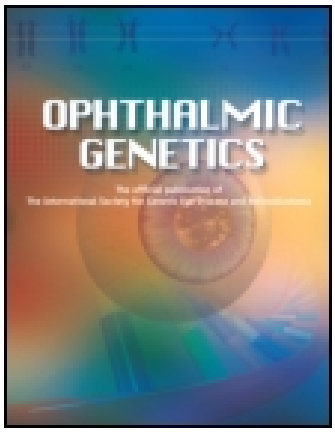

Ophthalmic Genetics
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/iopg20

Rescue of Sight by Gene Therapy—Closer than It May Appear

Tonia S. Rex[a]
[a] F.M. Kirby Center for Molecular Ophthalmology, University of Pennsylvania, PhiladelphiaUSA
Published online: 08 Jul 2009.

To cite this article: Tonia S. Rex (2007) Rescue of Sight by Gene Therapy—Closer than It May Appear, Ophthalmic Genetics, 28:3, 127-133

To link to this article: http://dx.doi.org/10.1080/13816810701503707

Ophthalmic Genetics, 28:127–133, 2007

ISSN: 1381-6810 (print) / 1744-5094 (online)
DOI: 10.1080/13816810701503707

informa
healthcare

REVIEW ARTICLE

Rescue of Sight by Gene Therapy—Closer than It May Appear

Tonia S. Rex
F.M. Kirby Center for Molecular Ophthalmology, University of Pennsylvania, Philadelphia, USA

This review will cover the state of the field in retinal degeneration and gene therapy with a focus on the great strides that have been made in retina gene therapy. Topics ranging from the development of animal models to clinical trials (for the treatment of Leber congenital amaurosis, age-related macular degeneration, and retinoblastoma) will be discussed. In addition, the results of gene therapy studies targeting the photoreceptors will be presented. Finally, strategies and progress in overcoming the challenges of photoreceptor-directed gene therapy will be presented.

Keywords Retina; gene therapy; retinoblastoma; AMD; retinal degeneration

INTRODUCTION

The eye is an ideal system for gene therapy for several reasons. The blood-retina barrier provides an immune protective environment.[1,2] The small volume of the eye prevents diffusion of vector resulting in a high concentration of virus at the area of injection. And, the optically clear nature of the eye allows for direct visualization—of the injection and the area transduced.

In some forms of retinal degeneration, the photoreceptors die as a response to a primary defect elsewhere. Examples include: mutations in retinal pigment epithelium (RPE)-specific transcripts [i.e., certain forms of Leber congenital amaurosis (LCA) and age-related macular degeneration (AMD)]; neovascularization (i.e.. the wet form of AMD, and retinopathy of prematurity); and retinal detachment. Gene therapy targeted to the RPE in order to prevent loss of photoreceptors has shown great promise. One reason for this success is due to the phagocytic nature of RPE cells. They actively take up viral particles injected into the subretinal space resulting in very efficient transduction.

The vast majority of inherited retinal degenerations are due to mutations in photoreceptor-specific transcripts (www.sph.uth.tmc.edu/Retnet/). In these cases it may be necessary to transduce the photoreceptors directly with a viral vector carrying the transgene of interest and a cell-specific promoter. There are unique challenges of photoreceptor-directed gene therapy. First, since it is impossible not to infect the RPE cells when viral particles are delivered by a subretinal injection, it may be necessary to use a cell-specific promoter in order to result in transgene expression in only the photoreceptors. Another problem is the low efficiency of transduction to these cells. Finally, and most importantly, under- or over-expression of some transgenes results in photoreceptor degeneration.[3,4] Therefore, in some cases it may be crucial to carefully regulate gene expression when using gene addition strategies to treat the photoreceptors directly.

The approach for gene therapy of retinal degenerations also depends on whether the genetic defect is autosomal recessive (AR) or autosomal dominant (AD). The majority of progress has been made in the arena of AR retinal degeneration, in which gene addition is performed. In gene addition, the cells of interest are transduced with a viral vector carrying the coding sequence for the wild-type gene and/or a neuroprotective factor. The thought is that once the wild-type protein is again present, the cell will use it and thus will not die, but rather, will function normally, resulting in improved vision and preservation of the retina. Neurotrophic factors are used as an attempt to prevent or slow photoreceptor cell death.

One potential problem of adding back the wild-type gene is that in the cases of null mutations, the body may see the introduced protein as foreign and this could elicit an immune response. However, in the retinal gene therapy studies that have thus far been performed in mice and dogs, no immune response has been detected to the transgene or viral vector (for review see[5]). This may be due to the immune privileged state of the subretinal space.[2] Some progress has been made in the treatment of AD retinal degenerations through the use of ribozyme or siRNA therapy delivered by viral vectors for longer-term treatment, as will be discussed later.

Received 5 April 2007; accepted 15 May 2007.
Address correspondence to Tonia S. Rex, Hamilton Eye Institute, University of Tennessee, Health Science Center, 930 Madison Ave., Ste. 750, Memphis, TN 38163. E-mail: trex@utmem.edu

GENE THERAPY FOR RETINOBLASTOMA

There are both sporadic and genetic (AD) forms of retinoblastoma (Rb). About one-third of all cases are due to mutations in the Rb gene.[6] Children with the inherited form of Rb present bilaterally. Current treatment strategies are radiation and/or chemotherapy. However, often enucleation is ultimately required.

