Published in final edited form as:

Curr Mol Med. 2012 March ; 12(3): 316–330.

AAV-mediated gene therapy in mouse models of recessive retinal degeneration

Ji-jing Pang^{1,2,*}, Lei Lei³, Xufeng Dai¹, Wei Shi², Xuan Liu², Astra Dinculescu², and J. Hugh McDowell²

¹Eye Hospital, School of Ophthalmology & Optometry, Wenzhou Medical College, Wenzhou, China

²Department of Ophthalmology, College of Medicine, University of Florida, Gainesville, FL

³Zhongshan Ophthalmic Center, Zhongshan University, Guangzhou, China

Abstract

In recent years, more and more mutant genes that cause retinal diseases have been detected. At the same time, many naturally occurring mouse models of retinal degeneration have also been found, which show similar changes to human retinal diseases. These, together with improved viral vector quality allow more and more traditionally incurable inherited retinal disorders to become potential candidates for gene therapy. Currently, the most common vehicle to deliver the therapeutic gene into target retinal cells is the adeno-associated viral vector (AAV). Following delivery to the immuno-privileged subretinal space, AAV-vectors can efficiently target both retinal pigment epithelium and photoreceptor cells, the origin of most retinal degenerations. This review focuses on the AAV-based gene therapy in mouse models of recessive retinal degenerations, especially those in which delivery of the correct copy of the wild-type gene has led to significant beneficial effects on visual function, as determined by morphological, biochemical, electrophoretographic and behavioral analysis. The past studies in animal models and ongoing successful LCA2 clinical trials, predict a bright future for AAV gene replacement treatment for inherited recessive retinal diseases.

1. Introduction

Inherited retinal degenerations affect approximately one in every 3000 individuals [1], although higher incidences were also reported in the elderly population and certain geographical areas [2,3]. It is a diverse group of conditions caused by mutations in genes predominantly expressed in either photoreceptors or RPE cells, and their mode of inheritance can be broadly divided into different forms, such as autosomal dominant, autosomal recessive, X linked recessive, digenic and mitochondrial. Significant progress in understanding the molecular and genetic basis of inherited retinal diseases, and the availability of an increasing number of animal models, has led to the development of a variety of therapeutic strategies aimed at preserving viable photoreceptor cells and rescuing the retinal function. Although stem cell therapy or neuroprotective factors offer hope for arresting the degenerative process, gene replacement therapy, especially when provided within an optimum window of time, is currently the only available method that can potentially cure monogenic recessive retinal degenerations, as shown by studies in animal models. Gene therapy directed at recessive diseases usually involves the introduction and

*Corresponding author: Ji-jing Pang, MD, PhD, Eye Hospital, School of Ophthalmology & Optometry, Wenzhou Medical College, Wenzhou, China, and Department of Ophthalmology, College of Medicine, University of Florida, Gainesville, FL; jpang@ufl.edu. The first 2 authors have equal contributions to this manuscript

expression of the correct gene to the RPE or photoreceptor cells, in order to ameliorate the progression of the disease pathology caused by an insufficiency or lack of functional wild-type protein. In addition to demonstrating its safety, the ongoing successful LCA2 clinical trials involving ocular RPE65 gene delivery, have shown that this form of therapy can lead to some visual improvement even in adults with late stages of disease [4–10].

In contrast to recessive diseases caused by deficiency of a functional wild-type gene protein product, dominant mutations present a more diverse set of conceptual and practical challenge for gene therapy, because the dominance can arise through loss-of-function mutations, gain-of-function mutations, and dominant-negative mutations [11]. Thus, gene therapy for dominant diseases uses a more complex approach depending on specific cases, which may require, for example, the simultaneous suppression of the mutant allele by specific siRNA targeting, and expression of the normal protein product by wild-type gene delivery to the appropriate target cells. In contrast to other vectors, rAAVs can efficiently transduce both RPE and mature photoreceptor cells by subretinal injection. The ability to target these non-dividing cells effectively, safely and in a long term manner has made the AAV a common vector for retinal gene transfer.

In this review, we focus on recent advances of AAV-mediated gene therapy for inherited retinal degenerations caused by monogenic recessive mutations. In the majority of these cases, delivery of the correct copy of the defective gene to the subretinal space using the right combination of AAV vector serotype and promoter has resulted in robust expression of the missing wild-type protein in its original target cell and subcellular localization, leading to amelioration of the disease pathology and even complete rescue of retinal function.

2. Gene therapy for retinal degeneration: An overview

2.1 The eye as a target for gene therapy

The eye has a combination of unique features, such as transparency and a highly compartmentalized anatomy, which make it particularly suitable as a target for gene therapy. After passing through three transparent structures within the eye, the cornea, lens and vitreous, light then reaches and interacts with the retina directly. This feature enables the visualization and accuracy of vector delivery and the subsequent non-invasive imaging and examination in vivo, by funduscopy, electroretinogram and optical coherence tomography, for example. The highly compartmentalized and enclosed anatomy of the eye makes the delivery of vectors to particular subsets of ocular cell types possible with minimal risk of vector dissemination to the rest of the body. The subretinal space has a relatively high degree of immunoprivilege and is thus being considered an ideal route for the delivery of vectors [12,13]. Tight junctions that form the blood-retina barrier separate the subretinal space from the blood supply, thus protecting it from immune-mediated damage that could lead to inflammatory processes and prevent vector-mediated transgene expression [12,13]. Inherited retinal degenerations are mainly characterized by a progressive dysfunction and death of photoreceptor cells, leading to loss of vision. Since the RPE and photoreceptor cells form an intimately interactive, functional unit, a great majority of the genes associated with retinal degeneration are expressed in either rods, cones or RPE cells, which can be efficiently targeted following subretinal delivery.

The vertebrate retina has a highly ordered laminar structure, made up of ten layers which can be observed histologically. Rod and cone photoreceptor cells are responsible for the conversion of input light into a neural signal. Both cell types contain 11-cis retinal as a chromophore covalently linked to an opsin protein in their outer segments, but they differ with respect to morphology, light absorption properties and sensitivity. Rod cells contain the photopigment rhodopsin and mediate dim light and peripheral vision in humans. They

contain a stack of membranous disks within the plasma membrane and are able to respond to a single photon of light. Cone cells contain one of three pigment proteins identified in humans, the long wavelength (L), middle wavelength (M) and short wavelength (S) sensitive opsins (red, green and blue cones, respectively), which are important for central and color vision in bright light. The variation in the amino acid composition of each opsin protein gives rise to its unique absorption spectral differences. The human retina has approximately 100 million rod photoreceptors and 6 million cone photoreceptors [14,15]. Cones are primarily concentrated in the central macula comprising nearly 100% of the fovea, where most of the inner retina is absent. The structure of the macula, especially the central fovea, makes the human retina very sensitive but also fragile to the potential subretinal injection-related damage [15]. In contrast, mice do not have a macula, and contain only two types of cones, green and blue, which are distributed throughout the whole retina with relatively more green cones in dorsal, and blue cones in ventral parts [16,17]. Furthermore, mouse blue cones are sensitive to shorter wavelengths than human blue cones, which means that mice can see into the ultraviolet [18]. A single subretinal injection containing only one microliter of vector can transfect almost 100% of the mouse retina. This allows the full rescue potential of the gene therapy to be evaluated in one eye following transgene expression in the majority of target cells, while the contralateral eye can be used as an untreated control within the same animal. In addition, since many retinal degenerative disorders lead to extensive retinal remodeling, the effects of gene therapy on the overall structural organization of the retina can also be accurately evaluated due to its highly organized laminar structure. The development of fast-acting, high-titer AAV vectors of different capsid serotypes and retinal cell specific promoters capable of efficiently transfecting the majority of retinal target cells following established surgical procedures, has expanded the potential of gene therapy for successful treatment of inherited ocular disorders.

2.2 Recombinant AAV vectors as tools for gene delivery

The AAV genome consists of a 4.7 kb linear single-stranded DNA molecule which is composed of two open reading frames, the *rep* and *cap* genes, encoding 4 replication (Rep) and 3 capsid proteins (VP1-3), respectively, flanked by two 145 bp inverted terminal repeats (ITRs). The genome is enclosed in a capsid protein with icosahedral symmetry, which determines the cellular tropism and the neutralizing antibody profile in its host. Wild type AAVs are not associated with any pathological conditions in humans. Recombinant AAV vectors used for gene therapy are derived from the wild type virus by deleting the entire viral coding region (rep and cap) thus leaving only the AAV ITRs flanking the cDNA for the therapeutic transgene, and its promoter.

At present, at least nine AAV serotypes have been evaluated for ocular use, AAV-1 to AAV-9, which differ from each other in their capsid protein sequences [19,20]. Capsid variations influence the extracellular events related to the recognition of specific receptors that determine the target tissue and cell affinities. The AAV capsid also affects intracellular processes related to AAV trafficking and uncoating, and thus plays an essential role in transduction kinetics and intensity of transgene expression. The majority of AAV vectors used for gene therapy are pseudotyped, containing a transgene of interest flanked by the ITRs of AAV2 within capsids from other AAV serotypes, thus obtaining rAAV2/n, where the first digit defines the ITRs and the second digit defines the capsid. AAV2 is the most common serotype found in humans. It has gained the most interest because of its use as a successful gene transfer vector in a variety of rodent and animal models, in spite of its limited packaging capacity. It can not only transduce RPE and photoreceptors via subretinal delivery, but also retinal ganglion cells efficiently by intravitreal injection. AAV5, 7, 8 and 9 exhibit faster transgene expression and more efficient transduction than AAV2 following subretinal delivery. However these wild-type capsid serotypes are not able to transduce

ganglion cells following intravitreal delivery [21]. New generations of AAVs allow for the transduction efficiencies to become higher and higher. For example, self-complementary AAV vectors (scAAVs) overcome the rate-limiting step required for the conversion of the vector single stranded DNA into double stranded DNA by the host cell [22,23] and mediate a quicker and more efficient transgene expression than standard AAV vectors in mouse retina [24]. scAAV-mediated gene therapy has been used in the treatment of disorders which require fast, high level intervention, particularly in some mouse models with very rapid retinal degeneration [16,17]. The other new approach is mutating highly conserved capsid tyrosine residues to phenylalanine and generating AAV capsid mutants able to escape proteasomal degradation, thus allowing more transgene protein to be expressed. This method prevents phosphorylation of surface capsid tyrosine residues on AAV viral vectors, decreasing their ubiquitination, and leading to significantly higher transgene expression levels and an increase in transduction kinetics relative to standard AAV [25]. A serotype 8 AAV containing a single point mutation (Y733F) in a surface-exposed tyrosine residue confers earlier onset and stronger transgene expression in photoreceptor cells than standard AAV8 [21]. This strong and fast-acting AAV8-733 vector delivering PDE β transgene has been shown to significantly slow down the process of retinal degeneration and to restore retinal function in the *rd10* mouse, a model with early photoreceptor degeneration, starting from postnatal day 16 [26]. This was the first demonstration of long-term restoration of vision by gene therapy in an animal model of *Pde6b*-retinitis pigmentosa.

