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Review

REVIEW

Clinical applications of retinal gene therapies

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Abstract

Retinal degenerative diseases are a major cause of blindness. Retinal gene therapy is a trail-blazer in the human gene therapy field, leading to the first FDA approved gene therapy product for a human genetic disease. The application of Clustered Regularly Interspaced Short Palindromic Repeat/Cas9 (CRISPR/Cas9)-mediated gene editing technology is transforming the delivery of gene therapy. We review the history, present, and future prospects of retinal gene therapy.

Introduction

The eye is an intricate sensory organ, loosely designed like a camera, in which the retina captures high resolution images functioning similar to the film (Fig. 1). The retina is composed of multiple layers of neurons which convert visible light images into electrical signals and transmit them to the brain (Fig. 2). The retinal photoreceptors are the primary light detecting cells (Fig. 2), whereas pigment epithelial cells play a crucial role in supporting photoreceptor cell functions including phagocytosis and regeneration of visual pigments via the visual cycle.

Loss of vision caused by inherited or acquired retinal diseases can significantly impact on quality of life. In the past several decades, scientists and physicians have begun to unravel the underlying molecular and genetic factors contributing to the onset and progression of these diseases. This review discusses recent early-stage clinical trials and a variety of preclinical studies in which the gene editing techniques resulted in significant functional improvement in the retina. It focuses mostly on the gene therapies that have showed the most progress and the greatest clinical potential. We list all retinal gene therapy trials that have been registered in ClinicalTrial.org in Table 1.

RPE65-Leber Congenital Amaurosis

RPE65 is a retinal pigment epithelial-specific protein (65 kDa), which is almost exclusively found in the retinal pigment epithelium (RPE) as a retinoid isomerase and is

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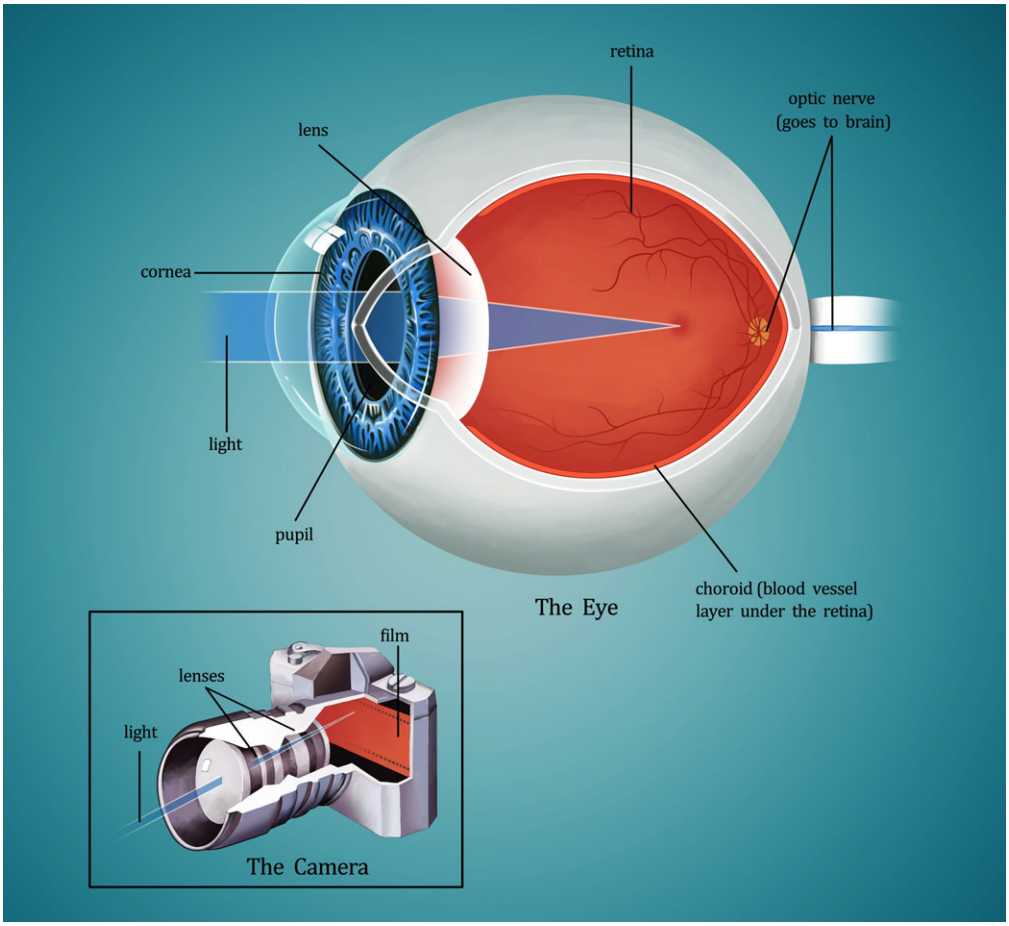