Animal Studies

In the animal model of Rb, cells from a human Rb cell line were injected into the vitreal cavities of adult mice.[7] Ten days later, eyes were injected with an adenovirus (Ad) carrying the coding sequence for thymidine kinase (Ad.TK) followed by injections of ganciclovir one and four days later.[8] Efficient transduction of the tumor cells was demonstrated after injection of the viral vector. In some animals, treatment resulted in complete annihilation of the vitreal tumor.

Clinical Trial

A phase I clinical trial has been completed in which Ad.TK was injected into the vitreous and then ganciclovir was delivered systemically.[9] All patients treated had bilateral retinoblastoma, had experienced treatment failure with other modalities, and were facing imminent enucleation. Importantly, they all still had some visual function so that they might benefit from treatment. Six out of eight patients exhibited ocular inflammation and were treated. All patients treated with at least 1×10^{10} viral particles exhibited a decrease in vitreous tumor seeds. It is exciting that therapeutic benefits were detected in a trial that was designed to test safety, not efficacy. Unfortunately, the viral vector did not diffuse through the viscous vitreous and therefore multiple injections were required to target all vitreal tumor seeds. Also, this therapy did not target or treat the primary tumor. However, it may be a very useful treatment along with traditional treatments in order to avoid enucleation.

GENE THERAPY FOR AMD

AMD occurs in 10–20% of all people over the age of 65.[10] As the name suggests, it is a progressive disease that is more common in older populations. AMD is a complex disorder involving some genetic linkages along with environmental risk factors including inducers of oxidative stress such as light exposure and smoking (for review see[11]). There are two forms of AMD, exudative (wet) and non-exudative (dry). In the dry form, there is an accumulation of material between the RPE and Bruch's membrane, termed drusen. This ultimately leads to photoreceptor cell death. Much work has been done to characterize drusen and to generate animal models of this very common form of AMD (for review see[11]). In the wet form of AMD, breaks in Bruch's membrane lead to choroidal neovascularization, retinal detachment, and scarring of the retina. Ultimately there is a dramatic loss of central vision due to photoreceptor cell death.

Animal Studies

One model for the oxidative stress aspect of dry AMD is light damage. In this model, rodents are exposed to bright light for several hours and photoreceptor cell death is assessed one day later.[12] Transduction of the RPE by Ad.catalase prior to light exposure allows for onset of gene expression prior to light exposure and results in protection of the photoreceptors by consuming the H_2O_2 produced in that oxidative environment.[12] Neuroprotective agents are also able prevent photoreceptor cell death in this model; examples include treatment with AAV.erythropoietin,[13] and Ad.PEDF.[14]

However, this is not a great model for the human disease in part due to the acute nature of light damage, and also it does not take into account the presence of drusen. Recently, new mouse models of dry AMD have been developed in which drusen or drusen-like deposits form, much like is seen in patients with AMD (for review see[11]).

A common model for the wet form of AMD is induction of choroidal neovascularization by laser directed disruption of Bruch's membrane (for review see[11]). Another model is overexpression of VEGF in the RPE by injection of Ad.VEGF or AAV.VEGF.[15–18] In the laser model of CNV, subretinal, intravitreal, or periocular injection of Ad.pigment epithelial derived factor (Ad.PEDF) significantly decreases the extent of neovascularization in mice.[19,20] As a next step towards a clinical trial, laser-induced CNV and gene therapy were performed in Yorkshire pigs.[21] The pig eye is an excellent model of the human eye since they are similar in size and vasculature. Importantly, periocular injections of Ad.PEDF were also successful in limiting the extent of CNV in this animal model.

Clinical Trial

Ad.PEDF was injected into the vitreal cavity of patients with neovascular AMD.[22,23] In their phase I trial, the researchers found a dose-related inflammatory response that was mild and reversible at or below a dose of 1×10^9 particle units. To qualify for the study, patients had to have a severe case of the wet form of AMD and low visual acuity in at least one eye, and be a minimum of 50 years old. The ongoing goals of the trial are to assess safety and feasibility of this treatment, identify the maximum tolerated dose of Ad.PEDF, and look for signs of PEDF activity or treatment.

RPE-DIRECTED GENE THERAPY FOR LCA

LCA is a group of AR retinal degenerations with an embryonic onset and is due to mutations in several different genes. Ten percent of all cases are due to mutations in the RPE-specific transcript, Rpe65. RPE65 was recently found to be the retinal isomerase, an essential enzyme in the visual cycle.[24,25] In its absence, 11-cis-retinal is not produced and therefore, rhodopsin regeneration is blocked. Mutations in lecithin:retinol acyl transferase (Lrat) can also cause LCA, although it is a relatively rare form of the disease (www.sph.uth.tmc.edu/Retnet/). LRAT is

located in the RPE and is necessary for storage of retinyl esters in structures called retinosomes. The time course and phenotype of retinal degeneration in the available mouse models closely resemble that found in humans with these forms of LCA.

Animal Studies

Lrat$^{-/-}$ mice were treated with an AAV2/1.Lrat.[26] The AAV serotype 2/1 was chosen due to its high specificity for the RPE and rapid onset of gene expression. Good transduction of the RPE was achieved by a single subretinal injection as shown by immunohistochemistry. Treated eyes had improved electroretinogram (ERG) and pupillary constriction responses as compared to the contralateral, untreated eyes.