2.3 The role of mouse models for the study of inherited retinal degeneration

A variety of well-characterized mouse models have been essential for retinal gene therapy studies. Since genetic defects play an important role in the pathogenesis of retinal diseases, the discovery and development of reproducible animal models provide insights into the various etiologies of human retinal disorders and also offer invaluable help in designing therapeutic strategies. Mouse models of recessive inherited retinal degeneration are the most widely used, and have provided good initial templates for gene transfer and pharmacological therapies. Animal models of spontaneous retinal degeneration also provide insight into the pathological mechanism(s) of disease progression and can be used to test proof of concept experiments before moving into human clinical trials. Moreover, identification of disease-associated genes in mice has led to the discovery of corresponding genes that cause retinal degeneration in humans. For example, several genes, including the β -phosphodiesterase gene mutation in retinal degeneration 1 (*rd1*) [27], the photoreceptor-specific nuclear receptor gene mutation in *rd7* [28], the PDE6c gene mutation in *cpfl1* [29,30] and the peripherin-rds gene mutation in retinal degeneration slow (*rds*) [31] were first identified in mouse models, which then led to the identification of related inherited retinopathies in human. Some of these models with naturally occurring mutations come from The Jackson Laboratory (TJL) [29,30,32–41] while others are obtained by genetic engineering [42–47]. The entire mouse retina can be targeted following a single subretinal delivery of vector, versus less than 20% of the human retina. In this sense, the mouse is a better model to initially test whether the rescue can potentially be complete following a single treatment compared to larger animal models [16,48]. Proof-of-concept experiments have demonstrated successful gene therapy in a number of mouse models, and following extensive preclinical evaluation in larger animals, some of those studies have progressed into clinical trials, such as the LCA2 form caused by RPE65 mutations. In many cases, human patients with inherited retinal disorders can present clinically with a highly variable retinal phenotype, even when the disease is associated with defects in the same gene product. It is interesting to note that a characteristic fundus pathological change, as developed in the *rd12* mice [35], a naturally occurring mouse model of human Leber congenital amaurosis with RPE65 mutation, does not appear in the RPE65 knockout mice [45], which have a similar phenotype as the *rd12* mice with respect to other features. Thus, mouse models also play a

crucial role in basic research studies aimed at understanding the variability in the phenotype caused by similar genetic defects.

3. Inherited recessive retinal degenerations

3.1 Leber congenital amaurosis (LCA)

Leber congenital amaurosis (LCA) is one of the most severe forms of inherited retinal degeneration. It is characterized by an early onset, rod-cone dystrophy causing congenital blindness or severely impaired vision, with an estimated prevalence of 1 in 81,000 [49]. There were no treatments available for LCA patients prior to gene therapy. Now, it is one of the most extensively studied diseases for retinal gene therapy and there are several ongoing clinical trials involving one form of LCA caused by mutations in the RPE65 gene [4,6–8,50].

Mutations identified in 15 different genes have been reported, which could explain approximately 70% of the LCA cases [51–53]. These genes act in various functional pathways, including retina development (CRB1 and CRX) [54–56], phototransduction (GUCY2D2 and AIPL1) [57], vitamin A metabolism (RPE65[58,59] LRAT [11], and RDH12 [60,61]), protein transport (TULP1[62], RPGRIP1[63,64], CEP290 [51], Lebercilin (LCA5) [65], and outer segment phagocytosis (MERTK [66]). In addition, the function of RD3 [65,67] and SPATA7 [53] remains unclear. Interestingly, mutations in some of these LCA genes also lead to other retinal diseases: RP (CRX, CRB1, RPE65, AIPL1, RPGRIP1 and TULP1), cone rod dystrophy (CRX, AIPL1, GUCY2D, RPE65 and RPGRIP1), Bardet-Biedl syndrome (CEP290). Therefore, research on LCA has provided meaningful insight into other retinal dystrophies. To date, successful gene therapy treatments were used in mouse models with different forms of LCA caused by mutations in the GUCY2D gene (retinal guanylate cyclase1, retGC1; LCA1) [68], the RPE65 gene (retinal pigment epithelium-specific; LCA2) [16,17,48,69], the AIPL1 gene (arylhydrocarbon receptor-interacting protein-like 1; LCA4) [70,71], the LRAT (lecithin retinol acyl transferase) gene [72] and the RPGRIP1 gene (retinitis pigmentosa GTPase regulator interacting protein 1; LCA6) [73,74].

3.1.1 The GUCY2D gene mutation—GUCY2D was the first gene implicated in LCA and was therefore designated as LCA1. About 20% of LCA cases are related to GUCY2D [75]. GUCY2D encodes photoreceptor-specific guanylate cyclase-1 (RETGC-1) which is found in cone and rod photoreceptor disc membranes. It is important in the phototransduction cascade because it plays an essential role in the regulation of cGMP and Ca^{2+} levels within the cells. Two animal models carrying null mutations in GC1 gene have been used in LCA1 studies: the naturally occurring GUCY1*B chicken and the guanylate cyclase 1 (GC1) knockout (KO) mouse [47,68,76,77]. Unlike retinal degeneration in LCA1 patients that involves both rod and cone cells, the degeneration in the GC1KO mouse is limited to cones with only slight changes in rods. The structure of rod cells appears to remain normal; however, an obvious reduction in the maximum ERG response and a faster recovery from a light flash were observed in rods. In cone cells, both function and morphology were disrupted. Loss of cone function based on ERG precedes cone degeneration. Cone numbers are initially normal but cone arrestin and cone alpha-transducin fail to properly translocate between photoreceptor outer and inner segments in response to light. The majority of cones degenerate by 6 months [47]. The rate of cone cell loss in the GC1KO mouse is comparable to that presented in LCA1 patients [76].

So far, two gene therapies have been performed in the GC1KO mouse model using the same serotype AAV5 vector, but with different promoters and species cDNA [68,77]. In an initial study, subretinal injections of AAV5-bGC1 (bovine GC1) were performed on 3 week old

GC1KO mice. Transgene expression and interaction with other proteins in the cones were confirmed by 5 weeks, though restoration of cone ERG response was not observed. The relatively low numbers and distribution of cones were initially thought to be the reason for the difficulty in detecting signals from the transduced cone cells by full-field ERG [77]. Recently, the same group used the same AAV5 vector but delivered the species-specific (murine) GC1 cDNA in 2-week old GC1KO mice by subretinal injection. Treatment efficacy was evaluated for 3 months post injection, resulting in both the restoration of correct light activated cone arrestin translocation, and robust improvement in cone-mediated ERG function and visual behavior [68].

3.1.2 The RPE65 gene mutation—Approximately 6% of LCA cases may be attributed to mutations in the RPE65 gene [9,78]. Among the visual cycle's important enzymes, RPE65 plays a crucial role as an isomerohydrolase. The RPE65 gene encodes an abundant and evolutionarily conserved 61-kDa microsomal protein expressed almost exclusively in the retinal pigment epithelium cells. The protein is associated with the smooth endoplasmic reticulum of the RPE and is necessary for the synthesis of the 11-cis retinal chromophore of photoreceptor cell visual pigments. Loss of RPE65 function results in a block in the retinoid cycle, hence affecting photoreceptor function. Mutations in RPE65 are linked to LCA2 and have provided the most successful example of gene therapy intervention in the treatment of an ocular disease.

Three mouse models of RPE65-deficiency have been reported to date: Rpe65^{-/-} knockout mouse model [45], Rpe65^{rd12} mouse model [35] and Rpe65^{R91W} a knockin mouse model [79]. Rpe65^{-/-} knockout mice display a slow, progressive retinal degeneration where cones show a faster rate of cell loss than rod photoreceptors. The lack of RPE65 function blocks the conversion of all-trans-retinyl esters to 11-cis-retinol within the RPE cells. The resulting accumulation of all-trans-retinyl esters is observed as lipid droplets in Rpe65^{-/-} mice by electron microscopy [78]. Early studies focused on restoration of rod function, since the remaining ERG signal in Rpe65^{-/-} mice was reported to originate from rod cells [80]. However, subsequent studies showed that cone function could be documented in young animals prior to the onset of cone degeneration [81]. It is possible that the remaining ERG in Rpe65 deficient mice includes a cone signal although it is abnormal [16]. AAV1 was first used for *in utero* delivery of human RPE65, driven by a CMV promoter [82]. Gene transfer into E14 fetuses resulted in RPE65 expression in RPE cells, and restoration of rhodopsin content as well as partial ERG recovery. AAV2 was also used to rescue the retinal function in this model [83]. Although both vectors resulted in some rod function restoration, the rescue effects were limited, perhaps due to methodology limitations. It is also interesting that no fundus abnormalities in the Rpe65 knockout mouse model were reported. In contrast, a naturally occurring animal model, *rd12 mouse*, which exhibits both fundus abnormalities and other characteristics of LCA2, was characterized by Pang and his colleagues [35]. In this animal, fundoscopic examination revealed evenly distributed yellowish-white spots throughout the whole retina from 5 months onward. The recessive nonsense mutation in the Rpe65 gene led to the absence of RPE65 protein and undetectable 11-cis-retinal or rhodopsin at any age. Retinyl esters increased dramatically in RPE cells with 10 fold higher than normal level by 5 months. Although cone degeneration started earlier [16,17], some small lipid-like droplets in RPE cells were first detected by electron microscopy at the age of 3 weeks. By 3 months of age, some gradually increasing voids were present, but the outer nuclear layer (ONL) showed near-normal thickness. By 7 months of age, 6–8 layers of ONL remained, and by 27 months, the ONL was reduced to 3–4 layers with the OS nearly absent. While histological examination indicated a relatively slow retinal degeneration, the scotopic ERG response was profoundly diminished and the photopic ERG response was recordable but severely attenuated even at 3 weeks of age with delayed peak times. In addition, *rd12*

mice showed substantially smaller amplitudes and lower sensitivities than wild-type mice for all measured ERG b-waves and photoresponse parameters [84].

Initially, *rd 12* mice were injected subretinally at postnatal day 14 with rAAV5-CBA-hRPE65 vector [48] and were evaluated 7 months after treatment, when fundus and retinal morphology remained normal, and retinyl ester levels were decreased in treated mice. Rhodopsin levels were restored with nearly normal ERG recovery, and all the parameters, including behavioral performance remained similar to wild-type for at least 7 months. Visual function was evaluated by ERG and VEP after the AAV5-hRPE65 vector delivery at different ages in the same mouse model [69]. The cortical visual function corresponded to the degree of retinal rescue for luminance information modulated at low temporal frequency, while VEPs to high frequency spatially and temporally modulated stimuli were impaired even at the earliest age [69]. Later, a self-complementary AAV5-smCBA-hRPE65 vector was injected into *rd12* eyes at P14 and P35 to test whether early compared to late delivery of RPE65 would make differences in the functional rescue. Evaluation showed that rod vision and function were restored with either P14 or P35 treatment, while cone vision and function were only detected after P14 treatment. The self-complementary AAV showed advantages over traditional AAV vectors and could restore function more rapidly (within 4 days post-treatment), hence, scAAV vectors may be more useful in diseases that require rapid and robust transgene expression [17,24]. Although there was no statistical difference for the amplitudes of cone-related ERG between P35 treated and untreated *rd12* eyes, the peak time in P35 treated *rd12* eyes was restored to normal level. Since cone degeneration starts around P14 in *rd12* mice, most cones have disappeared by P35, leaving only a few M-cones remaining in the peripheral dorsal and temporal quadrants [17]. Hence, it is also possible that the injections did not transfect enough RPE underlying the remaining M cones located in a small area of the peripheral retina. In order to test this hypothesis, a new experiment was designed and only those *rd12* mice that exhibited more than 95% retinal detachment following subretinal injection were selected to evaluate whether remaining peripheral cone structure and function could be restored using the same vector at P14 and P90 [16]. The results proved the hypothesis that even delayed treatment at P90 can restore both function and morphology of the remaining M cones. This has important implications for the ongoing LCA2 clinical trials [16]. In addition, the tolerance of readministration of AAV-RPE65 to the contra-lateral eye was not only observed in *rd12* mice [17], but also in the dog model [85] and in humans (Bennett et al, 2011 Translational Science Training Course and 14th ASGCT Annual Meeting, <http://www.asgct.org/am11/>).

The two animal models above represent a “null situation” for RPE65 mutations, in which the visual cycle was totally dysfunctional. However, more than 50% of RPE65 mutations in patients are missense mutation, thus the Rpe65R91W knockin mouse model was generated [79]. In contrast to the RPE65 mouse models presented above, the RPE65R91W model has less severe morphological and functional disturbances, a low but substantial presence of 11-cis-retinal and rhodopsin. This model suggests that there might be a longer window for gene therapy on those LCA2 patients with RPE65 missense mutation [86].