Figure 1. Structure of the eye comparing to a camera. Illustration adapted from American Academy of Ophthalmology.

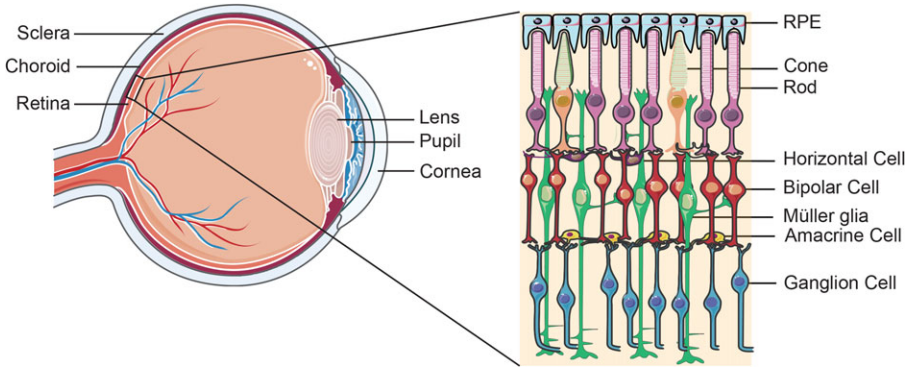

Figure 2. Schematic of the eye and retina structure. The magnified area represents different cell types in the retina. Most of retinal gene therapy trials are directed to defective genes affecting photoreceptors or RPE.

responsible for converting all-*trans* retinoid to 11-*cis* retinal during pigment regeneration.[1–3] Absence or deactivation of RPE65 results in an accumulation of all-*trans*-retinyl esters, which promotes pigment degeneration, disabling the formation of visual pigments, rhodopsin and cone opsin because of a lack of 11-*cis* retinal.[4–6] Therefore, mutations in *Rpe65* associate strongly with RPE65-Leber Congenital Amaurosis (LCA2), retinitis pigmentosa (RP), and early-onset severe retinal dystrophies.[7–10]

The absence of this isomerase activity along with pigment degeneration and significant visual impairment were observed in *Rpe65* knockout mice, mutant knock-in mice, and naturally occurring *Rpe65* mutant mouse.[11–13] Gene therapies using adeno-associated virus (AAV), adenovirus, and lentivirus-mediated *Rpe65* delivery have all resulted in improvement in electroretinogram (ERG) response and visual function test in RPE65 deficient mice models (Fig. 3).[14,15] Subretinal

Table 1. Information on recent gene therapy clinical trials adapted from ClinicalTrials.gov.