There are three animal models for LCA due to mutations in Rpe65. Two of these are the Rpe65$^{-/-}$ and the rd12 mouse.[7,28] The rd12 mouse has a nonsense mutation in the Rpe65 gene.[28] Like patients with LCA, both Rpe65$^{-/-}$ and rd12 mice exhibit severe and early vision impairment, but the photoreceptors have a slower rate of cell death. Therefore, studies have been performed in post-natal mice in order to rescue remaining photoreceptors.[29,30]

In all reports, rescue has been successful based on histological, behavioral, biochemical, and electrophysiological measurements. However, because early intervention may be necessary for complete recovery of visual function, Dejneka et al. (2003) treated knockout mice *in utero* with AAV2/1.hRpe65.[31] AAV2/1 was chosen because this serotype has the highest specificity for the RPE, which is where Rpe65 is normally expressed. A single subretinal injection resulted in production of RPE65 in the RPE after delivery to both fetal and adult mouse eyes. Treated eyes elicited ERG responses equivalent to those in normal, control eyes. In addition, rhodopsin was able to regenerate in the treated eyes. There was no adverse effect of the gene delivery on retinal development. This was the first demonstration of rescue of a congenital blinding disease by fetal gene therapy.

The third animal model of LCA is the Briard dog, which has a naturally occurring null mutation in Rpe65. Successful treatment has been demonstrated using AAV2/2.Rpe65. A single treatment results in long-term rescue.[32–34]

Clinical Trials

Phase I clinical trials for the treatment of LCA due to mutations in Rpe65 are currently underway,[35](R. Ali, personal communication). A third trial has been approved for the treatment of children with this blinding disease. It is expected that the first pediatric patient will be treated this summer at the Children's Hospital of Philadelphia (J. Bennett, personal communication).

PHOTORECEPTOR DIRECTED GENE THERAPY IN ANIMAL MODELS

LCA Due to Mutations in Photoreceptor-specific Transcripts

RPGRIP is a photoreceptor-specific protein, associated with the connecting cilium. AR mutations in the RPGRIP gene are responsible for another form of LCA. Pawlyk et al. (2005) treated *RPGRIP*$^{-/-}$ mouse retinas with an AAV containing a fragment of the mouse opsin promoter and the *RPGRIP* cDNA.[36] They were able to induce a large number of photoreceptors to produce RPGRIP and RPGR, a protein that closely associates with RPGRIP in the cilium, and localize them appropriately.

In addition, these transduced photoreceptors had longer and more organized outer segments than the contralateral, untreated eye. Finally, the thickness of the outer nuclear layer was improved. While they did detect an improved ERG in the treated eyes, there was still a decline in function over time although at a 72% lower rate than in the untreated eyes.

Another form of LCA is due to mutations in guanylate cyclase-1 (GC1). A model of this disease is the GC1 knockout mouse. This mouse was treated with an AAV carrying the GC1 coding sequence and either the mouse opsin promoter or the chicken beta actin promoter.[4] The authors achieved efficient transduction of the photoreceptors in the area of injection. However, there was no rescue of the photoreceptors either morphologically or physiologically.

The GUCY1B chicken has a deletion rearrangement in the retGC1 gene resulting in retina degeneration. Mutations in retGC1, a photoreceptor-specific protein important in regulating cGMP levels, underlie 10–20% of all cases of LCA. GC1 was delivered to the neural tubes of embryonic GUCY1B chicks via a lentivirus. Full recovery was achieved in 5 out of 6 animals that were treated.[37] Treated chicks elicited small responses by ERG, retina thickness was improved in the superior and inferior optic nerve regions, and the protein could be detected in some outer segments. The authors suggest that the slow, progressive retinal degeneration in otherwise successfully treated chicks may be due to the low number of transduced photoreceptors.

Other studies have demonstrated the ability of neighboring dysfunctional cells to negatively impact healthy cells. Therefore, they suggest injecting with a higher titer of virus, in order to introduce more viral particles and increase the number of cells transduced. Alternatively, they suggest co-injecting with a virus carrying a neurotrophic factor in order to block the neighboring cells from undergoing cell death.

Retinitis Pigmentosa

Some cases of retinitis pigmentosa are due to mutations in the peripherin/retinal degeneration slow (rds) gene. There is a naturally occurring mouse model of this disease, the rds mouse. After demonstrating limited success in rescuing photoreceptors in the rds mouse retina by treatment with AAV.prph2,[38–40] Buch et al (2006) sought to improve treatment success by co-injecting with an AAV carrying a neurotrophic factor.[41] They chose AAV.glial derived neurotrophic factor (GDNF) because of its ability to delay photoreceptor degeneration in the S334ter rat.[42] Eyes of rds/prph2 mice injected with both AAV.prph2 and AAV.GDNF had better ERG responses and histological preservation than did eyes injected with only one of the viral vectors.