3.1.3 The AIPL1 gene mutation—AIPL1 (Aryl hydrocarbon interacting protein like 1) mutations have been estimated to cause approximately 7% of LCA and have also been associated with cone-rod dystrophy and retinitis pigmentosa [57]. In the retina, AIPL1 is expressed in rod and cone photoreceptor cells. The function of AIPL1 is not fully understood, but it is thought to play a role as a molecular chaperone involved in the folding and assembly of the rod and cone PDE6 phosphodiesterases, and therefore it is indirectly involved in the mechanism of the phototransduction cascade [57,87].

AAV-mediated *Aipl1* gene replacement studies were performed in three mouse models displaying different rates of photoreceptor degeneration. One is the null *AIPL1*^{-/-} mouse, in which no *AIPL1* protein is produced and the photoreceptor cell loss is almost complete by 3 weeks of age [88]. Although these mice experience normal development of the outer nuclear layer and photoreceptors, they lack both rod and cone electroretinogram responses at any age and light intensity conditions. Effective gene therapy results in this rapidly degenerating model were obtained only by early delivery at post-natal day 9 of a fast acting AAV8-RK-h*AIPL1* vector, which led to transgene expression in both rods and cones, and substantial functional and morphological improvement [70]. The other 2 mouse models are deficient in the *AIPL1* protein, and thus display a slower photoreceptor degeneration rate compared to the null. One is the *AIPL1*^{h/h} hypomorphic mutant, which produces a lower *AIPL1* expression of approximately 20% of the wild type (WT) level, and loses photoreceptors at a very slow rate under low light conditions, but a much faster rate under increased illumination. The third mouse line *AIPL1*^{hypo}, is a crossbred between the null and the *AIPL1*^{h/h}, and displays a slightly faster onset of disease than the *AIPL1*^{h/h} mouse under low light conditions [70,71,87,88]. *AIPL1* gene replacement therapy in the hypomorphic mutant mice with a slower retinal degeneration was achieved for a long term even with a less effective AAV5 serotype, which had failed to work in the null mouse [70].

3.1.4 The RPGRIP1 gene mutation and mouse model—RPGRIP1 gene, which encodes the retinitis pigmentosa GTPase regulator interacting protein 1, is expressed in photoreceptor cells. Mutations in RPGRIP1 are estimated to cause around 4.1% LCA [63,64,89]. The RPGRIP1 protein is involved in the ciliary transport mechanisms in photoreceptors and outer segment disk morphogenesis by regulating actin [90]. The mutation of this gene will cause deregulation of protein trafficking across the connecting cilium and subsequently photoreceptor loss. The RPGRIP1 knockout mouse is the only available model with early and rapidly progressing photoreceptor degeneration [73,74]. Photoreceptor abnormalities (e.g. expanded disk diameters and loss of photoreceptors) are seen as early as postnatal day 15 and most photoreceptors are lost by 3 months. However, both rod and cone photoreceptors are able to respond to light as assayed by ERG, with mildly impaired ERG responses for at least 3 weeks.

Initially, the subretinal injection of an AAV2 vector containing the murine RPGRIP1 gene at postnatal days 18–20 resulted in effectively reconstituting RPGRIP function and ameliorated the degeneration of photoreceptor morphology and function as assessed by histology and ERG [73]. However, the functional rescue was incomplete and there was decreased functional recovery at five months. This might be due to the relatively slow onset of transgene expression by AAV2 as it usually takes 2–4 weeks to reach effective protein levels after injection. Recently, an AAV2/8 vector containing the human RPGRIP1 gene was administered subretinally at postnatal days 14 into RPGRIP1 knockout mice. Preservation of both visual function and retinal morphology was obtained over a period of 5 months [74].

3.1.5 The LRAT gene mutation and mouse model—Lecithin retinol acyl transferase (LRAT) is an important visual cycle enzyme that catalyzes the esterification of all-*trans* retinol (vitamin A) to all-*trans* retinyl esters. Mutations in LRAT are found in about 1.1% of LCA cases [89,91]. The *LRAT*^{-/-} mouse model [42] shows a similar pattern of retinal degeneration as the *Rpe65*^{-/-} one, with rapid cone degeneration and cone opsin mislocalized to the inner segment, relatively slow rod degeneration, with correct rod opsin localization, and a retarded pupillary response. AAV-mediated gene replacement therapy successfully restored ERG responses to 50% of the normal level, and the pupillary light responses of *LRAT*^{-/-} mice increased approximately 2.5 log units [72].

3.1.6 Other mouse models of LCA—The *rd16* mouse model, which carries an in-frame deletion in a novel centrosomal protein, CEP290, has been implicated as a model of LCA due to its progressive and early-onset photoreceptor degeneration and ERG changes [36]

Crx (cone rod homeobox) is an otx-family homeobox gene expressed predominantly in photoreceptors and it plays a critical role in the development of photoreceptor cells [92,93]. Nearly 1.5% LCA is related to Crx gene [89]. Crx deficient mice exhibit abnormal development of photoreceptors followed by slow degeneration which is indistinguishable from the *rd* mouse [93]. Additionally, outer segment morphogenesis was found to be blocked at the elongation stage, leading to a failure in production of the phototransduction apparatus and abnormal synaptic endings in the outer plexiform layer [92]. These two models can also be used for gene therapy studies of LCA [94].

3.2 Retinitis Pigmentosa (RP)

Autosomal-recessive retinitis pigmentosa (arRP) accounts for about 20% of RP and gene therapy is relatively more straightforward to perform compared to dominant RP, since it is mainly focused on replacing a missing or insufficient gene product. There are 20 loci known for arRP, and 14 of them have been cloned [95]. More and more genes have been identified, and more related mouse models have been used for gene therapy studies. Some genes are shared by both LCA and RP (see section 3.1). Here we focus on several different genes and animal models examined in gene therapy studies.

3.2.1 Mertk gene and gene therapy—The Royal College of Surgeons (RCS) rat is one of the most commonly used spontaneous rodent models of arRP due to a defect resulting from a mutation in the Mertk gene which is normally expressed in the RPE [96]. The Mertk gene encodes a receptor tyrosine kinase (MER protein) required for proper phagocytosis of shed rod outer segments by RPE. The mutation of Mertk gene causes the dysfunction of RPE phagocytosis that results in accumulation of outer segment debris in the subretinal space, which subsequently leads to progressive loss of photoreceptor cells. In this animal model, photoreceptor cell loss occurs from postnatal day 18 onward and subretinal accumulation of debris is apparent at that time. The degeneration is almost complete after 2–3 months when little ERG signal is detectable [97,98]. Despite photoreceptor degeneration, morphologic studies show that the inner retinal architecture remains fairly normal [99]. Later, the Mertk knockout mouse, which displays a striking similarity to the RCS rat, was developed and reported [100,101]. The Mertk knockout mouse exhibited rapid and progressive photoreceptor degeneration, absence of phagosomes in the RPE at the peak of outer segment disc shedding, accumulation of debris in the outer segment-RPE interface, and slow removal of pyknotic photoreceptor nuclei [100]. Also, the ablation of MER function in mice resulted in decreased scotopic ERG readings and a similar retinal phenotype as the RCS rat on histology. However, the onset and rate of degeneration in Mertk mice is slightly faster than RCS rat.

A recombinant replication-deficient adenovirus (Ad) encoding rat Mertk was delivered to young RCS rats [96]. The results showed that the RPE phagocytosis defect in areas surrounding the injection site was corrected, photoreceptors were preserved and an increased sensitivity of treated eyes to low intensity light was also indicated by ERG. However, the relatively short duration of transgene expression is the disadvantage of Ad vector. Thus, an AAV2 vector expressing the murine Mertk gene was delivered in the same RCS rat by Smith AJ [102]. Significantly prolonged photoreceptor cell survival was observed for up to 7 months and functional photoreceptor signals were still present at 9 weeks. Histological analysis of treated eyes revealed a decrease in the amount of debris in the subretinal space, suggesting that RPE function was restored. Considering the possibility that the 2–3 weeks

delay for the onset of classical AAV-mediated transgene expression may compromise the efficacy, this research group tried to deliver another lentiviral-mediated vector (HIV-SSFV-Merk) in 10 days old RCS rats [103]. Compared to the AAV2 treatment, the lentiviral vector-mediated treatment showed improved retinal function for up to 27 weeks (9 weeks in AAV2) and with obvious higher ERG b-wave amplitudes. Thus, the use of lentiviral vectors achieved the most significant rescue for the treatment of such disorders. However, as more progress is made in the new generation of faster and more efficient AAV vectors, such as self complementary AAV [16,17,24] or capsid tyrosine-mutant AAVs [20,21,26] which shorten the onset period of transgene expression [19,48,104], there is still debate on the use of lentiviral vectors or AAV vector based on their advantages and disadvantages.

3.2.2 Usher syndrome—Usher syndrome is an autosomal recessive disorder causing both deafness and blindness which has characteristics of retinitis pigmentosa. Thus, from a visual perspective, the Usher syndrome can be seen as part of the RP family of inherited retinal disorders. There are three clinical subtypes, USH1, USH2 and USH3, based on the severity of the clinical phenotype and the genes involved. Abnormalities in ciliary cells or basement membrane are seen in USH1 and USH2, respectively, while a synaptic defect is thought to be related to USH3 [105]. However, the functions of many Usher proteins in the retina are still unclear. Different hypotheses of multiple and complex Usher protein interactions in retina, especially in the photoreceptor cilium, have been proposed [106–108]. Currently, various mouse models for Usher syndrome have been identified and generated [105] to match different subtypes of usher syndromes, although some of them failed to demonstrate the progressive retinal degeneration with reduced ERG amplitudes. Gene therapy is thought to be potentially suitable for usher syndrome because it is inherited recessively and caused by loss of function. In addition, associated hearing impairment at birth makes it possible for the usher syndrome to be identified prior to the onset of retinal degeneration. One study involved lentiviral-mediated MYO7A vector delivered to the eyes of MYO7A-null mice by Hashimoto and his colleagues [109]. Following treatment, melanosome motility, phagosome digestion and opsin clearance from the connecting cilium were observed. However, this vector did not efficiently transduce photoreceptors. One previous study has shown that AAV5 capsids are capable of packaging up to 8.9 kb of single-stranded DNA more efficiently than other serotypes, and thus developing AAV-based strategies for expression of large transgenes might be more appropriate for the treatment of Usher syndrome for efficient targeting of photoreceptor cells.

3.2.3 PDE β gene and rd mouse and gene therapy—Mutations in the human ortholog of Pde6b have been linked to autosomal recessive RP. The Pde6b gene encodes the β -subunit of rod photoreceptor cGMP phosphodiesterase, β -PDE, an essential part of the phototransduction cascade. The mutation in Pde6b results in a nonfunctional Pde6b and an accumulation of cGMP, which is a key messenger molecule that links absorption of photons of light energy to neural signaling in photoreceptors. The rd1 mouse was the first well-characterized model of arRP and has a very fast retinal degeneration [27,29]. Photoreceptor degeneration begins at 1 week of age with rods degenerating first, followed by cone cell death. The majority of the photoreceptors cells are lost by 4 weeks. Native β -PDE in the normal mouse retina is expressed by postnatal day 5 to 6. So, due to the early and rapid rate of photoreceptor cell loss it is difficult to provide effective gene therapy in time for the rd1 mouse. Subretinal injection of adenoviral, retroviral, or adeno-associated viral vectors encoding the Pde6b gene to neonatal rd1 mice resulted in partial preservation of photoreceptor structure but little, if any, ERG rescue [110–113].