Condition	Identifier	Status	Study Title	Interventions	Sponsor
RPE65 - Leber Congenital Amaurosis	NCT00643747	Completed	Safety Study of RPE65 Gene Therapy to Treat Leber Congenital Amaurosis	rAAV 2/2.hRPE65p. hRPE65 (tgAAG76)	University College, London
	NCT00749957	Completed	Phase 1/2 Safety and Efficacy Study of AAV-RPE65 Vector to Treat Leber Congenital Amaurosis	rAAV2-CB-hRPE65	Applied Genetic Technologies Corp
	NCT00821340	Completed	Clinical Trial of Gene Therapy for Leber Congenital Amaurosis Caused by RPE65 Mutations	rAAV2-hRPE65	Hadassah Medical Organization
	NCT01496040	Completed	Clinical Gene Therapy Protocol for the Treatment of Retinal Dystrophy Caused by Defects in RPE65	rAAV2-hRPE65	Nantes University Hospital
	NCT00481546	Active	Phase I Trial of Gene Vector to Patients With Retinal Disease Due to RPE65 Mutations	rAAV2-CBSB-hRPE65	University of Pennsylvania
	NCT00516477	Active	Safety Study in Subjects with Leber Congenital Amaurosis	AAV2-hRPE65v2	Spark Therapeutics
	NCT00999609	Active	Safety and Efficacy Study in Subjects With Leber Congenital Amaurosis	AAV2-hRPE65v2	Spark Therapeutics
	NCT01208389	Active	Phase 1 Follow-on Study of AAV2-hRPE65v2 Vector in Subjects With Leber Congenital Amaurosis (LCA) 2	AAV2-hRPE65v2	Spark Therapeutics
	NCT02946879	Recruiting	Long-Term Follow-Up Gene Therapy Study for Leber Congenital Amaurosis OPTIRPE65 (Retinal Dystrophy Associated with Defects in RPE65)	AAV OPTIRPE65	MeiraGTx UK II Ltd
	NCT02781480	Recruiting	Clinical Trial of Gene Therapy for the Treatment of Leber Congenital Amaurosis (LCA)	AAV RPE65	MeiraGTx UK II Ltd
MERTK-associated retinitis pigmentosa	NCT01482195	Recruiting	Trial of Ocular Subretinal Injection of a Recombinant Adeno-Associated Virus (rAAV2-VMD2-hMERTK) Gene Vector to Patients With Retinal Disease Due to MERTK Mutations	rAAV2-VMD2-hMERTK	Fowzan Alkuraya
Usher syndrome	NCT01505062	Recruiting	Study of UshStat in Patients With Retinitis Pigmentosa Associated With Usher Syndrome Type 1B	EIAV-CMV-MYO7A (UshStat)	Sanofi
	NCT02065011	Enrolling by invitation	A Study to Determine the Long-Term Safety, Tolerability and Biological Activity of UshStat® in Patients With Usher Syndrome Type 1B	EIAV-CMV-MYO7A (UshStat)	Sanofi
Stargardt disease	NCT01367444	Recruiting	Phase I/II Study of SAR422459 in Patients With Stargardt's Macular Degeneration	EIAV-ABCA4 (SAR422459)	Sanofi
	NCT01736592	Recruiting	Phase I/II Follow-up Study of SAR422459 in Patients With Stargardt's Macular Degeneration	EIAV-ABCA4 (SAR422459)	Sanofi
Choroideremia	NCT01461213	Completed	Gene Therapy for Blindness Caused by Choroideremia	rAAV2.REP1	University of Oxford
	NCT02553135	Completed	Choroideremia Gene Therapy Clinical Trial	AAV2-REP1	Byron Lam
	NCT02077361	Completed	An Open Label Clinical Trial of Retinal Gene Therapy for Choroideremia	rAAV2.REP1 vector	Ian M. MacDonald
	NCT02671539	Active	THOR - Tübingen Choroideremia Gene Therapy Trial	rAAV2.REP1	STZ eyetrial
	NCT02341807	Active	Safety and Dose Escalation Study of AAV2-hCHM in Subjects with CHM (Choroideremia) Gene Mutations	AAV2-hCHM	Spark Therapeutics
	NCT02407678	Recruiting	REP1 Gene Replacement Therapy for Choroideremia	AAV-REP1	University of Oxford
	NCT03496012	Recruiting	Efficacy and Safety of AAV2-RPE1 for the Treatment of Choroideremia	AAV2-RPE1	Nightstar Therapeutics
	NCT03507686	Recruiting	A Safety Study of Retinal Gene Therapy for Choroideremia (GEMINI)	AAV2-REP1	Nightstar Therapeutic