Mutations in phosphodiesterase (PDEβ) underlie additional forms of retinitis pigmentosa, and this disease is recapitulated in the rd/rd mouse.[43] Treatment with AAV.PDEβ resulted in production of PDEβ in the retina, and there are indications of some very limited rescue by histological and ERG analysis.[44] This was the first demonstration of gene transfer to the photoreceptors from an AAV. Delivery of PDEβ through recombinant adenovirus or lentivirus has also led to a transient delay in photoreceptor death.[45–47]

One animal model of AD retinitis pigmentosa is the P23H rat. Since gene addition strategies will not be sufficient to treat AD disease, one alternative is treatment with a neuroprotective or anti-apoptotic factor to block photoreceptor cell death. A number of virally delivered neurotrophic factors have been used to block photoreceptor cell death in this model, including GDNF and ciliary neurotrophic factor (CNTF) .[42,48] Recently, X-linked inhibitor of apoptosis (XIAP) was delivered to the photoreceptors in this animal model via a subretinal injection of AAV2/5.[49] This serotype of AAV was chosen due to its ability to transduce photoreceptors more efficiently than other available serotypes.[50] Good transduction of the photoreceptors in the area of injection was achieved, and the outer nuclear layer was significantly thicker in treated eyes from either animal model.

Retinoschisis

Retinoschisis is an X-linked, recessive retinal degeneration due to mutations in retinoschisin (*RS1*). RS1 is secreted from both photoreceptors and bipolar cells and it seems to play a role in cellular adhesion and cell-cell interactions. In its absence, the inner retina splits starting in the fovea. An AAV was used to deliver the RS1 transgene to the photoreceptors of Rs1-deficient mice.[51] Expression was driven from the mouse opsin promoter thus providing cellular specificity of transgene expression. A high efficiency of transduction was achieved within the injected area. And, importantly there was significant improvement of retinal structure and function as determined by ophthalmoscopy and ERG recordings.

CHALLENGES AND STRATEGIES

Animal Models

One limitation in the development of gene therapy strategies for retinal degenerations is the need for accurate animal models. There are excellent models for some human inherited retina diseases (see review[52]). However, oftentimes either no model exists or current models do not truly recapitulate the human disease. With the advent of transgenic technologies, various transgenic, knock-out, and knock-in mice are continually being generated and much progress has been made. However, for complex diseases such as AMD, in which there is not a single causative gene, recapitulation of the human disease is particularly challenging.

In addition, in translating data from animal models to clinical studies, the issue of species-species variability needs to be addressed. For example, a recent gene therapy clinical trial illustrated the fact that the mouse immune system is different from the human immune system. While in animal studies no immune response was detected after gene therapy directed to the muscle, a large immune response was elicited during a phase I clinical trial.[53] Fortunately, this specific example is less of a concern in retinal gene therapy,[1,2,5] particularly where doses are orders of magnitude smaller than those used systemically, but it does illustrate the importance of testing in large animal models prior to the onset of clinical trials.

Even strain-strain differences within the inbred mouse population can alter results. A genetic defect or damage protocol in one mouse strain will result in a faster or more severe degeneration than the same defect or damage protocol in a mouse of a different background strain. For example, different levels of photoreceptor cell death occur in two strains of mice after exposure to the same light exposure protocol. In comparing the two strains it was found that they carry different isoforms of a protein involved in the visual cycle, and one isoform results in faster recycling of the visual pigment.[54]

Another example is the oxygen-induced model of retinopathy of prematurity. This model only reproducibly results in significant neovascularization in the C57Bl/6 mouse strain.[55] In fact, the relevance of strain-strain differences in the development, treatment, and comparison of animal models has become so evident that databases of this information have been developed. These issues become increasingly important as researchers approach translation of gene therapy from rescue in a mouse model to clinical trials in humans.

Viral Vectors

Packaging Capacity

The packaging capacity for AAV2 is about 4.8 kb, making delivery of large genes a challenge. This is an important issue because Stargardt's disease, an early onset macular degeneration, as well as at least one form of LCA, and some forms of Usher's syndrome are all due to mutations in photoreceptor-specific transgenes that are much longer than this limit. These transgenes can be packaged into lentivirus or adenovirus, however these vectors do not transduce the photoreceptors. Another option is the development of trans-splicing viral vectors and this has shown some promise,[56] although the efficiency is low.

Transduction Efficiency

A recent advance in gene therapy is the development of novel AAVs. Some have a quicker onset of gene expression and improved transduction efficiency.[57] This may also be true in the retina and needs to be tested. The issue of the rate of onset is particularly relevant to congenital and acute degenerations. In these cases, since it may not be possible to treat prior to or at the onset of symptoms, it may be crucial to initiate treatment as soon as possible. The issue of transduction efficiency in the retina is

directed to the neurons and, in particular, mostly the photoreceptors. Since many retinal degenerations are due to mutations in genes expressed in these cells and current vectors have relatively low transduction efficiencies. In addition, recent studies have indicated that dying cells can cause surrounding, treated cells to die. So, treatment success is limited by this "toxicity" effect. Therefore, it may be necessary to co-treat with a secreted survival factor. An example of this approach is the co-treatment with AAV.prph2 and AAV.GDNF (see below and[41]).

AD Retinal Degenerations

Treatment of AD retinal degeneration is also making headway. One proposed method is to deliver a ribozyme or siRNA (delivered by AAV) targeting both mutant and wild-type forms of rhodopsin to the retina.[58–60] This is followed by treatment with an AAV carrying a modified form of rhodopsin that is fully functional but impervious to cleavage by the ribozyme. So far the ribozyme siRNA, and modified form of rhodopsin are being developed and tested.[59–64] With this method, one treatment strategy could be used for all AD retinitis pigmentosa patients with mutations in rhodopsin. This is obviously easier and more cost effective than attempting to develop a ribozyme for each specific rhodopsin mutation.

