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Adv Exp Med Biol. Author manuscript; available in PMC 2016 July 12.

Published in final edited form as:

Adv Exp Med Biol. 2016 ; 854: 487–493. doi:10.1007/978-3-319-17121-0_65.

Gene Therapy for *MERTK*-Associated Retinal Degenerations

Matthew M. LaVail,
Beckman Vision Center, UCSF School of Medicine, 10 Koret Way, San Francisco, CA 941430730, USA

Douglas Yasumura,
Beckman Vision Center, UCSF School of Medicine, 10 Koret Way, San Francisco, CA 941430730, USA

Michael T. Matthes,
Beckman Vision Center, UCSF School of Medicine, 10 Koret Way, San Francisco, CA 941430730, USA

Haidong Yang,
Beckman Vision Center, UCSF School of Medicine, 10 Koret Way, San Francisco, CA 941430730, USA

William W. Hauswirth,
Department of Ophthalmology, College of Medicine, University of Florida, Gainesville, FL 326100284, USA

Wen-Tao Deng, and
Department of Ophthalmology, College of Medicine, University of Florida, Gainesville, FL 326100284, USA

Douglas Vollrath
Department of Genetics, Stanford University School of Medicine, Stanford, CA 94305, USA

Matthew M. LaVail: matthew.lavail@ucsf.edu; Douglas Yasumura: matthew.lavail@ucsf.edu; Michael T. Matthes: Michael.Matthes@ucsf.edu; Haidong Yang: yang.harvey@gmail.com; William W. Hauswirth: hauswrth@ufl.edu; Douglas Vollrath: Vollrath@stanford.edu

Abstract

MERTK-associated retinal degenerations are thought to have defects in phagocytosis of shed outer segment membranes by the retinal pigment epithelium (RPE), as do the rodent models of these diseases. We have subretinally injected an RPE-specific AAV2 vector, AAV2-VMD2-h*MERTK*, to determine whether this would provide long-term photoreceptor rescue in the RCS rat, which it did for up to 6.5 months, the longest time point examined. Moreover, we found phagosomes in the RPE in the rescued regions of RCS retinas soon after the onset of light. The same vector also had a major protective effect in *Mertk*-null mice, with a concomitant increase in ERG response amplitudes in the vector-injected eyes. These findings suggest that planned clinical trials with this vector will have a favorable outcome.

Correspondence to: Matthew M. LaVail, matthew.lavail@ucsf.edu.

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Keywords

Gene therapy; Retinal degeneration; MERTK; Phagocytosis; Treatment

65.1 Introduction

Retinitis pigmentosa is a family of diseases that affects approximately one in 3500 people worldwide and is a major cause of inherited blindness in the Western world. More than 50 genes have been identified in which mutations lead to retinitis pigmentosa (http://www.sph.uth.tmc.edu/retnet/). Vision loss results from the degeneration of rod and cone photoreceptors due to mutation of genes expressed either in these cells, or in the closely interacting retinal pigment epithelial (RPE) cells.

The RCS rat is a widely studied retinal degeneration (RD) model in which photoreceptor cells begin to degenerate at postnatal day (P) 20, with most disappearing by about P60 (Dowling and Sidman 1962). It has been known since the 1970s that this degeneration has a defect in the ability of the RPE to phagocytize rod outer segment tips, leading to an accumulation of outer segment debris in the subretinal space (Bok and Hall 1971; Mullen and LaVail 1976). The gene responsible for the defect in RCS rats was identified as the mer proto-oncogene tyrosine kinase (*Mertk*) (D'Cruz et al. 2000), which encodes a transmembrane receptor tyrosine kinase (Strick and Vollrath 2010).

Once the mutated gene was identified, proof of concept of gene replacement therapy was obtained in RCS rats using an adenovirus vector by Vollrath et al. (2001). Subsequently, a number of studies using different vectors, including adeno-associated virus (AAV) (Smith et al. 2003; Deng et al. 2012) and lentivirus (Tschernutter et al. 2005) were effective to different degrees, each showing improvement in photoreceptor survival, electroretinographic responses and RPE phagocytic function.

Numerous studies have described individuals with inherited RD due to *MERTK* mutations (Gal et al. 2000; Thompson et al. 2002; Tschernutter et al. 2006; Charbel Issa et al. 2009; Mackay et al. 2010; Shahzadi et al. 2010; Ostergaard et al. 2011), emphasizing the critical need for appropriate vectors for gene replacement therapy. Recombinant AAV (rAAV) in particular has gained prominence in the treatment of inherited retinal disorders in recent years (Boye et al. 2013). Three separate Phase I clinical trials for Leber congenital amaurosis type 2 have demonstrated the safety of AAV2 in human patients (Jacobson et al. 2006; Bainbridge et al. 2008; Cideciyan et al. 2008; Maguire et al. 2008).