Continued

Table 1. Continued

Condition	Identifier	Status	Study Title	Interventions	Sponsor
Wet age-related macular degeneration	NCT01494805	Completed	Safety and Efficacy Study of rAAV.sFlt-1 in Patients With Exudative Age-Related Macular Degeneration	rAAV.sFlt-1	Lions Eye Institute
	NCT00109499	Completed	Study of AdGVPEDF.11D in Neovascular Age-related Macular Degeneration (AMD)	AdGVPEDF.11D	GenVec
	NCT01301443	Completed	Phase I Dose Escalation Safety Study of RetinoStat in Advanced Age-Related Macular Degeneration (AMD) (GEM)	RetinoStat (EIAV-CMV-hEndo-hAngio)	Oxford BioMedica
	NCT01024998	Active	Safety and Tolerability Study of AAV2-sFLT01 in Patients with Neovascular Age-Related Macular Degeneration (AMD)	AAV2-sFLT01	Genzyme, a Sanofi Company
	NCT03066258	Recruiting	RGX-314 Gene Therapy for Neovascular AMD Trial	AAV-VEGF (RGX-314)	Regenxbio Inc.
	NCT01678872	Enrolling by invitation	A Follow-up Study to Evaluate the Safety of RetinoStat® in Patients With Age-Related Macular Degeneration	RetinoStat (EIAV-CMV-hEndo-hAngio)	Oxford BioMedica
Achromatopsia	NCT03278873	Recruiting	Long-Term Follow-Up Gene Therapy Study for Achromatopsia CNGB3	AAV - CNGB3	MeiraGTx UK II Ltd
	NCT02599922	Recruiting	Safety and Efficacy Trial of AAV Gene Therapy in Patients With CNGB3 Achromatopsia	rAAV2tYF-PR1.7-hCNGB3	Applied Genetic Technologies Corp
	NCT03001310	Recruiting	Gene Therapy for Achromatopsia (CNGB3)	AAV-CNGB3	MeiraGTx UK II Ltd
	NCT02935517	Recruiting	Safety and Efficacy Trial of AAV Gene Therapy in Patients With CNGA3 Achromatopsia	AGTC-402	Applied Genetic Technologies Corp
	NCT02610582	Active	Safety and Efficacy of a Single Subretinal Injection of rAAV.hCNGA3 in Patients with CNG3-linked Achromatopsia	rAAV.hCNGA3	STZ eyetrial
X-linked retinoschisis	NCT02317887	Recruiting	Study of RS1 Ocular Gene Transfer for X-linked Retinoschisis	AAV RS1	National Eye Institute (NEI)
	NCT02416622	Recruiting	Safety and Efficacy of rAAV-hRS1 in Patients With X-linked Retinoschisis (XLRS)	rAAV2tYF-CB-hRS1	Applied Genetic Technologies Corp

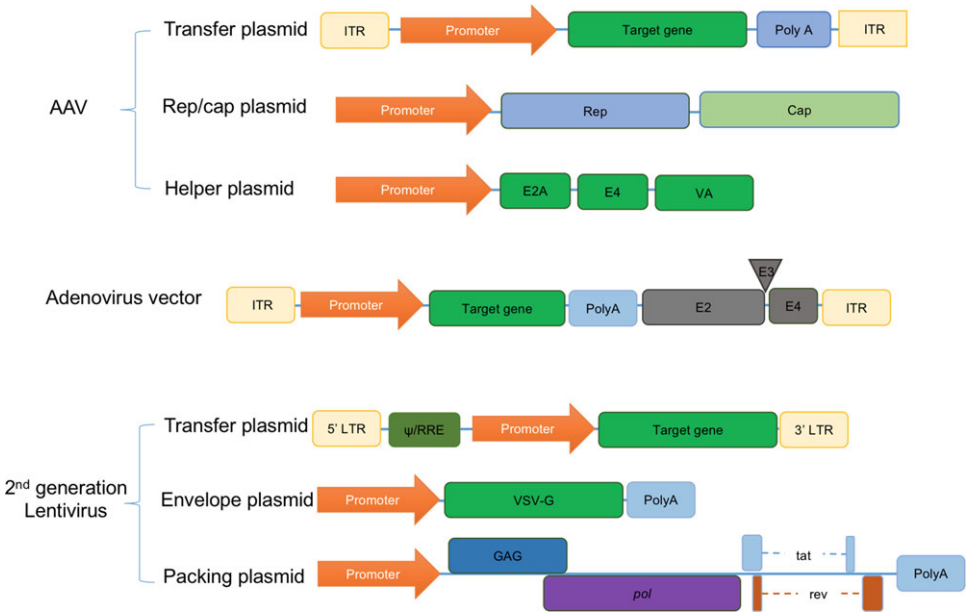

Figure 3. Diagrams of gene delivery vectors including adeno-associated virus (AAV), adenovirus, and second-generation lentivirus. ITR, inverted terminal repeats; Rep, Replication; Cap, Capsid; E2A/E3/E4/VA, adenovirus genes that mediate replication. LTR, long terminal repeats; ψ/RRE, Rev response element; VSV-G, vesicular stomatitis virus G protein; GAG, Group-specific antigen; *pol*, DNA polymerase; tat, Trans-Activator of Transcription.