Regulation of Transgene Expression

One method for regulating gene expression, a key component to successful gene therapy in the photoreceptor is through the use of regulatable promoter systems. While these have been around for several years they have been much improved recently. Modifications to the tetracycline system have resulted in less basal transgene expression (tet-on; for review see.[65] New analogs of rapamycin have been developed that do not suppress the immune system and can be used reproducibly to activate gene expression in the eye.[66] These systems may be the best option for situations that only require short-term treatment and where long-term treatment with the transgene may be deleterious or in cases where transgene expression levels need to be tightly controlled for effective treatment without negative consequences.

SUMMARY

The data so far from the few clinical trials that have been carried out are very encouraging. The fact that two clinical trials have now demonstrated that first generation recombinant adenoviral vectors are safe to use in the eye is quite exciting. Also exciting is the hints at efficacy in these phase I safety trials. Hopefully treatment success will be demonstrated in the phase II trials. In addition, the progress in gene therapy targeted to the photoreceptors is dramatic. It is a great time to be in the field, the vectors are continually improving in efficacy and specificity, promoters are being refined, there are more animal models available, and systems for regulating transgene expression are improved. It is very likely that within the next ten years there will be even more clinical trials using intraocular gene therapy.

ACKNOWLEDGMENT

The author thanks Dr. Jean Bennett for helpful comments.

REFERENCES

1. Anand V, Duffy B, Yang Z, Dejneka NS, Maguire AM, Bennett J. A deviant immune response to viral proteins and transgene product is generated on subretinal administration of adenovirus and adeno-associated virus. *Mol Ther*. 2002;5(2):125–132.
2. Bennett, J. Immune response following intraocular delivery of recombinant viral vectors. *Gene Ther*. 2003;10(11):977–982.
3. Chen J, Flannery JG, LaVail MM, Steinberg RH, Xu J, Simon MI. bcl-2 overexpression reduces apoptotic photoreceptor cell death in three different retinal degenerations. *Proc Nat Acad Sci. USA*. 1996;93:7042–7047.
4. Haire SE, Pang J, Boye SL, Sokal I, Craft CM, Palczewski K, Hauswirth WW, Semple-Rowland SL. Light-driven cone arrestin translocation in cones of postnatal guanylate cyclase-1 knockout mouse retina treated with AAV.GC1. *Invest Ophthalmol Vis Sci*. 2006;47(9):3745–3753.
5. Bennett J. Immune response following intraocular delivery of recombinant viral vectors. *Gene Ther*. 2003;10(11):977–982.
6. Francke U, Kung, F. Sporadic bilateral retinoblastoma and 13q-chromosomal deletion. *Med Ped Oncol*. 1976;2(4):379–385.
7. Chevez-Barrios P, Hurwitz MY, Louie K, Marcus KT, Holcombe VN, Schafer P, Aguilar-Cordova CE, Hurwitz RL. Metastatic and nonmetastatic models of retinoblastoma. *Amer J Pathol*. 2000;157(4):1405–1412.
8. Hurwitz MY, Marcus KT, Chevez-Barrios P, Louie K, Aguilar-Cordova E, Hurwitz RL. Suicide gene therapy for treatment of retinoblstoma in a murine model. *Hum Gene Ther*. 1999;10:441–448.
9. Chevez-Barrios P, Chintagumpala M, Mieler W, Paysse E, Boniuk M, Kozinetz C, Hurwitz MY, Hurwitz RL. Response of retinoblastoma with vitreous tumor seeding to adenovirus-mediated delivery of thymidine kinase followed by ganciclovir. *J Clin Oncol*. 2005;23(31):7927–7935.
10. Klein R, Klein B, Linton K. Prevalence of age-related maculopathy. The Beaver Dam Eye Study. *Ophthalmol*. 1992;99(6):933–943.
11. Edwards A, Malek G. Molecular genetics of AMD and current animal models. *Angiogenesis*. 2007;10:119–132.
12. Rex TS, Tsui I, Hahn P, Maguire AM, Duan D, Bennett J, Dunaief JL. Adenovirus-mediated delivery of catalase to retinal pigment epithelial cells protects neighboring photoreceptors from photo-oxidative stress. *Hum Gene Ther*. 2004;15:960–967.
13. Rex TS, Allocca M, Domenici L, Surace EM, Maguire AM, Lyubarsky A, Cellerino A, Bennett J, Auricchio A. Systemic but not intraocular Epo gene transfer protects the retina from light- and genetic-induced degeneration. *Mol Ther*. 2004;10(5):855–861.
14. Imai D, Yoneya S, Gehlbach PL, Wei LL, Mori K. Intraocular gene transfer of pigment epithelium-derived factor rescues photoreceptors from light-induced cell death. *J Cell Physiol*. 2005;202:570–578.
15. Wang F, Rendahl KG, Manning WC, Quiroz D, Coyne M, Miller SS. AAV-mediated expression of vascular endothelial growth factor induces choroidal neovascularization in rat. *Invest Ophthalmol Vis Sci*. 2003;44:781–790.
16. Spilsbury K, Garrett KL, Shen W-Y, Constable IJ, Rakoczy PE. Overexpression of vascular endothelial growth factor (VEGF)