A series of preclinical potency and safety evaluations of an AAV2 vector expressing human *MERTK* cDNA driven by an RPE-specific VMD2 (Bestrophin) promoter that was planned for human patients was recently carried out (Conlon et al. 2013). The −585/+38 bp version of the human VMD2 promoter had previously been shown to drive efficient and exclusive transgene expression in the RPE (Alexander and Hauswirth 2008). The effectiveness of the vector in RCS rats was demonstrated by electroretinogram (ERG) analysis done 60 days after injection at P9. The potential toxicity of the vector was assessed in Sprague–Dawley

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(SD) rats by electrophysiology, retinal morphology, and GLP-compliant experiments based on clinical observations and histopathology.

For the assessment of this RPE-specific vector on RDs for clinical trial application, it would be useful to know whether the vector is effective in long-term reversal of the defect in RPE phagocytosis and in rescue of photoreceptors in RCS rats. In addition, it would be important to demonstrate that the vector can rescue photoreceptors in a *MERTK*-associated RD in a different species with a different gene mutation. In this study, we have addressed both of these issues.

65.2 Materials and Methods

65.2.1 Animals

All studies were conducted in accordance with the ARVO Statement for the Use of Animals and the IACUC at UCSF. Inbred, pink-eyed RCS rats with inherited retinal dystrophy due to a deletion in the *Mertk* gene (D'Cruz et al. 2000) were characterized previously (Dowling and Sidman 1962; LaVail and Battelle 1975). *Mertk* knockout mice with an RCS-like retinal dystrophy phenotype were described earlier (Duncan et al. 2003).

65.2.2 Vector Injections, ERG Procedure and Histological Analysis

Subretinal injections of the AAV2-VMD2-h*MERTK* vector were made at P10 for RCS rats and at P4 for *Mertk* knockout mice using a previously described method (Lewin et al. 1998).

ERG analysis was carried out as previously described (Lewin et al. 1998).

For histologic studies to quantify the outer nuclear layer (ONL) thickness, methods previously described were used (LaVail and Battelle 1975; LaVail et al. 1987; Faktorovich et al. 1992).

65.3 Results

65.3.1 Long-Term Photoreceptor Rescue and Reversal of Phagocytosis Defect in RCS Rats

Comparison at P196 of the retinal structure of eyes of RCS rats injected subretinally with AAV2-VMD2-h*MERTK* and uninjected contralateral control eyes revealed a remarkable difference, equal to that seen by Conlon et al. (2013) for younger rats. In the uninjected eyes, most of the photoreceptor nuclei in the ONL had degenerated and disappeared, and an outer segment debris layer characteristic of retinal dystrophy in RCS rats was evident (Fig. 65.1a). By contrast, the vector-injected eyes appeared virtually normal in the areas of maximal rescue (Fig. 65.1b). The extent of photoreceptor rescue was typically about half of the full retinal length as shown in a retinal spidergram (Fig. 65.1c). When the RCS retinas were taken soon after the onset of light in the morning, large packets of outer segment disc membranes (phagosomes) were abundant in the RPE cell processes and internally within the RPE cell bodies (Fig. 65.1d).

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65.3.2 Photoreceptor Rescue in the MERTK-null Mouse

The differences at P52 in retinal structure between eyes of *Mertk* knockout mice injected subretinally with AAV2-VMD2-h*MERTK* at P4 and uninjected contralateral eyes were also remarkable. In the uninjected eyes, the ONL had been reduced to less than one complete row (Fig. 65.2a). By contrast, the vector-injected eyes appeared virtually normal in the areas of maximal rescue (Fig. 65.2b). The extent of photoreceptor rescue typically was most of the full retinal length, as viewed in a retinal spidergram of ONL thickness (Fig. 65.2c). The ERG responses were dramatically different for each of the waveforms; the uninjected eyes showed no scotopic a- or b-waves, and only minimal photopic b-waves, but the vector-injected eyes had responses that were 40–60% of normal (Fig. 65.2d).