injection with AAV1-RPE65 to a *Rpe65* knockout (*Rpe65*$^{-/-}$) mouse model of LCA2 as early as *in-utero* resulted in substantial improvements in ERG responses lasting as late as 24 months of age.[16] Another study in which AAV5-RPE65 was subretinally delivered to a naturally occurring *Rpe65* mutant mouse model, *rd12*, also showed improvements in ERG responses and visual guided behaviors.[17] Additional experiments with the *rd12* mice receiving subretinal AAV2-RPE65 delivery attempted to establish an *in vivo* bioassay to evaluate the stability of vectors used in clinical trials of LCA2.[18]

Although cone photoreceptors use a pathway independent of the RPE for chromophore recycling that enables them to function in continuous bright light, studies of patients affected with LCA2 suggest that cone survival is still dependent on RPE65 isomerase activity, regardless of the residual cone activity in the absence of the enzyme.[19–21] This is consistent with observations of rapid cone degeneration in *Rpe65*$^{-/-}$ and *rd12* models.[3,22] Self-complementary AAV-RPE65 vectors have been shown to be capable of restoring cone function and preventing cone degeneration in both *rd12* mice and *Rpe65* and *Rhodopsin* double knockout (*Rpe65*$^{-/-}$ *Rho*$^{-/-}$) mice.[23,24]

The first gene therapy for LCA2 in a large animal model gained widespread attention in 2001 using a recombinant adeno-associated virus (rAAV2) vector containing *Rpe65* cDNA to treat three *Rpe65* mutant dogs (Fig. 3).[25] In this study, a subretinal delivery of the canine *Rpe65* gene carried by the rAAV2 vector, under the control of the hybrid cytomegalovirus/chicken β-actin (CBA) promoter, resulted in substantial visual improvements as assessed by ERG (Fig. 4). Follow-up studies found that subretinal delivery of recombinant AAV1, AAV4, and AAV5-mediated RPE65 expression driven by a human promoter were also capable of restoring visual function, which remained stable over time.[26–33] Additional follow-ups found that cortical responses, assessed by functional magnetic resonance imaging (fMRI), were significantly improved and visually guided behavior was recovered in treated dogs, suggesting that retinal signals were correctly propagated to the visual processing centers of the brain.[28,31,32,34]

Unlike most conventional treatment methods, the efficacy of gene therapies for LCA2 is not heavily affected by the disease stage. The previously mentioned studies, which demonstrated significant therapeutic efficacy, included animals exhibiting mid-to-late stage disease, such as dogs treated at 30 months of age and *rd12* mice treated at 3 months of age.[26,35] Even *Rpe65*$^{-/-}$ mice treated as late as 24 months of age resulted in mild but significant (16%) ERG improvements.[36] These results indicate that an adult patient qualified for Phase I trials would have a reasonable chance of obtaining improved visual function after treatment.

Previous animal studies laid a foundation of proof-of-concept studies that allowed researchers to conduct multiple phase I/II trials which eventually led to an FDA-approved gene therapy after a successful phase III trial in late 2017/early 2018.[37–43] The studies delivered an AAV2 vector carrying a normal human *Rpe65* cDNA through subretinal injection. All trials reported clinically measurable visual improvement, albeit with varied magnitude and significance. In addition, no vector-related adverse events or toxic immune responses were elicited despite differences in postoperative steroid use, doses delivered, promoters used, vector specifics, and anesthesia during delivery.[41] Improvement in vision was maintained 3 years after treatment,[42,44] but progressively declined