in the retinal pigment epithelium leads to the development of choroidal neovascularization. *Amer J Pathol*. 2000;157(1):135–144.
17. Oshima Y, Oshima S, Nambu H, Kachi S, Hackett SF, Melia M, Kaleko M, Connelly S, Esumi N, Zack DJ, Campochiaro PA. Increased expression of VEGF in retinal pigmented epithelial cells is not sufficient to cause choroidal neovascularization. *J Cell Physiol*. 2004;01:393–400.
18. Baffi J, Byrnes G, Chan CC, Csaky KG. Choroidal neovascularization in the rat induced by adenovirus mediated expression of vascular endothelial growth factor. *Invest Ophthalmol Vis Sci*. 2000;41(11):3582–3589.
19. Gehlbach P, Demetriades AM, Yamamoto S, Deering T, Duh EJ, Yang HS, Cingolani C, Lai H, Wei L, Campochiaro PA. Periocular injection of an adenoviral vector encoding pigment epithelium-derived factor inhibits choroidal novascularization. *Gene Ther*. 2003;10:637–646.
20. Mori K, Duh E, Gehlbach P, Ando A, Takahashi K, Pearlman J, Mori K, Yang HS, Zack DJ, Ettyreddy D, Brough DE, Wei LL, Campochiaro PA. Pigment epithelium-derived factor inhibits retinal and choroidal neovascularization. *J Cell Physiol*. 2001;188:253–263.
21. Saishin Y, Silva RL, Saishin Y, Kachi S, Aslam S, Gong YY, Lai H, Carrion M, Harris B, Hamilton M, Wei L, Campochiaro PA. Periocular gene transfer of pigment epithelium-derived factor inhibits choroidal neovascularization in a human-sized eye. *Hum Gene Ther*. 2005;16:473–478.
22. Campochiaro P, Nguyen QD, Shah SM, Klein ML, Holz E, Frank RN, Saperstein DA, Gupta A, Stout JT, Macko J, DiBartolomeo R, Wei LL. Adenoviral vector-delivered pigment epithelium-derived factor for neovascular age-related macular degeneration: Results of a phase I clinical trial. *Hum Gene Ther*. 2006;17:167–176.
23. Rasmussen H, Chu KW, Campochiaro P, Gehlbach PL, Haller JA, Handa JT, Nguyen QD, Sung JU. Clinical protocol. An open-label, phase I, single administration, dose-escalastion study of ADGVPEDF.11D (ADPEDF) in neovascular age-related macular degeneration (AMD). *Hum Gene Ther*. 2001;2(16):2029–2032.
24. Jin M, Li S, Moghrabi WN, Sun H, Travis GH. Rpe65 is the retinoid isomerase in bovine retinal pigment epithelium. *Cell*. 2005;122(3):449–459.
25. Moiseyev G, Chen Y, Takahashi Y, Wu BX, Ma JX. RPE65 is the isomerohydrolase in the retinoid visual cycle. *Proc Nat Acad Sci. USA*. 2005;102(35):12413–12418.
26. Batten ML, Imanishi Y, Tu DC, Doan T, Zhu L, Pang J, Glushakova L, Moise AR, Baehr W, Van Gelder RN, Hauswirth WW, Rieki F, Palczewski K. Pharmacological and rAAV gene therapy rescue of visual functions in a blind mouse model of Leber Congenital Amaurosis. *PLoS Med*. 2005;2(11):1177–1189.
27. Redmond TM, Yu S, Lee E, Bok D, Hamasaki D, Chen N, Goletz P, Ma JX, Crouch RK, Pfeifer K. Rpe65 is necessary for production of 11-cis-vitamin A in the retinal visual cycle. *Nat Genet*. 1998;20(4):344–351.
28. Pang JJ, Chang B, Hawes NL, Hurd RE, Davisson MT, Li J, Noorwez SM, Malhotra R, McDowell JH, Kaushal S, Hauswirth WW, Nusinowitz S, Thompson DA, Heckenlively JR. Retinal degeneration 12 (rd12): a new, spontaneously arising mouse model for human Leber congenital amaurosis (LCA). *Mol Vis*. 2005;11:152–162.