65.4 Discussion

In this study, we found that when the RPE-specific AAV2-VMD2-h*MERTK* vector was injected subretinally, it protected photoreceptors from degeneration in the RCS rat for up to 6.5 months of age, the oldest examined. Moreover, the absence in phagocytosis imparted by the *Mertk* gene defect in the RCS rats (Bok and Hall 1971) was clearly reversed, as large phagosomes were abundant when the eyes were taken soon after the onset of light, typical of circadian outer segment disc shedding in the rat (LaVail 1976, 1980).

We also found that in the *Mertk* knockout mouse, which exhibits rapid loss of most photoreceptors (Duncan et al. 2003), subretinal injection of the AAV2-VMD2-h*MERTK* vector protected a majority of photoreceptor cells from degenerating. As a consequence, the electrical activity of the photoreceptors in response to light was significantly increased over that in the uninjected control eyes, where the responses were almost abolished.

These findings strongly suggest that the RPE-specific AAV2-VMD2-h*MERTK* vector that is being used in a clinical trial of different forms of *MERTK*-associated RDs (FS Alkuraya, personal communation) will prove to be effective.

Acknowledgments

This study was supported by NIH grants EY001919, EY006842 and EY002162 (MML), The Foundation Fighting Blindness (MML, DV, WWH) and Unrestricted Awards to UCSF and the University of Florida from Research to Prevent Blindness.

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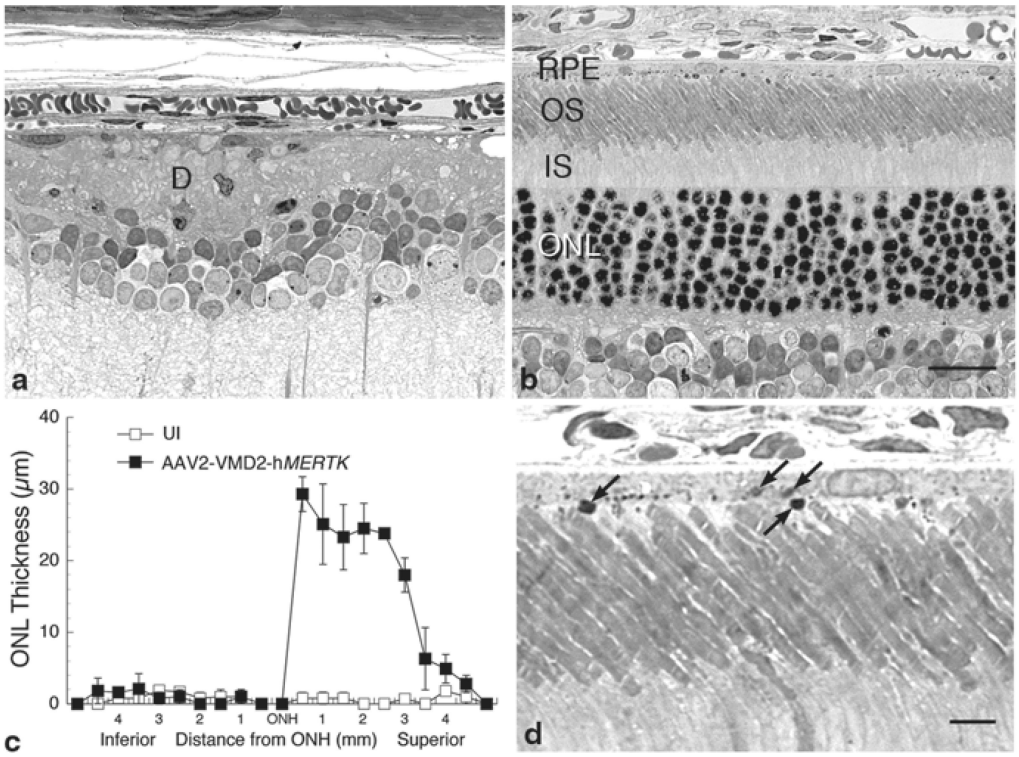

Fig. 65.1.

Structural analysis of RCS rats injected subretinally into one eye with AAV2-VMD2-h*MERTK* compared with uninjected (UI) contralateral eyes of the same rats, **a, b** Light micrographs of 1-μm plastic sections of the posterior retina of the UI eye (**a**), where most photoreceptor nuclei in the ONL have degenerated and disappeared, and an outer segment debris (**d**) zone is present. The retina of the opposite eye from the eye injected with vector is shown (**b**), which is comparable in appearance to that of normal rat retinas. **c** Retinal spidergram showing the ONL thickness along the vertical meridian of UI and vector-injected eyes (each data point is the mean ± SD from 2 rats). **d** Higher magnification of a vector-injected eye showing phagosomes (*arrows*) at the apical surface and intracellularly in the RPE. *IS* inner segments. Scale bars: **b** = 20 μm; **d** = 5 μm