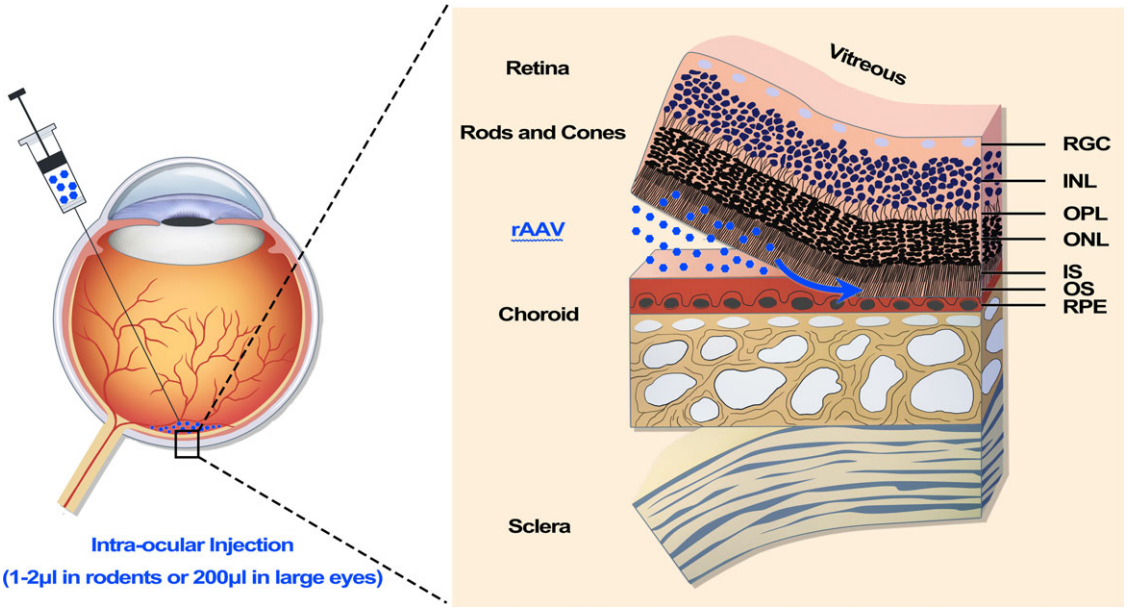

Figure 4. Delivery of a viral vector via intraocular injection. Maximal 1–2 µl of viral mixture in rodents or 200 µl in large eyes can be injected into the subretinal space through a small scleral incision. A successful injection was judged by creation of a small subretinal fluid bleb.

after 6 years because of photoreceptor degeneration in the treated retina as in the untreated retina.[45] Moreover, in cases where vector was delivered subfoveally and caused a foveal detachment, patients with LCA2 typically reported no change in foveal light sensitivity but instead reported improvement extrafoveally.[39,46] In a 3-year study, nearly half of the patients experienced a detached fovea caused by a vector bleb and further foveal thickness loss.[44] A follow-up with optical coherence tomography (OCT) analysis suggested that the loss of thickness resulted from foveal cone loss, which can occur from potentially damaging effects of subretinal vector-mediated foveal detachment,[45] suggesting that subretinal vector delivery in this locale should be approached cautiously. Notably, in this study, a few patients with improved extrafoveal function experienced a shift of their visual fixation away from the fovea into the superior-temporal retina, known as the 'pseudo-fovea', that coincided with the locale of the vector bleb.[47] This phenomenon results in a change in cortical control of the ocular muscles such that images are positioned on this new, more light-sensitive pseudo-fovea.

In summary, human gene therapy for RPE65-LCA2 has been shown to be safe, free of serious complications, and effective at improving impaired vision, yet still needs more investigation and exploration.