29. Rakoczy P, Lai CM, Yu MJ, Daniels DM, Brankov M, Rae BC, Stoddart CW, Barnett NL, Martin-Iverson MT, Redmond TM, Narfstrom K, Zhou X, Constable IJ. Assessment of rAAV-mediated gene therapy in the Rpe65−/− mouse. *Adv Exp Med Biol*. 2003;533:431–438.
30. Pang JJ, Chang B, Kumar A, Nusinowitz S, Noorwez SM, Li J, Rani A, Foster TC, Chiodo VA, Doyle T, Li H, Malhotra R, Teusner JT, McDowell JH, Min S-H, Li Q, Kaushal S, Hauswirth WW. Gene therapy restores vision-dependent behavior as well as retinal structure and function in a mouse model of RPE65 Leber congenital amaurosis. *Mol Ther*. 2006;13(3):565–572.
31. Dejneka NS, Surace EM, Aleman TS, Cideciyan AV, Lyubarsky A, Savchenko A, Redmond TM, Tang W, Wei Z, Rex TS, Glover E, Maguire AM, Pugh EN Jr, Jacobson SG, Bennett J. In utero gene therapy rescues vision in a murine model of congenital blindness. *Mol Ther*. 2004;9(2):182–188.
32. Acland GM, Aguirre GD, Ray J, Zhang Q, Aleman TS, Cideciyan AV, Pearce-Kelling SE, Anand V, Zeng Y, Maguire AM, Jacobson SG, Hauswirth WW, Bennett J. Gene therapy restores vision in a canine model of childhood blindness. *Nat Genet*. 2001;28(1):92–95.
33. Acland GM, Aguirre GD, Bennett J, Aleman TS, Cideciyan AV, Bennicelli J, Dejneka NS, Pearce-Kelling SE, Maguire AM, Palczewski K, Hauswirth WW, Jacobson SG. Long-term restoration of rod and cone vision by single dose rAAV-mediated gene transfer to the retina in a canine model of childhood blindness. *Mol Ther*. 2005;12(6):1072–1082.
34. Narfstrom K, Katz ML, Ford M, Redmond TM, Rakoczy E, Bragadottir. In vivo gene therapy in young and adult Rpe65−/− dogs produces long-term visual improvement. *J Heredity*. 2003;94(1):31–37.
35. Jacobson SG, Acland GM, Aguirre GD, Aleman TS, Schwartz SB, Cideciyan AV, Zeiss CJ, Komaromy AM, Kaushal S, Roman AJ, Windsor EA, Sumaroka A, Pearce-Kelling SE, Conlon TJ, Chiodo VA, Boye SL, Flotte TR, Maguire AM, Bennett J, Hauswirth WW. Safety in nonhuman primates of ocular AAV2-RPE65, a candidate treatment for blindness in Leber congenital amaurosis. *Hum Gene Ther*. 2006;17:845–858.
36. Pawlyk B Smith AJ, Buch PK, Adamian M, Hong D-H, Sandberg MA, Ali RR, Li T. Gene replacement therapy rescues photoreceptor degeneration in a murine model of Leber congenital amaurosis lacking RPGRIP. *Invest Ophthalmol & Vis Sci*. 2005;46(9):3039–3045.
37. Williams ML, Coleman JE, Haire SE, Aleman TS, Cideciyan AV, Sokal I, Palczewski K, Jacobson SG, Semple-Rowland SL. Lentiviral expression of retinal guanylate cyclase-1 (RetGC1) restores vision in an avian model of childhood blindness. *PLoS Med*. 2006;3(6):904–917.
38. Schlichtenbrede F, da Cruz L, Stephens C, Smith AJ, Georgiadis A, Thrasher AJ, Bainbridge JWB, Seeliger MW, Ali RR. Long-term evaluation of retinal function in $Prph2^{Rd2/Rd2}$ mice following AAV-mediated gene replacement therapy. *J Gene Med*. 2003;5:757–764.
39. Sarra G-M, Stephens C, de Alwis M, Bainbridge JWB, Smith AJ, Thrasher AJ, Ali RR. Gene replacement therapy in the retinal degeneration slow (rds) mouse: the effect on retinal degeneration following partial transduction of the retina. *Hum Mol Genet*. 2001;10(21):2353–2361.