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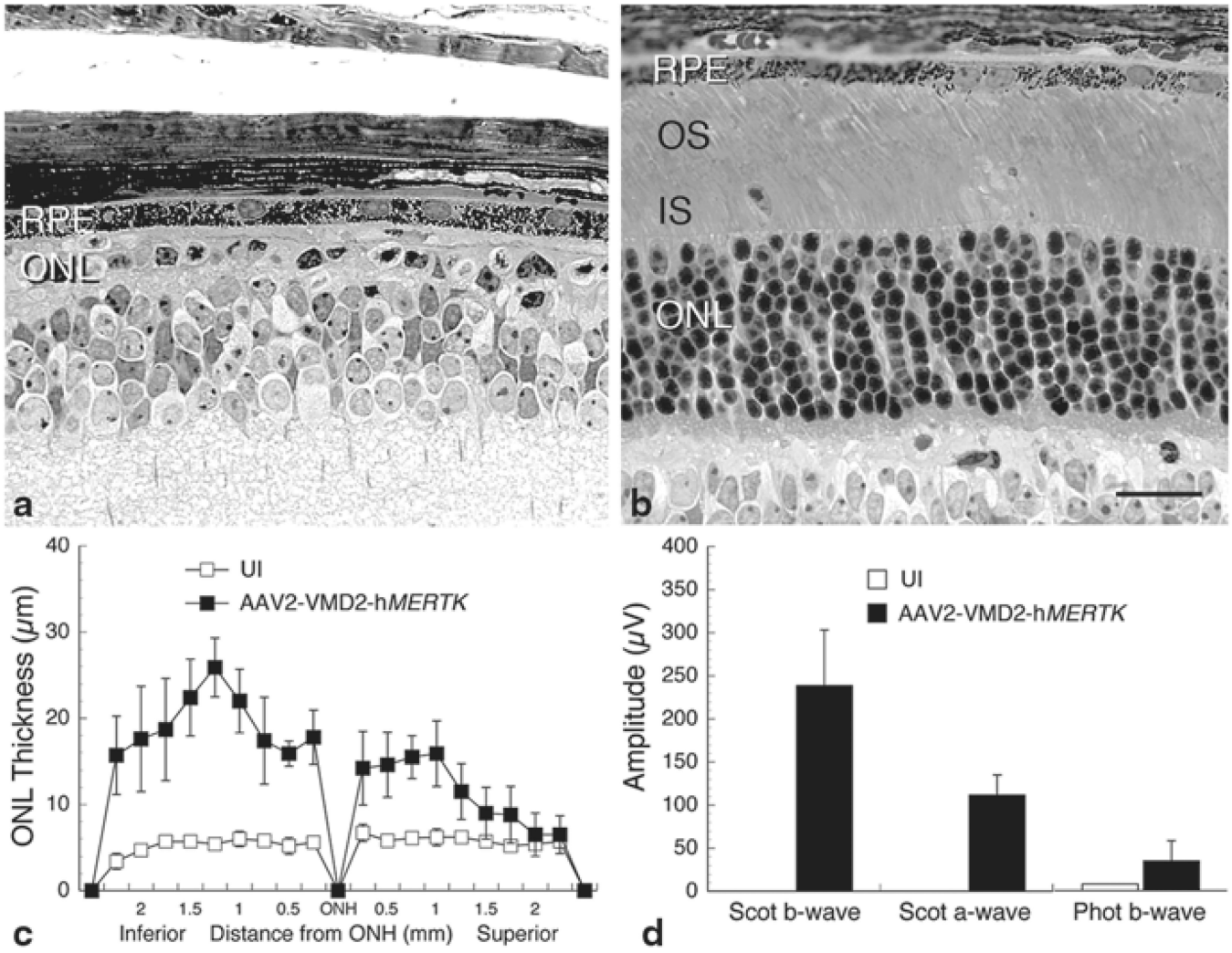

Fig. 65.2.

Structural and functional analysis of *Mertk* knockout mice injected subretinally into one eye with AAV2-VMD2-h*MERTK* (**b**) compared with uninjected (UI) contralateral eyes of the same mice (**a**). Labeling as described in Fig. 65.1 and in the text. **c** Retinal spidergram showing the ONL thickness along the vertical meridian of UI and vector-injected eyes (each data point is the mean±SD from 5 mice). **d** Electroretinographic response amplitudes from the same mice as in **c**. Scale bar=20 μm