MERTK-associated autosomal recessive retinitis pigmentosa

Retinitis pigmentosa (RP) is a retinal rod photoreceptor specific disease characterized by primary rod photoreceptor death and degeneration, followed by secondary cone death.[48] RP is one of the most common inherited blinding retinal diseases, affecting more than one million patients worldwide.[49,50] The mer receptor tyrosine kinase (MERTK) is a member of the Axl/Mer/Tyro3 receptor tyrosine kinase family and is necessary for proper phagocytosis of photoreceptor outer segments by the RPE. The MERTK-associated form of autosomal recessive retinitis pigmentosa (arRP) is caused by an absence of functional MERTK expression, leading to significant degeneration of the retina.[51] This disease is very rare; it is only found in isolated populations identified in the Middle East and the Faroe Islands. Nevertheless, the profound impact it has on a patient's quality of life has attracted the attention of the scientific community.[52–54] Since then, several groups have reported numerous isolated cases of MERTK-associated arRP in other parts of the world.[51,55] Studies simultaneously identifying patients with RP and homozygous mutations in *Mertk* conclusively linked this gene to the disease.[56] Degeneration of the retina is caused by accumulation of subretinal debris of shed photoreceptor outer segments resulting from inability of the RPE to perform phagocytosis, leading to consequent apoptosis of retinal cells and progressive deterioration of visual function as evaluated by ERG.[57–59]

The most successful gene-replacement study for MERTK-associated arRP used a lentivirus expressing MERTK and was successful at preserving retinal structure and function, as observed by microscopy and ERG up to 7 months post-injection (Fig. 3).[60] A later study used a fast-acting AAV8 Y733F capsid mutant vector as early treatment for long-term preservation of retinal function in a mouse model; this treatment method can quickly restore MERTK expression before a significant debris field can incite apoptosis in photoreceptors.[61] The effect of *Mertk* gene therapy was shown to be improved with a co-administration of AAV expressing glial cell derived neurotropic factor (GDNF).[62] Most recently, a new method, homology-independent targeted integration (HITI), was reported to treat a Royal College of Surgeons (RCS) rat, a well-established animal model for RP resulting from a homozygous 1.9-kb deletion from intron 1 to exon 2 in the *Mertk* gene.[63] The HITI used CRISPR/Cas9-mediated gene editing to endogenously insert a wild-type exon 2 at *Mertk* locus. Subretinal injection of HITI-AAVs led to statistically significant increases in *Mertk* mRNA and protein expression levels, preservation of the retina outer nuclear layer (ONL) thickness, and significant improvement in ERG b-wave responses.[63]

A phase I clinical trial of six patients showed no complications that could be attributed to the gene vector and resulted in improved visual acuity in three of the patients (ClinicalTrials.gov Identifier: NCT01482195). However, at 2-year follow-up, two of these patients lost these improvements, although disease progression could have caused this. Based on the established safety profile, the trials are still recruiting to assess the efficacy of this approach, especially in the population with higher starting visual acuity.[64]

Usher syndrome

Usher syndrome (USH) is a heterogeneous collection of autosomal recessive disorders, causing a combination of deafness and blindness in people with an estimated three-to-six-person prevalence per 100 000 individuals.[65–67] It accounts for 15–20% of RP cases, and 50% of combined blindness and deafness cases.[68,69] The three clinical subtypes of USH (USH1, USH2, USH3) are distinguished by severity and the progression of hearing loss, presence or absence of vestibular dysfunction, and vision loss from RP, with USH1 being the most severe in terms of onset, extent of hearing loss, and RP.[70–72] Currently, there are 11 protein-encoding genes associated with USH reported in the literature. They are considered to be important for stability and development of the inner hair bundle, photoreceptor cilium, and phagocytosis of the RPE.[73–75]

The most prevalent causative gene for USH is myosin VIIa (*Myo7A*), which encodes a critical actin-base protein functioning in the inner ear and retina.[70,76] Mutations in *Myo7A* (USH1b gene) account for approximately 60% of

all USH1 cases, causing deafness, vestibular dysfunction, and retinal degeneration with onset during childhood.[71,77] MYO7A has been found to be expressed in the RPE, the photoreceptor connecting cilia and synapses. It is proposed to play a role in intracellular transport, endocytosis, and cell-cell adhesion.[78–81]

There are several reported mouse models containing mutations in the *Myo7A* gene.[82] While they all display deafness and vestibular dysfunction phenotypes, their photoreceptors do not undergo degeneration as do human ones.[83,84] However, *shaker1* mice, which carry a mutated *Myo7A* gene, have been shown to exhibit retinal degeneration when exposed to cycles of bright light.[85,86] This is thought to be caused by pathologies in melanosome localization, opsin transport through the collecting cilium, and dysregulation of transducin translocation.[87–90]