Recently, the rd10 mouse [29,39,114], a hypomorphic Pde6b mutant with a missense mutation in exon 13, is considered a better model than rd1 for RP because rd10 displays a later onset and milder retinal degeneration, more similar to the related human arRP with the

same mutation. The mutation causes partial loss of β -PDE activity and a mild phenotype compared to rd1 mice. Loss of photoreceptor cells begins after P16 and most of the photoreceptors disappear by 5 weeks [29,39,114]. Dark-rearing rd10 mice further slows the rate of degeneration by as much as 4 weeks [39]. The maximal ERG response occurs at 3 weeks and is non-detectable at 2 months of age. Dark-reared 14-day-old rd 10 mice were treated with AAV-5-smCBA-PDE β vector resulting in prolonged photoreceptor survival and improved ERG and vision-guided performance for at least 3 weeks after subretinal injection [104]. However, the therapeutic effects had faded 6 weeks after treatment, since the AAV5 could not provide sufficient PDE β in time to stop the apoptosis of photoreceptors in rd10 mice. Once the apoptosis process starts, it is hard to stop it, especially after repeated exposure to strong light during ERG examination. A tyrosine-capsid mutant AAV8 (Y733F) vector, which can transduce most of the photoreceptor cells in a couple of days following in vivo delivery [20,21,26], had the earliest onset and highest transduction efficiency of photoreceptor cells. Thus, Pang et al also showed that the extent of retinal function rescue increased when the mutant AAV8 vector encoding the same PDE β gene was used. Subretinal injection of the tyrosine-capsid mutant AAV8 (Y733F)-smCBA-PDE6b to the same dark-reared P14 rd 10 mice [26] led to restored retinal function and improved visual behavioral performance plus preservation of more than half of the photoreceptors for at least six months as determined by ERG, optomotor behavioral tests, spectral domain optical coherence tomography (SD-OCT) and histology. Secondary retinal remodeling was also prevented in treated rd10 eyes [26]. Although the early photoreceptor degeneration would suggest that treatment even earlier than P14 in the *rd10* mouse might lead to even more rescue, it is difficult to detach a significant fraction of the mouse retina following transcornea subretinal injection with minimal injection-related damage before the mouse eye opens around P14 [24,104]. Although trans-sclera subretinal injection can be used for neonatal mouse treatment, it can only transduce a small part of the retina with one injection and multiple injections cause damage [19,24,115]. Furthermore, the extent of retinal coverage by the vector is important for maximal and stable rescue. For example, in rd10 mice, AAV5-mediated rescue after P14 treatment [104] is more robust than that following a similar AAV5 vector but initiating treatment at P2 [116] when both cases are evaluated at P35. The long-term rescue effect in rd10 mice as mediated by the AAV8-733 vector [26] provides a promising approach for the gene therapy for RP patients with Pde6b mutations and paves the way for potential clinical trials later on.

3.2.4 Gene therapy strategy for late stage retinal degeneration—Although photoreceptor cells degenerate in retinal disease such as RP, other neurons, including bipolar and retinal ganglion cells (RGCs) are usually preserved for a longer time. Thus, a new strategy for restoring vision in late stage retinal degeneration by utilizing the remaining retinal neurons has emerged: transduction of the channelrhodopsin-2 (ChR2) into genetically blind mice. ChR2 (Chop2 opsin with an attached chromophore) is a microbial-type rhodopsin cloned from the green alga *Chlamydomonas reinhardtii* [117,118]. It functions as a light-driven cation selective channel [118] and its delivery has been reported to convert retinal neurons such as RGCs [119,120] and ON bipolar cells [121,122] into photosensitive cells.

Initially, by intravitreal injection of the rAAV2-CAG-ChR2-GFP in the *rd1/rd1* mouse, Bi et al. found that the RGC expression of Chop2 gene was robust in ganglion cells and it restored the visually evoked cortical responses [120]. However, the Chop2 expression appeared to target both ON- and OFF-type ganglion cells. Tomita et al. tested the effects of the retinal expression of AAV2 delivered Chop2 on aged RCS rats [119,123]. Thirty percent of the retinal ganglion cells were transduced and the RCS rat vision was restored as determined by visually evoked potential (VEP) as well as behavioral testing [123]. On the other hand, AAV-mediated ChR2 gene expression in ON bipolar cells was successfully achieved in

different mouse models of RP, such as rd1 and rd10 [121,122]. Restored photosensitivity and behavioral responses were observed. These results are important for the potential treatment of late stage retinitis pigmentosa.

3.3 Stargardt disease(STGD)

Stargardt disease is the most common hereditary macular dystrophy whose features include progressive central visual loss that begins in early teenage years, and macular atrophy (estimated frequency of 1:8000–1:10000 in the US). It has an autosomal recessive mode of inheritance and is caused by mutations in the ABCA4 gene (also known as ABCR) [124]. The ABCA4 protein, which corresponds to a previously identified rod outer segment protein called rim protein (RmP), is responsible for the translocation of N-retinylidene-PE, an intermediate in the visual cycle, from the lumen of the disc to the photoreceptor cytoplasm [125,126]. Following ABCA4 dysfunction, N-retinylidene-PE will accumulate in the lumen of the outer segment disc and react with all-trans-retinal leading to the formation of A2E, a major autofluorescent component of lipofuscin. High levels of lipofuscin accumulation in RPE can cause photoreceptor degeneration as seen in Stargardt disease. ABCA4 mutations have also been identified in autosomal recessive RP [127,128], cone-rod dystrophy [128] and fundus flavimaculatus [129].

3.3.1 Rodent models for Stargardts disease—To date, the ABCA4^{-/-} mouse is the only genetic model for Stargardts disease and was first reported by Weng and colleagues [130]. This mouse model with a knock-out of ABCA4 gene and consequent loss of RmP harbors two major features observed in humans: (1) Delayed rod dark adaptation [124], (2) Delayed clearance of all-trans-retinal and increased phosphatidylethanolamine and N-Retinylidene-Phosphatidylethanolamine. In addition, there is an increased accumulation of lipofuscin and A2E within the RPE cells.

3.3.2 Gene therapy for Stargardts disease—As discussed before (see section 2), the traditional rAAV2 vector has a limited package capacity of only 4.7kb. The ABCA4 cDNA was considered too large (8.9kb) to be packaged into this vector. By testing the ability of eight different rAAV serotypes to package a large expression cassette containing either ABCA4 or MYO7A, Allocca M and colleagues [131] found that rAAV2/5 worked the most efficiently. The rAAV2/5-CMV- ABCA4 vector was administered via subretinal injection to 1-month-old ABCA4^{-/-} mice, resulting in a reduced number of lipofuscin granules and a decreased RPE thickness. This suggests that rAAV2/5 mediated-ABCA4 gene transfer ameliorates the RPE ultrastructural abnormalities in this ABCA4 knock-out mouse. Furthermore, levels of the fluorophores were significantly reduced and the ability of ABCA4^{-/-} photoreceptors to recover was also improved. This use of rAAV2/5 for ABCA4 delivery provides a promising therapeutic strategy for recessive Stargardt disease and expands the therapeutic potential for large genes.

3.4 Achromatopsia

Achromatopsia is a rare, recessive genetic disease characterized by cone dysfunction, leaving the patient with only rod mediated vision. Two types of achromatopsia are recognized: complete (typical) and incomplete (atypical). The two forms bear phenotypic resemblance, with the only differences being that incomplete achromatopsia patients tend to retain some residual color vision and have slightly better visual acuity. The complete form results in serious visual deficits and affects approximately 1:30,000 Americans [132]. No treatments or cure were available to correct cone function for achromats, and the only medical care given was to manage symptoms by limiting retinal light exposure with tinted contact lenses.

To date, four genes have been found to be implicated in achromatopsia-associated mutations: cyclic nucleotide-gated channel beta-3 (CNGB3), cyclic nucleotide-gated channel alpha-3 (CNGA3), guanine nucleotide-binding protein (GNAT2) [132–137] and phosphodiesterase 6C (PDE6C) [30,138]. The proteins encoded by these four genes are specifically expressed in cone photoreceptors cells. CNGA3 encodes the alpha subunit and CNGB3 encodes the beta subunit of the cone cyclic nucleotide-gated (CNG) cation channel. GNAT2 encodes the cone specific alpha subunit of transducin. Transducin mediates an initial step in phototransduction, whereas the cGMP-gated channels CNGA3 and CNGB3 moderate the final steps in the cascade. PDE6C encodes the cone alpha subunit of cyclic guanosine monophosphate (cGMP) phosphodiesterase, which converts cGMP to 5'-GMP, and thus plays an important role in cone phototransduction [138]. Of all mutations that cause achromatopsia, those in CNGB3 account for about 45–50% of cases and CNGA3 mutations account for about 23%, whereas only a few families have been reported to have GNAT2 or PDE6C mutations [30,132,134,136,138,139]. Naturally occurring mouse models with CNGA3, GNAT2 and PDE6C mutation have been discovered [15,30,37]. CNGA3 [140] and CNGB3 knockout mice were also created [43]. AAV mediated gene therapy successfully restored vision in most of them, indicating that this treatment model might soon enter the clinical stage.

3.4.1 The mutant GNAT2 mouse and gene therapy—The GNAT2^{cpfl3} mouse is the only reported mouse model related to the mutation of GNAT2 gene [37]. Homozygous *cpfl3* mice exhibit progressive vacuolization of the photoreceptor outer segments and signs of early retinal degeneration (retinal vein dilation and arteriole constriction) at 8 months. ERG results showed abnormal photopic responses which decreased progressively and were extinguished by 9 months. Scotopic responses have some diminution with time but were near normal at 9 months. However, cones appear to retain structural integrity by PNA staining.

Using Gnat2^{cpfl3} mice, Alexander and colleagues performed subretinal injections of an AAV serotype 5 vector (4×10^{10} vector genome containing particles) carrying a wild type mouse GNAT2 cDNA under control of a human red cone opsin promoter that targets vector transgene expression to cones [141]. In treated eyes, light-adapted (cone specific) ERG amplitudes were restored and maintained at normal levels for at least 7 months [141]. Cone ERG amplitudes in untreated eyes remained undetectable. Furthermore, visual acuity was restored to normal levels by optomotor behavioral testing. These encouraging results suggest that long term, effective cone-targeted therapy is possible, providing a basis for treating a variety of related diseases. Additional testing has revealed that treated eyes also respond to cone isolating flicker ERG stimuli more robustly than untreated contralateral eyes, further confirming the efficacy to cone-target gene therapy in this model of achromatopsia.

3.4.2 The CNGA3 Mutant Mouse and Gene Therapy—CNGA3^{-/-} mouse model was generated using homozygous knockout of the CNGA3 by targeted deletion of exon 7 of the CNGA3 gene [140]. The genetic inactivation of CNGA3 in mice introduces progressive cone degeneration with normal rod function and structure. Electron microscopy revealed disorganized cone outer segments and immunohistochemistry showed decreased cone photoreceptors with age. ERG results showed that while the a-wave amplitude was normal, the b-wave at high light intensities was decreased which suggested that the deletion of CNGA3 specifically disrupted cone function whereas the rod pathway was intact.

In this CNGA3^{-/-} mouse model, Michalskis et al. successfully restored cone function for up to 3 months by using rAAV5 vectors [142]. Specifically, the vector was driven by a 0.5kb fragment of the mouse blue opsin promoter and then packaged with Y719F AAV5 mutant capsid serotype for higher resistance to proteasomal degradation. A total of $6-9 \times 10^9$ rAAV

genomic particles were delivered into the subretinal space of P12-P14 *CNGA3*^{-/-} mice. ERG results showed that functional restoration of cone photoreceptor began at 10 weeks post-treatment. Furthermore, ganglion cells from the treated *CNGA3*^{-/-} mice displayed cone-driven, light-evoked, spiking activity indicating that signals generated were transmitted to the brain.

Another mouse with a cone function loss, *Cpfl5* (Cone photoreceptor function Loss 5), which has an ocular phenotype similar to human achromatopsia, was discovered in The Jackson Laboratory. *Cpfl5* is a naturally occurring mouse model of autosomal recessive achromatopsia as defined by a missense mutation in exon 5 of the *CNGA3* gene [15]. Functional studies found no cone ERG response. Histological and immunohistochemical analysis revealed a migration of cone cell bodies into the outer plexiform layer of the retina from as early as postnatal week 3 suggesting early pathogenesis.

