should use viral vectors that target both photoreceptors and RPE. This would include adeno-associated virus (AAV) serotype 2, which has been shown to be safe and effective after subretinal delivery in several patients in preliminary reports from...
three ongoing clinical trials.35–37 Future studies should address the hypotheses suggested by the present study. Additionally, it will be important to study the involvement of the choroid, particularly the choriocapillaris, in the pathogenesis of CHM.

Acknowledgments

The authors thank Anton Berns and Lionel Larue for transgenic mouse strains, Richard Marais and Robert Hayward for the help with obtaining the animals, Glen Jeffery, Marcus Fruttiger, and Jaimie Hoh Kam for valuable discussions on microglia cells and help with the whole mount preparation, Dhani Tracey-White for assistance with animal care and genotyping, and Abul Tarafder for stimulating discussions.

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