40. Ali RR, Sarra G-M, Stephens C, de Alwis M, Bainbridge JWB, Munro PM, Fauser S, Reichell MB, Kinnon C, Hunt DM, Bhattacharya SS, Thrasher AJ. Restoration of photoreceptor ultrastructure and function in retinal degeneration slow mice by gene therapy. *Nat Genet*. 2000;25:306–310.
41. Buch PK, MacLaren RE, Duran Y, Balaggan KS, MacNeil A, Schlichtenbrede FC, Smith AJ, Ali RR. In contrast to AAV-mediated *Cntf* expression, AAV-mediated *Gdnf* expression enhances gene replacement therapy in rodent models of retinal degeneration. *Mol Ther*. 2006;14(5):700–709.
42. McGee Sanftner LH, Abel H, Hauswirth WW, Flannery JG. Glial cell line derived neurotrophic factor delays photoreceptor degeneration in a transgenic rat model of retinitis pigmentosa. *Mol Ther*. 2001;4(6):622–629.
43. Farber D, Flannery J, Bowes-Rickman C. The rd mouse story; seventy years of research on an animal model of inherited retinal degeneration. *Prog Ret Eye Res*. 1994;13(1):31–64.
44. Jomary C, Vincent KA, Grist J, Neal MJ, Jones SE. Rescue of photoreceptor function by AAV-mediated gene transfer in a mouse model of inherited retinal degeneration. *Gene Ther*. 1997;4:683–690.
45. Bennett J, Tanabe T, Sun D, Zeng Y, Kjeldbye H, Gouras P, Maguire AM. Photoreceptor cell rescue in retinal degeneration (rd) mice by in vivo gene therapy. *Nat Med*. 1996;2(6):649–654.
46. Kumar-Singh R, Farber DB. Encapsidated adenovirus minichromosome-mediated delivery of genes to the retina: application to the rescue of photoreceptor degeneration. *Hum Mol Genet*. 1998;(12):1893–1900.
47. Takahashi M, Miyoshi H, Verma IM, Gage FH. Rescue from photoreceptor degeneration in the rd mouse by human immunodeficiency virus vector-mediated gene transfer. *J Virol*. 1999;73(9):7812–6.
48. Liang FQ, Aleman TS, Dejneka NS, Dudus L, Fisher KJ, Maguire AM, Jacobson SG, Bennett J. Long-term protection of retinal structure but not function using RAAV.CNTF in animal models of retinitis pigmentosa. *Mol Ther*. 2001;4(5):461–472.
49. Leonard KC, Petrin D, Coupland SG, Baker AN, Leonard BC, LaCasse EC, Hauswirth WW, Korneluk RG, Tsilfidis C. XIAP protection of photoreceptors in animal models of retinitis pigmentosa. *PLoS Med*. 2007;2(3):1–8.
50. Auricchio A, Kobinger G, Anand V, Hildinger M, O'Connor E, Maguire AM, Wilson JM, Bennett J. Exchange of surface proteins impacts on viral vector cellular specificity and transduction characteristics: Rhe retina as a model. *Hum Mol Genet*. 2001;10(26):3075–3081.
51. Min S-H, Molday LL, Seeliger MW, Dinculescu A, Timmers AM, Janssen A, Tonagel F, Tanimoto N, Weber BHF, Molday RS, Hauswirth WW. Prolonged recovery of retinal structure/function after gene therapy in an *Rs1h*-deficient mouse model of X-linked juvenile retinoschisis. *Mol Ther*. 2005;12(4):644–651.
52. Budzynski E, Lee Y, Sakamoto K, NAggert JK, Nishina PM. From vivarium to bedside: Lessons learned from animal models. *Ophthal Genet*. 2006;27:123–137.
53. Manno CS, Pierce GF, Arruda VR, Glader B, Ragni M, Rasko JJ, Ozelo MC, Hoots K, Blatt P, Konkle B, Dake M, Kaye R, Razavi M, Zajko A, Zehnder J, Rustagi PK, Nakai H, Chew A, Leonard D, Wright JF, Lessard RR, Sommer JM, Tigges M, Sabatino D, Luk A, Jiang H, Mingozzi F, Couto L, Ertl HC, High KA, Kay MA. Successful transduction of liver in hemophilia by AAV-Factor IX and limitations imposed by the host immune response. *Nat Med*. 2006;2(3):342–347.
54. Wenzel A, Reme CE, Williams TP, Hafezi F, Grimm C. The Rpe65 Leu450Met variation increases retinal resistance against light-induced degeneration by slowing rhodopsin regeneration. *J Neurosci*. 2001;21(1):53–58.
55. Smith LE, Wesolowski E, McLellan A, Kostyk SK, D'Amato R, Sullivan R, D'Amore PA. Oxygen-induced retinopathy in the mouse. *Invest Ophthalmol Vis Sci*. 1994;35(1):101–111.
56. Reich SJ, Auricchio A, Hildinger M, Glover E, Maguire AM, Wilson JM, Bennett J. Efficient trans-splicing in the retina expands the utility of adeno-associated virus as a vector for gene therapy. *Hum Gene Ther*. 2003;14(1):37–44.
57. Gao G, Vandenberghe LH, Wilson JM. New recombinant serotypes of AAV vectors. *Curr Gene Ther*. 2005;5(3):285–297.
58. Millington-Ward S, O'Neill B, Tuohy G, Al-Jandal N, Kiang A-S, Kenna PF, Palfi A, Hayden P, Mansergh F, Kennan A, Humphries P, Farrar GJ. Strategems in vitro for gene therapies directed to dominant mutations. *Hum Mol Genet*. 1997;6(9):1415–1426.
59. Gorbatyuk MS, PAng J-J, Thomas Jr J, Hauswirth WW, Lewin AS. Knockdown of wild-type mouse rhodopsin using an AAV vectored ribozyme as part of an RNA replacement approach. *Mol Vis*. 2005;11:648–656.
60. Kiang A-S, Palfi A, Ader M, Kenna PF, Millington-Ward S, Clark G, Kennan A, O'Reilly M, Tam LCT, Ahern A, McNally N, Humphries P, Farrar GJ. Toward a gene therapy for dominant disease: validation of an RNA interference-based mutation- independent approach. *Mol Ther*. 2005;12(3):555–561.
61. Gorbatyuk M, Justilien V, Liu J, Hauswirth WW, Lewin AS. Preservation of photoreceptor morphology and function in P23H rats using an allele independent ribozyme. *Exper Eye Res*. 2007;84:44–52.
62. Cashman SM, Binkley EA, Kumar-Singh R. Towards mutation-independent silencing of genes involved in retinal degeneration by RNA interference. *Gene Ther*. 2005;12:1223–1228.
63. Palfi A, Ader M, Kiang A-S, Millington-Ward S, Clark G, O'Reilly M, McMahon HP, Kenna PF, Humphries P, Farrar GJ. RNAi-based suppression and replacement of rds-peripherin in retinal organotypic culture. *Hum Mut*. 2006;7(3):260–268.
64. Sullivan JM, Pietras KM, Shin BJ, Misasi JN. Hammerhead ribozymes designed to cleave all human rod opsin mRNAs which cause autosomal dominant retinitis pigmentosa. *Mol Vis*. 2002;8:102–113.
65. Goverdhana S, Puntel M, Xiong W, Zirger JM, Barcia C, Curtin JF, Soffer EB, Mondkar S, King GD, Hu J, Sciascia SA, Candolfi M, Greengold DS, Lowenstein PR, Castro MG. Regulatable gene expression systems for gene therapy applications: Progress and future challenges. *Curr Gene Ther*. 2006;6(4):421–438.
66. Lebherz C, Auricchio A, Maguire AM, Rivera VM, Tang W, Grant RL, Clackson T, Bennett J, Wilson JM. Long-term inducible gene expression in the eye via adeno-associated virus gene transfer in nonhuman primates. *Hum Gene Ther*. 2005;16(2):178–186.