To test whether AAV-mediated *CNGA3* gene therapy could restore cone function to *Cpfl5* mice [142], an AAV vector encoding the wild type mouse *CNGA3* gene driven by a human blue cone promoter (HB570) that preferentially targeted transgene expression to cones was delivered to one eye of P14 *Cpfl5* mice and the untreated contralateral eyes were used as controls. Dark- and light-adapted ERGs were recorded periodically from 3 to 10 weeks after injection. In the treated eyes, measurable light-adapted ERG signals were observed at 3 weeks after vector administration. This restored light-adapted ERG remained stable for at least 10 weeks after treatment with b-wave amplitudes about half of that recorded in normal C57BL/6 J mice. In the contralateral untreated eyes, cone-driven ERGs remained unrecordable. Dark-adapted ERG analysis confirmed that the rod function is normal and unaffected by vector treatment in *Cpfl5* mice at this age.

3.4.3 The CNGB3 and PDE6C Mutant Mouse Models—Recently, *CNGB3* knockout mice have been produced [43,143]. Unlike *CNGA3*^{-/-} mice which have no recordable cone ERGs, *CNGB3*^{-/-} mice have a residual cone response (~25% of the wild-type level) [144] and the cone degeneration is relatively slow. AAV2/8-mediated gene therapy also rescued this model [144] with a wider treatment window.

Significant improvement of function between treated and untreated eyes was observed in all age groups from P15 to P180, although an ideal window for treatment is present around P15–30. Treatment at P30 restored near normal ERGs, which lasted for at least 9 months and corrected the mis-localization of M-cone opsin. Furthermore, AAV-mediated human *CNGB3* expression also restored the *CNGA3* level although the exact mechanism is unknown [144].

The spontaneous mouse mutant *Cpfl1* (Cone photoreceptor function loss 1), featuring a lack of cone function and rapid cone photoreceptor degeneration, represents a homologous mouse model for PDE6C associated achromatopsia. The mice have a normal fundus with overall normal retinal structure except for progressive degeneration of cone cells. The ERG shows no cone-mediated photoresponse but normal rod-mediated photoresponses from 3 weeks to 15 months of age [30].

3.5 X-linked retinoschisis

X-linked juvenile retinoschisis (XLRS), a leading cause of macular degeneration in young male patients, is caused by mutations in the *RS1* gene encoding a 224 amino acid secreted protein, retinoschisin, implicated in cell adhesion and maintenance of retinal integrity [145]. XLRS is a recessively inherited retinal disorder with a worldwide prevalence ranging from 1:5,000 to 1:25,000 [146]. The disease is characterized by foveo-macular schisis within multiple retinal layers, the formation of cystic cavities within the central retina, and a

reduced electroretinogram b-wave relative to the a-wave, leading to progressive loss of photoreceptors. Patients with XLRS are more prone to complications, such as retinal detachment, vitreous hemorrhage, and neovascular glaucoma, which may cause severe visual impairment [147].

3.5.1 Mouse models for XLRS disorder—Currently, there are 3 mouse models of retinoschisis, which share some of the morphological and functional retinal abnormalities found in the human disease, such as areas of schisis within the inner nuclear layer, an abnormal dark adapted ERG b-wave, and disorganization of retinal layers [46,148]. The effects of gene therapy in two of the knockout models of retinoschisis were successfully tested using various AAV serotypes and promoters.

3.5.2 Gene therapy for XLRS disorder—AAV-mediated gene replacement therapy in two of the knockout mouse models of XLRS showed that either subretinal or intravitreal delivery led to expression of robust RS1 protein, improved retinal integrity and increased ERG b-wave [149,150]. In contrast to humans, mice lack a foveal region, and thus a less invasive surgical procedure such as intravitreal delivery may be the preferred route for future human clinical trials, since the fragile foveal region is severely affected in most XLRS patients [151].

4. Conclusion

Based on a great amount of evidence, it is clear that AAV vectors can offer long-term and robust expression of transgenes in targeted retinal cells, with a suitable combination of AAV capsid serotype, promoter, and injection site. Many well-characterized mouse models with monogenic recessive mutations are currently available for testing potential treatments related to human diseases. With the new generation of tyrosine mutant AAV vectors, substantial morphological and functional retinal improvements have been achieved not only in those mouse models with relatively slow photoreceptor cell loss, but also in those with a quick and extensive retinal degeneration.

These proof of concept experiments have led to successful ongoing clinical trials of AAV-mediated gene therapy for human LCA2 with RPE65 mutations. It is possible that disorders with other recessive mutations including MERTK-RP, achromatopsia with CNGB3 mutations, and X-linked juvenile retinoschisis with RS-1 mutation, could also move into clinical trials in the near future. Since the majority of these disorders leave the retina fragile, and prone to damage following subretinal surgical detachment, mouse models also offer the possibility to test the efficiency of the outer retinal transduction following a safer intravitreal approach. Although the mouse eye is different from human, it has enabled the evaluation of AAV-mediated gene replacement therapy yielding a potentially bright clinical future for recessive inherited retinal degenerations.

Reference List

1. Bessant DA, Ali RR, Bhattacharya SS. Molecular genetics and prospects for therapy of the inherited retinal dystrophies. *Curr Opin Genet Dev.* 2001; 11(3):307–316. [PubMed: 11377968]
2. Xu L, Hu L, Ma K, Li J, Jonas JB. Prevalence of retinitis pigmentosa in urban and rural adult Chinese: The Beijing Eye Study. *Eur J Ophthalmol.* 2006; 16(6):865–866. [PubMed: 17191195]
3. Sen P, Bhargava A, George R, Ve RS, Hemamalini A, Prema R, et al. Prevalence of retinitis pigmentosa in South Indian population aged above 40 years. *Ophthalmic Epidemiol.* 2008; 15(4): 279–281. [PubMed: 18780262]

4. Bainbridge JW, Smith AJ, Barker SS, Robbie S, Henderson R, Balaggan K, et al. Effect of gene therapy on visual function in Leber's congenital amaurosis. *N Engl J Med*. 2008; 358(21):2231–2239. [PubMed: 18441371]
5. Hauswirth WW, Aleman TS, Kaushal S, Cideciyan AV, Schwartz SB, Wang L, et al. Treatment of leber congenital amaurosis due to RPE65 mutations by ocular subretinal injection of adeno-associated virus gene vector: short-term results of a phase I trial. *Hum Gene Ther*. 2008; 19(10): 979–990. [PubMed: 18774912]
6. Maguire AM, Simonelli F, Pierce EA, Pugh EN Jr, Mingozzi F, Bennicelli J, et al. Safety and efficacy of gene transfer for Leber's congenital amaurosis. *N Engl J Med*. 2008; 358(21):2240–2248. [PubMed: 18441370]
7. Cideciyan AV, Aleman TS, Boye SL, Schwartz SB, Kaushal S, Roman AJ, et al. Human gene therapy for RPE65 isomerase deficiency activates the retinoid cycle of vision but with slow rod kinetics. *Proc Natl Acad Sci U S A*. 2008; 105(39):15112–15117. [PubMed: 18809924]
8. Cideciyan AV, Hauswirth WW, Aleman TS, Kaushal S, Schwartz SB, Boye SL, et al. Human RPE65 gene therapy for Leber congenital amaurosis: persistence of early visual improvements and safety at 1 year. *Hum Gene Ther*. 2009; 20(9):999–1004. [PubMed: 19583479]
9. Cideciyan AV. Leber congenital amaurosis due to RPE65 mutations and its treatment with gene therapy. *Prog Retin Eye Res*. 2010; 29(5):398–427. [PubMed: 20399883]
10. Simonelli F, Maguire AM, Testa F, Pierce EA, Mingozzi F, Bennicelli JL, et al. Gene therapy for Leber's congenital amaurosis is safe and effective through 1.5 years after vector administration. *Mol Ther*. 2010; 18(3):643–650. [PubMed: 19953081]
11. Wilson JH, Wensel TG. The nature of dominant mutations of rhodopsin and implications for gene therapy. *Mol Neurobiol*. 2003; 28(2):149–158. [PubMed: 14576453]
12. Li W, Kong F, Li X, Dai X, Liu X, Zheng Q, et al. Gene therapy following subretinal AAV5 vector delivery is not affected by a previous intravitreal AAV5 vector administration in the partner eye. *Mol Vis*. 2009; 15:267–275. [PubMed: 19190735]
13. Li Q, Miller R, Han PY, Pang J, Dinculescu A, Chiodo V, et al. Intraocular route of AAV2 vector administration defines humoral immune response and therapeutic potential. *Mol Vis*. 2008; 14:1760–1769. [PubMed: 18836574]
14. Ahnelt PK, Kolb H. The mammalian photoreceptor mosaic-adaptive design. *Prog Retin Eye Res*. 2000; 19(6):711–777. [PubMed: 11029553]
15. Pang JJ, Alexander J, Lei B, Deng W, Zhang K, Li Q, et al. Achromatopsia as a potential candidate for gene therapy. *Adv Exp Med Biol*. 2010; 664:639–646. [PubMed: 20238068]
16. Li X, Li W, Dai X, Kong F, Zheng Q, Zhou X, et al. Gene therapy rescues cone structure and function in the 3-month-old rd12 mouse: a model for midcourse RPE65 leber congenital amaurosis. *Invest Ophthalmol Vis Sci*. 2011; 52(1):7–15. [PubMed: 21169527]
17. Pang J, Boye SE, Lei B, Boye SL, Everhart D, Ryals R, et al. Self-complementary AAV-mediated gene therapy restores cone function and prevents cone degeneration in two models of Rpe65 deficiency. *Gene Ther*. 2010; 17(7):815–826. [PubMed: 20237510]
18. Jacobs GH, Neitz J, Deegan JF. Retinal receptors in rodents maximally sensitive to ultraviolet light. *Nature*. 1991; 353(6345):655–656. [PubMed: 1922382]
19. Pang JJ, Lauramore A, Deng WT, Li Q, Doyle TJ, Chiodo V, et al. Comparative analysis of in vivo and in vitro AAV vector transduction in the neonatal mouse retina: effects of serotype and site of administration. *Vision Res*. 2008; 48(3):377–385. [PubMed: 17950399]
20. Petrs-Silva H, Dinculescu A, Li Q, Deng WT, Pang JJ, Min SH, et al. Novel Properties of Tyrosine-mutant AAV2 Vectors in the Mouse Retina. *Mol Ther*. 2011; 19(2):293–301. [PubMed: 21045809]
21. Petrs-Silva H, Dinculescu A, Li Q, Min SH, Chiodo V, Pang JJ, et al. High-efficiency transduction of the mouse retina by tyrosine-mutant AAV serotype vectors. *Mol Ther*. 2009; 17(3):463–471. [PubMed: 19066593]
22. McCarty DM. Self-complementary AAV vectors; advances and applications. *Mol Ther*. 2008; 16(10):1648–1656. [PubMed: 18682697]

23. McCarty DM, Fu H, Monahan PE, Toulson CE, Naik P, Samulski RJ. Adeno-associated virus terminal repeat (TR) mutant generates self-complementary vectors to overcome the rate-limiting step to transduction in vivo. *Gene Ther.* 2003; 10(26):2112–2118. [PubMed: 14625565]
24. Kong F, Li W, Li X, Zheng Q, Dai X, Zhou X, et al. Self-complementary AAV5 vector facilitates quicker transgene expression in photoreceptor and retinal pigment epithelial cells of normal mouse. *Exp Eye Res.* 2010; 90(5):546–554. [PubMed: 20138034]
25. Zhong L, Li B, Mah CS, Govindasamy L, Agbandje-McKenna M, Cooper M, et al. Next generation of adeno-associated virus 2 vectors: point mutations in tyrosines lead to high-efficiency transduction at lower doses. *Proc Natl Acad Sci U S A.* 2008; 105(22):7827–7832. [PubMed: 18511559]
26. Pang JJ, Dai X, Boye SE, Barone I, Boye SL, Mao S, et al. Long-term Retinal Function and Structure Rescue Using Capsid Mutant AAV8 Vector in the rd10 Mouse, a Model of Recessive Retinitis Pigmentosa. *Mol Ther.* 2011; 19(2):234–242. [PubMed: 21139570]
27. Bowes C, Li T, Danciger M, Baxter LC, Applebury ML, Farber DB. Retinal degeneration in the rd mouse is caused by a defect in the beta subunit of rod cGMP-phosphodiesterase. *Nature.* 1990; 347(6294):677–680. [PubMed: 1977087]
28. Akhmedov NB, Piriev NI, Chang B, Rapoport AL, Hawes NL, Nishina PM, et al. A deletion in a photoreceptor-specific nuclear receptor mRNA causes retinal degeneration in the rd7 mouse. *Proc Natl Acad Sci U S A.* 2000; 97(10):5551–5556. [PubMed: 10805811]
29. Chang B, Hawes NL, Hurd RE, Davisson MT, Nusinowitz S, Heckenlively JR. Retinal degeneration mutants in the mouse. *Vision Res.* 2002; 42(4):517–525. [PubMed: 11853768]
30. Chang B, Grau T, Dangel S, Hurd R, Jurkles B, Sener EC, et al. A homologous genetic basis of the murine cpfl1 mutant and human achromatopsia linked to mutations in the PDE6C gene. *Proc Natl Acad Sci U S A.* 2009; 106(46):19581–19586. [PubMed: 19887631]
31. Travis GH, Brennan MB, Danielson PE, Kozak CA, Sutcliffe JG. Identification of a photoreceptor-specific mRNA encoded by the gene responsible for retinal degeneration slow (rds). *Nature.* 1989; 338(6210):70–73. [PubMed: 2918924]
32. Boatright JH, Moring AG, McElroy C, Phillips MJ, Do VT, Chang B, et al. Tool from ancient pharmacopoeia prevents vision loss. *Mol Vis.* 2006; 12:1706–1714. [PubMed: 17213800]
33. Hawes NL, Chang B, Hageman GS, Nusinowitz S, Nishina PM, Schneider BS, et al. Retinal degeneration 6 (rd6): a new mouse model for human retinitis punctata albescens. *Invest Ophthalmol Vis Sci.* 2000; 41(10):3149–3157. [PubMed: 10967077]
34. Heckenlively JR, Hawes NL, Friedlander M, Nusinowitz S, Hurd R, Davisson M, et al. Mouse model of subretinal neovascularization with choroidal anastomosis. *Retina.* 2003; 23(4):518–522. [PubMed: 12972764]
35. Pang JJ, Chang B, Hawes NL, Hurd RE, Davisson MT, Li J, et al. Retinal degeneration 12 (rd12): a new, spontaneously arising mouse model for human Leber congenital amaurosis (LCA). *Mol Vis.* 2005; 11:152–162. [PubMed: 15765048]
36. Chang B, Khanna H, Hawes N, Jimeno D, He S, Lillo C, et al. In-frame deletion in a novel centrosomal/ciliary protein CEP290/NPHP6 perturbs its interaction with RPGR and results in early-onset retinal degeneration in the rd16 mouse. *Hum Mol Genet.* 2006; 15(11):1847–1857. [PubMed: 16632484]
37. Chang B, Dacey MS, Hawes NL, Hitchcock PF, Milam AH, Atmaca-Sonmez P, et al. Cone photoreceptor function loss-3, a novel mouse model of achromatopsia due to a mutation in Gnat2. *Invest Ophthalmol Vis Sci.* 2006; 47(11):5017–5021. [PubMed: 17065522]
38. Chang B, Heckenlively JR, Bayley PR, Brecha NC, Davisson MT, Hawes NL, et al. The nob2 mouse, a null mutation in Cacna1f: anatomical and functional abnormalities in the outer retina and their consequences on ganglion cell visual responses. *Vis Neurosci.* 2006; 23(1):11–24. [PubMed: 16597347]
39. Chang B, Hawes NL, Pardue MT, German AM, Hurd RE, Davisson MT, et al. Two mouse retinal degenerations caused by missense mutations in the beta-subunit of rod cGMP phosphodiesterase gene. *Vision Res.* 2007; 47(5):624–633. [PubMed: 17267005]
40. Chang B, Heckenlively JR, Hawes NL, Roderick TH. New mouse primary retinal degeneration (rd-3). *Genomics.* 1993; 16(1):45–49. [PubMed: 8486383]

41. Won J, Shi LY, Hicks W, Wang J, Hurd R, Naggert JK, et al. Mouse model resources for vision research. *J Ophthalmol.* 2011; 2011:391384. [PubMed: 21052544]
42. Batten ML, Imanishi Y, Maeda T, Tu DC, Moise AR, Bronson D, et al. Lecithin-retinol acyltransferase is essential for accumulation of all-trans-retinyl esters in the eye and in the liver. *J Biol Chem.* 2004; 279(11):10422–10432. [PubMed: 14684738]
43. Ding XQ, Harry CS, Umino Y, Matveev AV, Fliesler SJ, Barlow RB. Impaired cone function and cone degeneration resulting from CNGB3 deficiency: down-regulation of CNGA3 biosynthesis as a potential mechanism. *Hum Mol Genet.* 2009; 18(24):4770–4780. [PubMed: 19767295]
44. Lem J, Krasnoperova NV, Calvert PD, Kosaras B, Cameron DA, Nicolo M, et al. Morphological, physiological, and biochemical changes in rhodopsin knockout mice. *Proc Natl Acad Sci U S A.* 1999; 96(2):736–741. [PubMed: 9892703]
45. Redmond TM, Yu S, Lee E, Bok D, Hamasaki D, Chen N, et al. Rpe65 is necessary for production of 11-cis-vitamin A in the retinal visual cycle. *Nat Genet.* 1998; 20(4):344–351. [PubMed: 9843205]
46. Zeng Y, Takada Y, Kjellstrom S, Hiriyanna K, Tanikawa A, Wawrousek E, et al. RS-1 Gene Delivery to an Adult Rslh Knockout Mouse Model Restores ERG b-Wave with Reversal of the Electronegative Waveform of X-Linked Retinoschisis. *Invest Ophthalmol Vis Sci.* 2004; 45(9):3279–3285. [PubMed: 15326152]
47. Yang RB, Robinson SW, Xiong WH, Yau KW, Birch DG, Garbers DL. Disruption of a retinal guanylyl cyclase gene leads to cone-specific dystrophy and paradoxical rod behavior. *J Neurosci.* 1999; 19(14):5889–5897. [PubMed: 10407028]
48. Pang JJ, Chang B, Kumar A, Nusinowitz S, Noorwez SM, Li J, et al. 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. [PubMed: 16223604]
49. Stone EM. Leber congenital amaurosis - a model for efficient genetic testing of heterogeneous disorders: LXIV Edward Jackson Memorial Lecture. *Am J Ophthalmol.* 2007; 144(6):791–811. [PubMed: 17964524]
50. Cideciyan AV, Hauswirth WW, Aleman TS, Kaushal S, Schwartz SB, Boye SL, et al. Vision 1 year after gene therapy for Leber's congenital amaurosis. *N Engl J Med.* 2009; 361(7):725–727. [PubMed: 19675341]
51. den Hollander AI, Koenekoop RK, Yzer S, Lopez I, Arends ML, Voeseke KE, et al. Mutations in the CEP290 (NPHP6) gene are a frequent cause of Leber congenital amaurosis. *Am J Hum Genet.* 2006; 79(3):556–561. [PubMed: 16909394]
52. den Hollander AI, Roepman R, Koenekoop RK, Cremers FP. Leber congenital amaurosis: genes, proteins and disease mechanisms. *Prog Retin Eye Res.* 2008; 27(4):391–419. [PubMed: 18632300]
53. Wang H, den Hollander AI, Moayed Y, Abulimiti A, Li Y, Collin RW, et al. Mutations in SPATA7 cause Leber congenital amaurosis and juvenile retinitis pigmentosa. *Am J Hum Genet.* 2009; 84(3):380–387. [PubMed: 19268277]
54. Freund CL, Wang QL, Chen S, Muskat BL, Wiles CD, Sheffield VC, et al. De novo mutations in the CRX homeobox gene associated with Leber congenital amaurosis. *Nat Genet.* 1998; 18(4):311–312. [PubMed: 9537410]
55. Swaroop A, Wang QL, Wu W, Cook J, Coats C, Xu S, et al. Leber congenital amaurosis caused by a homozygous mutation (R90W) in the homeodomain of the retinal transcription factor CRX: direct evidence for the involvement of CRX in the development of photoreceptor function. *Hum Mol Genet.* 1999; 8(2):299–305. [PubMed: 9931337]
56. Jacobson SG, Cideciyan AV, Huang Y, Hanna DB, Freund CL, Affatigato LM, et al. Retinal degenerations with truncation mutations in the cone-rod homeobox (CRX) gene. *Invest Ophthalmol Vis Sci.* 1998; 39(12):2417–2426. [PubMed: 9804150]
57. Sohocki MM, Perrault I, Leroy BP, Payne AM, Dharmaraj S, Bhattacharya SS, et al. Prevalence of AIPL1 mutations in inherited retinal degenerative disease. *Mol Genet Metab.* 2000; 70(2):142–150. [PubMed: 10873396]
58. Marlhens F, Bareil C, Griffioen JM, Zrenner E, Amalric P, Eliaou C, et al. Mutations in RPE65 cause Leber's congenital amaurosis. *Nat Genet.* 1997; 17(2):139–141. [PubMed: 9326927]

59. Perrault I, Rozet JM, Ghazi I, Leowski C, Bonnemaïson M, Gerber S, et al. Different functional outcome of RetGC1 and RPE65 gene mutations in Leber congenital amaurosis. *Am J Hum Genet.* 1999; 64(4):1225–1228. [PubMed: 10090910]
60. Jancke AR, Thompson DA, Utermann G, Becker C, Hubner CA, Schmid E, et al. Mutations in RDH12 encoding a photoreceptor cell retinol dehydrogenase cause childhood-onset severe retinal dystrophy. *Nat Genet.* 2004; 36(8):850–854. [PubMed: 15258582]
61. Perrault I, Hanein S, Gerber S, Barbet F, Ducroq D, Dollfus H, et al. Retinal dehydrogenase 12 (RDH12) mutations in leber congenital amaurosis. *Am J Hum Genet.* 2004; 75(4):639–646. [PubMed: 15322982]
62. Hagstrom SA, North MA, Nishina PL, Berson EL, Dryja TP. Recessive mutations in the gene encoding the tubby-like protein TULP1 in patients with retinitis pigmentosa. *Nat Genet.* 1998; 18(2):174–176. [PubMed: 9462750]
63. Gerber S, Perrault I, Hanein S, Barbet F, Ducroq D, Ghazi I, et al. Complete exon-intron structure of the RPGR-interacting protein (RPGRIP1) gene allows the identification of mutations underlying Leber congenital amaurosis. *Eur J Hum Genet.* 2001; 9(8):561–571. [PubMed: 11528500]
64. Dryja TP, Adams SM, Grimsby JL, McGee TL, Hong DH, Li T, et al. Null RPGRIP1 alleles in patients with Leber congenital amaurosis. *Am J Hum Genet.* 2001; 68(5):1295–1298. [PubMed: 11283794]
65. den Hollander AI, Koenekoop RK, Mohamed MD, Arts HH, Boldt K, Towns KV, et al. Mutations in LCA5, encoding the ciliary protein lebercilin, cause Leber congenital amaurosis. *Nat Genet.* 2007; 39(7):889–895. [PubMed: 17546029]
66. Gal A, Li Y, Thompson DA, Weir J, Orth U, Jacobson SG, et al. Mutations in MERTK, the human orthologue of the RCS rat retinal dystrophy gene, cause retinitis pigmentosa. *Nat Genet.* 2000; 26(3):270–271. [PubMed: 11062461]
67. Friedman JS, Chang B, Kannabiran C, Chakarova C, Singh HP, Jalali S, et al. Premature truncation of a novel protein, RD3, exhibiting subnuclear localization is associated with retinal degeneration. *Am J Hum Genet.* 2006; 79(6):1059–1070. [PubMed: 17186464]
68. Boye SE, Boye SL, Pang J, Ryals R, Everhart D, Umino Y, et al. Functional and behavioral restoration of vision by gene therapy in the guanylate cyclase-1 (GC1) knockout mouse. *PLoS One.* 2010; 5(6):e11306. [PubMed: 20593011]
69. Nusinowitz S, Ridder WH III, Pang JJ, Chang B, Noorwez SM, Kaushal S, et al. Cortical visual function in the rd12 mouse model of Leber Congenital Amaurosis (LCA) after gene replacement therapy to restore retinal function. *Vision Res.* 2006; 46(22):3926–3934. [PubMed: 16814838]
70. Sun X, Pawlyk B, Xu X, Liu X, Bulgakov OV, Adamian M, et al. Gene therapy with a promoter targeting both rods and cones rescues retinal degeneration caused by AIPL1 mutations. *Gene Ther.* 2010; 17(1):117–131. [PubMed: 19710705]
71. Tan MH, Smith AJ, Pawlyk B, Xu X, Liu X, Bainbridge JB, et al. Gene therapy for retinitis pigmentosa and Leber congenital amaurosis caused by defects in AIPL1: effective rescue of mouse models of partial and complete Aipl1 deficiency using AAV2/2 and AAV2/8 vectors. *Hum Mol Genet.* 2009; 18(12):2099–2114. [PubMed: 19299492]
72. Batten ML, Imanishi Y, Tu DC, Doan T, Zhu L, Pang J, et al. Pharmacological and rAAV gene therapy rescue of visual functions in a blind mouse model of Leber congenital amaurosis. *PLoS Med.* 2005; 2(11):e333. [PubMed: 16250670]
73. Pawlyk BS, Smith AJ, Buch PK, Adamian M, Hong DH, Sandberg MA, et al. 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. [PubMed: 16123399]
74. Pawlyk BS, Bulgakov OV, Liu X, Xu X, Adamian M, Sun X, et al. Replacement gene therapy with a human RPGRIP1 sequence slows photoreceptor degeneration in a murine model of Leber congenital amaurosis. *Hum Gene Ther.* 2010; 21(8):993–1004. [PubMed: 20384479]
75. Perrault I, Rozet JM, Gerber S, Ghazi I, Ducroq D, Souied E, et al. Spectrum of retGC1 mutations in Leber's congenital amaurosis. *Eur J Hum Genet.* 2000; 8(8):578–582. [PubMed: 10951519]

76. Coleman JE, Zhang Y, Brown GA, Semple-Rowland SL. Cone cell survival and downregulation of GCAP1 protein in the retinas of GC1 knockout mice. *Invest Ophthalmol Vis Sci.* 2004; 45(10): 3397–3403. [PubMed: 15452041]
77. Haire SE, Pang J, Boye SL, Sokal I, Craft CM, Palczewski K, et al. 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. [PubMed: 16936082]
78. Katz ML, Redmond TM. Effect of Rpe65 knockout on accumulation of lipofuscin fluorophores in the retinal pigment epithelium. *Invest Ophthalmol Vis Sci.* 2001; 42(12):3023–3030. [PubMed: 11687551]
79. Samardzija M, von LJ, Tanimoto N, Oberhauser V, Thiersch M, Reme CE, et al. R91W mutation in Rpe65 leads to milder early-onset retinal dystrophy due to the generation of low levels of 11-cis-retinal. *Hum Mol Genet.* 2008; 17(2):281–292. [PubMed: 17933883]
80. Seeliger MW, Grimm C, Stahlberg F, Friedburg C, Jaissle G, Zrenner E, et al. New views on RPE65 deficiency: the rod system is the source of vision in a mouse model of Leber congenital amaurosis. *Nat Genet.* 2001; 29(1):70–74. [PubMed: 11528395]
81. Rohrer B, Lohr HR, Humphries P, Redmond TM, Seeliger MW, Crouch RK. Cone opsin mislocalization in Rpe65^{-/-} mice: a defect that can be corrected by 11-cis retinal. *Invest Ophthalmol Vis Sci.* 2005; 46(10):3876–3882. [PubMed: 16186377]
82. Dejneka NS, Surace EM, Aleman TS, Cideciyan AV, Lyubarsky A, Savchenko A, et al. In utero gene therapy rescues vision in a murine model of congenital blindness. *Mol Ther.* 2004; 9(2):182–188. [PubMed: 14759802]
83. Lai CM, Yu MJ, Brankov M, Barnett NL, Zhou X, Redmond TM, et al. Recombinant adeno-associated virus type 2-mediated gene delivery into the Rpe65^{-/-} knockout mouse eye results in limited rescue. *Genet Vaccines Ther.* 2004; 2(1):3. [PubMed: 15109394]
84. Roman AJ, Boye SL, Aleman TS, Pang JJ, McDowell JH, Boye SE, et al. Electroretinographic analyses of Rpe65-mutant rd12 mice: developing an in vivo bioassay for human gene therapy trials of Leber congenital amaurosis. *Mol Vis.* 2007; 13:1701–1710. [PubMed: 17960108]
85. Amado D, Mingozzi F, Hui D, Bennicelli JL, Wei Z, Chen Y, et al. Safety and efficacy of subretinal readministration of a viral vector in large animals to treat congenital blindness. *Sci Transl Med.* 2010; 2(21):21ra16.
86. Kostic C, Crippa SV, Pignat V, Bemelmans AP, Samardzija M, Grimm C, et al. Gene therapy regenerates protein expression in cone photoreceptors in rpe65 mice. *PLoS One.* 2011; 6(2):e16588. [PubMed: 21304899]
87. Dyer MA, Donovan SL, Zhang J, Gray J, Ortiz A, Tenney R, et al. Retinal degeneration in Aipl1-deficient mice: a new genetic model of Leber congenital amaurosis. *Brain Res Mol Brain Res.* 2004; 132(2):208–220. [PubMed: 15582159]
88. Ramamurthy V, Niemi GA, Reh TA, Hurley JB. Leber congenital amaurosis linked to AIPL1: a mouse model reveals destabilization of cGMP phosphodiesterase. *Proc Natl Acad Sci U S A.* 2004; 101(38):13897–13902. [PubMed: 15365178]
89. Li Y, Wang H, Peng J, Gibbs RA, Lewis RA, Lupski JR, et al. Mutation survey of known LCA genes and loci in the Saudi Arabian population. *Invest Ophthalmol Vis Sci.* 2009; 50(3):1336–1343. [PubMed: 18936139]
90. Zhao Y, Hong DH, Pawlyk B, Yue G, Adamian M, Grynberg M, et al. The retinitis pigmentosa GTPase regulator (RPGR)- interacting protein: subserving RPGR function and participating in disk morphogenesis. *Proc Natl Acad Sci U S A.* 2003; 100(7):3965–3970. [PubMed: 12651948]
91. Thompson DA, Li Y, McHenry CL, Carlson TJ, Ding X, Sieving PA, et al. Mutations in the gene encoding lecithin retinol acyltransferase are associated with early-onset severe retinal dystrophy. *Nat Genet.* 2001; 28(2):123–124. [PubMed: 11381255]
92. Morrow EM, Furukawa T, Raviola E, Cepko CL. Synaptogenesis and outer segment formation are perturbed in the neural retina of Crx mutant mice. *BMC Neurosci.* 2005; 6:5. [PubMed: 15676071]
93. Pignatelli V, Cepko CL, Strettoi E. Inner retinal abnormalities in a mouse model of Leber's congenital amaurosis. *J Comp Neurol.* 2004; 469(3):351–359. [PubMed: 14730587]

94. Fan J, Rohrer B, Frederick JM, Baehr W, Crouch RK. Rpe65^{-/-} and Lrat^{-/-} mice: comparable models of leber congenital amaurosis. *Invest Ophthalmol Vis Sci*. 2008; 49(6):2384–2389. [PubMed: 18296659]
95. Wang Q, Chen Q, Zhao K, Wang L, Wang L, Traboulsi EI. Update on the molecular genetics of retinitis pigmentosa. *Ophthalmic Genet*. 2001; 22(3):133–154. [PubMed: 11559856]
96. Vollrath D, Feng W, Duncan JL, Yasumura D, D'Cruz PM, Chappelaw A, et al. Correction of the retinal dystrophy phenotype of the RCS rat by viral gene transfer of Mertk. *Proc Natl Acad Sci U S A*. 2001; 98(22):12584–12589. [PubMed: 11592982]
97. Dowling JE, Sidman RL. Inherited retinal dystrophy in the rat. *J Cell Biol*. 1962; 14:73–109. [PubMed: 13887627]
98. LaVail MM, Battelle BA. Influence of eye pigmentation and light deprivation on inherited retinal dystrophy in the rat. *Exp Eye Res*. 1975; 21(2):167–192. [PubMed: 1164921]
99. Ball S, Hanzlicek B, Blum M, Pardue M. Evaluation of inner retinal structure in the aged RCS rat. *Adv Exp Med Biol*. 2003; 533:181–188. [PubMed: 15180263]
100. Duncan JL, LaVail MM, Yasumura D, Matthes MT, Yang H, Trautmann N, et al. An RCS-like retinal dystrophy phenotype in mer knockout mice. *Invest Ophthalmol Vis Sci*. 2003; 44(2):826–838. [PubMed: 12556419]
101. Camenisch TD, Koller BH, Earp HS, Matsushima GK. A novel receptor tyrosine kinase, Mer, inhibits TNF- α production and lipopolysaccharide-induced endotoxic shock. *J Immunol*. 1999; 162(6):3498–3503. [PubMed: 10092806]
102. Smith AJ, Schlichtenbrede FC, Tschernutter M, Bainbridge JW, Thrasher AJ, Ali RR. AAV-Mediated gene transfer slows photoreceptor loss in the RCS rat model of retinitis pigmentosa. *Mol Ther*. 2003; 8(2):188–195. [PubMed: 12907141]
103. Tschernutter M, Schlichtenbrede FC, Howe S, Balaggan KS, Munro PM, Bainbridge JW, et al. Long-term preservation of retinal function in the RCS rat model of retinitis pigmentosa following lentivirus-mediated gene therapy. *Gene Ther*. 2005; 12(8):694–701. [PubMed: 15660111]
104. Pang JJ, Boye SL, Kumar A, Dinculescu A, Deng W, Li J, et al. AAV-mediated gene therapy for retinal degeneration in the rd10 mouse containing a recessive PDEbeta mutation. *Invest Ophthalmol Vis Sci*. 2008; 49(10):4278–4283. [PubMed: 18586879]
105. Williams DS. Usher syndrome: animal models, retinal function of Usher proteins, and prospects for gene therapy. *Vision Res*. 2008; 48(3):433–441. [PubMed: 17936325]
106. Kremer H, van WE, Marker T, Wolfrum U, Roepman R. Usher syndrome: molecular links of pathogenesis, proteins and pathways. *Hum Mol Genet*. 2006; 15(Spec No 2):R262–R270. [PubMed: 16987892]
107. Reiners J, Nagel-Wolfrum K, Jurgens K, Marker T, Wolfrum U. Molecular basis of human Usher syndrome: deciphering the meshes of the Usher protein network provides insights into the pathomechanisms of the Usher disease. *Exp Eye Res*. 2006; 83(1):97–119. [PubMed: 16545802]
108. Reiners J, Wolfrum U. Molecular analysis of the supramolecular usher protein complex in the retina. Harmonin as the key protein of the Usher syndrome. *Adv Exp Med Biol*. 2006; 572:349–353. [PubMed: 17249595]
109. Hashimoto T, Gibbs D, Lillo C, Azarian SM, Legacki E, Zhang XM, et al. Lentiviral gene replacement therapy of retinas in a mouse model for Usher syndrome type 1B. *Gene Ther*. 2007; 14(7):584–594. [PubMed: 17268537]
110. Bennett J, Tanabe T, Sun D, Zeng Y, Kjeldbye H, Gouras P, et al. Photoreceptor cell rescue in retinal degeneration (rd) mice by in vivo gene therapy. *Nat Med*. 1996; 2(6):649–654. [PubMed: 8640555]
111. 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(7):683–690. [PubMed: 9282169]
112. 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):7812–7816. [PubMed: 10438872]

113. Kumar-Singh R, Farber DB. Encapsidated adenovirus mini-chromosome-mediated delivery of genes to the retina: application to the rescue of photoreceptor degeneration. *Hum Mol Genet.* 1998; 7(12):1893–1900. [PubMed: 9811932]
114. Gargini C, Terzibasi E, Mazzoni F, Strettoi E. Retinal organization in the retinal degeneration 10 (rd10) mutant mouse: a morphological and ERG study. *J Comp Neurol.* 2007; 500(2):222–238. [PubMed: 17111372]
115. Pang J, Cheng M, Haire SE, Barker E, Planelles V, Blanks JC. Efficiency of lentiviral transduction during development in normal and rd mice. *Mol Vis.* 2006; 12:756–767. [PubMed: 16862069]
116. Allocca M, Manfredi A, Iodice C, Di VU, Auricchio A. AAV-mediated gene replacement either alone or in combination with physical and pharmacological agents results in partial and transient protection from photoreceptor degeneration associated with {beta}PDE deficiency. *Invest Ophthalmol Vis Sci.* 2011; 52(8):5713–5719. [PubMed: 21273543]
117. Sineshchekov OA, Jung KH, Spudich JL. Two rhodopsins mediate phototaxis to low- and high-intensity light in *Chlamydomonas reinhardtii*. *Proc Natl Acad Sci U S A.* 2002; 99(13):8689–8694. [PubMed: 12060707]
118. Nagel G, Szellas T, Huhn W, Kateriya S, Adeishvili N, Berthold P, et al. Channelrhodopsin-2, a directly light-gated cation-selective membrane channel. *Proc Natl Acad Sci U S A.* 2003; 100(24):13940–13945. [PubMed: 14615590]
119. Tomita H, Sugano E, Yawo H, Ishizuka T, Isago H, Narikawa S, et al. Restoration of visual response in aged dystrophic RCS rats using AAV-mediated channelrhodopsin-2 gene transfer. *Invest Ophthalmol Vis Sci.* 2007; 48(8):3821–3826. [PubMed: 17652757]
120. Bi A, Cui J, Ma YP, Olshevskaya E, Pu M, Dizhoor AM, et al. Ectopic expression of a microbial-type rhodopsin restores visual responses in mice with photoreceptor degeneration. *Neuron.* 2006; 50(1):23–33. [PubMed: 16600853]
121. Doroudchi MM, Greenberg KP, Liu J, Silka KA, Boyden ES, Lockridge JA, et al. Virally delivered Channelrhodopsin-2 Safely and Effectively Restores Visual Function in Multiple Mouse Models of Blindness. *Mol Ther.* 2011; 19(7):1220–1229. [PubMed: 21505421]
122. Lagali PS, Balya D, Awatramani GB, Munch TA, Kim DS, Busskamp V, et al. Light-activated channels targeted to ON bipolar cells restore visual function in retinal degeneration. *Nat Neurosci.* 2008; 11(6):667–675. [PubMed: 18432197]
123. Tomita H, Sugano E, Isago H, Hiroi T, Wang Z, Ohta E, et al. Channelrhodopsin-2 gene transduced into retinal ganglion cells restores functional vision in genetically blind rats. *Exp Eye Res.* 2010; 90(3):429–436. [PubMed: 20036655]
124. Allikmets R, Singh N, Sun H, Shroyer NF, Hutchinson A, Chidambaram A, et al. A photoreceptor cell-specific ATP-binding transporter gene (ABCR) is mutated in recessive Stargardt macular dystrophy. *Nat Genet.* 1997; 15(3):236–246. [PubMed: 9054934]
125. Azarian SM, Travis GH. The photoreceptor rim protein is an ABC transporter encoded by the gene for recessive Stargardt's disease (ABCR). *FEBS Lett.* 1997; 409(2):247–252. [PubMed: 9202155]
126. Illing M, Molday LL, Molday RS. The 220-kDa rim protein of retinal rod outer segments is a member of the ABC transporter superfamily. *J Biol Chem.* 1997; 272(15):10303–10310. [PubMed: 9092582]
127. Martinez-Mir A, Paloma E, Allikmets R, Ayuso C, del RT, Dean M, et al. Retinitis pigmentosa caused by a homozygous mutation in the Stargardt disease gene ABCR. *Nat Genet.* 1998; 18(1): 11–12. [PubMed: 9425888]
128. Cremers FP, van de Pol DJ, van DM, den Hollander AI, van Haren FJ, Knoers NV, et al. Autosomal recessive retinitis pigmentosa and cone-rod dystrophy caused by splice site mutations in the Stargardt's disease gene ABCR. *Hum Mol Genet.* 1998; 7(3):355–362. [PubMed: 9466990]
129. Rozet JM, Gerber S, Souied E, Perrault I, Chatelin S, Ghazi I, et al. Spectrum of ABCR gene mutations in autosomal recessive macular dystrophies. *Eur J Hum Genet.* 1998; 6(3):291–295. [PubMed: 9781034]

130. Weng J, Mata NL, Azarian SM, Tzekov RT, Birch DG, Travis GH. Insights into the function of Rim protein in photoreceptors and etiology of Stargardt's disease from the phenotype in abcr knockout mice. *Cell*. 1999; 98(1):13–23. [PubMed: 10412977]
131. Allocca M, Doria M, Petrillo M, Colella P, Garcia-Hoyos M, Gibbs D, et al. Serotype-dependent packaging of large genes in adeno-associated viral vectors results in effective gene delivery in mice. *J Clin Invest*. 2008; 118(5):1955–1964. [PubMed: 18414684]
132. Kohl S, Baumann B, Rosenberg T, Kellner U, Lorenz B, Vadala M, et al. Mutations in the cone photoreceptor G-protein alpha-subunit gene GNAT2 in patients with achromatopsia. *Am J Hum Genet*. 2002; 71(2):422–425. [PubMed: 12077706]
133. Kohl S, Marx T, Giddings I, Jagle H, Jacobson SG, Apfelstedt-Sylla E, et al. Total colourblindness is caused by mutations in the gene encoding the alpha-subunit of the cone photoreceptor cGMP-gated cation channel. *Nat Genet*. 1998; 19(3):257–259. [PubMed: 9662398]
134. Kohl S, Baumann B, Broghammer M, Jagle H, Sieving P, Kellner U, et al. Mutations in the CNGB3 gene encoding the beta-subunit of the cone photoreceptor cGMP-gated channel are responsible for achromatopsia (ACHM3) linked to chromosome 8q21. *Hum Mol Genet*. 2000; 9(14):2107–2116. [PubMed: 10958649]
135. Sundin OH, Yang JM, Li Y, Zhu D, Hurd JN, Mitchell TN, et al. Genetic basis of total colourblindness among the Pingelapese islanders. *Nat Genet*. 2000; 25(3):289–293. [PubMed: 10888875]
136. Aligianis IA, Forshew T, Johnson S, Michaelides M, Johnson CA, Trembath RC, et al. Mapping of a novel locus for achromatopsia (ACHM4) to 1p and identification of a germline mutation in the alpha subunit of cone transducin (GNAT2). *J Med Genet*. 2002; 39(9):656–660. [PubMed: 12205108]
137. Kohl S, Varsanyi B, Antunes GA, Baumann B, Hoyng CB, Jagle H, et al. CNGB3 mutations account for 50% of all cases with autosomal recessive achromatopsia. *Eur J Hum Genet*. 2005; 13(3):302–308. [PubMed: 15657609]
138. Thiadens AA, den Hollander AI, Roosing S, Nabuurs SB, Zekveld-Vroon RC, Collin RW, et al. Homozygosity mapping reveals PDE6C mutations in patients with early-onset cone photoreceptor disorders. *Am J Hum Genet*. 2009; 85(2):240–247. [PubMed: 19615668]
139. Wissinger B, Gamer D, Jagle H, Giorda R, Marx T, Mayer S, et al. CNGA3 mutations in hereditary cone photoreceptor disorders. *Am J Hum Genet*. 2001; 69(4):722–737. [PubMed: 11536077]
140. Biel M, Seeliger M, Pfeifer A, Kohler K, Gerstner A, Ludwig A, et al. Selective loss of cone function in mice lacking the cyclic nucleotide-gated channel CNG3. *Proc Natl Acad Sci U S A*. 1999; 96(13):7553–7557. [PubMed: 10377453]
141. Alexander JJ, Umino Y, Everhart D, Chang B, Min SH, Li Q, et al. Restoration of cone vision in a mouse model of achromatopsia. *Nat Med*. 2007; 13(6):685–687. [PubMed: 17515894]
142. Michalakakis S, Muhlfriedel R, Tanimoto N, Krishnamoorthy V, Koch S, Fischer MD, et al. Restoration of Cone Vision in the CNGA3(–/–) Mouse Model of Congenital Complete Lack of Cone Photoreceptor Function. *Mol Ther*. 2010; 12:2057–2063. [PubMed: 20628362]
143. Xu J, Morris L, Fliesler SJ, Sherry DM, Ding XQ. Early-onset, slow progression of cone photoreceptor dysfunction and degeneration in CNG channel subunit CNGB3 deficiency. *Invest Ophthalmol Vis Sci*. 2011; 52(6):3557–3566. [PubMed: 21273547]
144. Carvalho LS, Xu J, Pearson RA, Smith AJ, Bainbridge JW, Morris LM, et al. Long-term and age-dependent restoration of visual function in a mouse model of CNGB3-associated achromatopsia following gene therapy. *Hum Mol Genet*. 2011; 20(16):3161–3175. [PubMed: 21576125]
145. Vijayasarathy C, Takada Y, Zeng Y, Bush RA, Sieving PA. Organization and molecular interactions of retinoschisin in photoreceptors. *Adv Exp Med Biol*. 2008; 613:291–297. [PubMed: 18188957]
146. Mooy CM, Van Den Born LI, Baarsma S, Paridaens DA, Kraaijenbrink T, Bergen A, et al. Hereditary X-linked juvenile retinoschisis: a review of the role of Muller cells. *Arch Ophthalmol*. 2002; 120(7):979–984. [PubMed: 12096974]
147. Sieving PA, MacDonald IM, Meltzer MR, Smaoui N. X-Linked Juvenile Retinoschisis. 1993

148. Sikkink SK, Biswas S, Parry NR, Stanga PE, Trump D. X-linked retinoschisis: an update. *J Med Genet.* 2007; 44(4):225–232. [PubMed: 17172462]
149. Min SH, Molday LL, Seeliger MW, Dinculescu A, Timmers AM, Janssen A, et al. 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. [PubMed: 16027044]
150. Zeng Y, Takada Y, Kjellstrom S, Hiriyanna K, Tanikawa A, Wawrousek E, et al. RS-1 Gene Delivery to an Adult *Rs1h* Knockout Mouse Model Restores ERG b-Wave with Reversal of the Electronegative Waveform of X-Linked Retinoschisis. *Invest Ophthalmol Vis Sci.* 2004; 45(9): 3279–3285. [PubMed: 15326152]
151. Park TK, Wu Z, Kjellstrom S, Zeng Y, Bush RA, Sieving PA, et al. Intravitreal delivery of AAV8 retinoschisin results in cell type-specific gene expression and retinal rescue in the *Rs1*-KO mouse. *Gene Ther.* 2009; 16(7):916–926. [PubMed: 19458650]