Before human trials began, some studies demonstrated both the requirement of MYO7A for the apical localization of melanosomes in human RPE cells and that RPE melanosome localization and opsin transport could be restored in the *shaker1* mouse using a lentivirus containing *Myo7A* delivered through subretinal injection.[91,92] After these findings, Oxford Biomedica UK launched a phase I clinical trial to evaluate the safety of subretinally delivered *Myo7A* using an equine infectious anemia virus (EAIV) with lentiviral vector (UshStat) in patients with USH1b (ClinicalTrials.gov Identifier: NCT01505062).[93] This was followed by a long-term study of UshStat safety, tolerability, and biological activity (ClinicalTrials.gov Identifier: NCT02065011). Concurrently with the ongoing clinical trials, the safety profile of the EAIV-based *Myo7A* gene therapy was assessed in rhesus monkeys.[93] However, lentiviral transduction is limited mostly to the RPE after subretinal delivery of postnatal retina. There is a clear need to effectively transduce photoreceptors in patients with USH1b as photoreceptors are the site of the earliest disease expression. Studies on mice showed that a photoreceptor mutant phenotype was corrected with HIV-MYO7A.[94,95] As AAV capsid capacity is only approximately 4.7 kb, one approach is to split the full-length *Myo7A* cDNA (6.6kb) into two and package them separately. Depending on the design, these incomplete cDNAs are reconstituted into the full gene through recombination between internal homologous sequences, or trans-splicing, or a hybrid mix of the two strategies.[96–99] Dual AAV vector delivery methods have since been used to deliver *Myo7A* to the subretinal space of C57BL/6 mice,[100] *shaker1* mice,[96] as well as pigs.[101] These approaches have shown promising results in terms of MYO7A expression in RPE and photoreceptors, but require further investigation to establish a long-term safety profile and therapeutic efficacy before clinical trials.

Stargardt disease

Stargardt disease, an inherited form of juvenile macular degeneration, is both clinically and genetically highly heterogeneous.[102] This disease is commonly caused by recessive mutations in *ATP-binding cassette, sub-family A, member 4* (*ABCA4*) gene, which encodes a transporter protein present in photoreceptors and RPE.[103] ABCA4 actively transports retinylidene phosphatidylethanolamine, and phosphatidylethanolamine from the lumen to the cytoplasm of photoreceptor outer segments, playing an important role in the visual cycle.[104] Mutations on *ABCA4* gene reduce or terminate this transporter activity, leading to a buildup of potentially toxic bisretinoid compounds in the lumen and outer segment membranes of photoreceptors.[105,106] The accumulation of toxic bisretinoid compounds leads to lipofuscin accumulation in the RPE,[107] followed by degeneration of RPE and later of photoreceptors.[108] Mice missing ABCA4 also exhibit formation of lipofuscin granules.[109]

There are several gene therapy approaches under investigation to treat Stargardt disease, including the use of AAV and lentiviruses. Although, similar to *Myo7A*, the size of *ABCA4* cDNA exceeds the usual packaging capacity of AAVs (4.7 kb) for gene replacement, different procedures have attempted to surmount this challenge. Successful expression of ABCA4 using oversized AAVs in photoreceptors of $Abca4^{-/-}$ mice resulted in improved morphology and function of retina.[110] Later, a dual AVV trans-splicing strategy that efficiently reconstituted *ABCA4* gene in mice was used and demonstrated significant phenotype improvement.[96] Moreover, lentivirus was also used to infect photoreceptors in $Abca4^{-/-}$ mice, which showed better results than AAV-based methods when subretinal injections of the vector were performed on postnatal Days 4 and 5.[111] Experiments by Binley *et al.* achieved even more efficient photoreceptor transduction in retinae of non-human primates using EIAV lentivectors.[112]

In light of these good results, currently there are two ongoing Phase I/II clinical trials using gene therapy to treat Stargardt disease (ClinicalTrials.gov Identifiers: NCT01367444, NCT01736592). Since 2012, Oxford BioMedica has performed subretinal injections of EIAV lentivirus to deliver the ABCA4 gene to patients with homozygotic mutation of *ABCA4* and significant visual impairment, to assess safety and tolerability of ascending doses of the virus in both the short and long term.

Choroideremia

Choroideremia is an X-linked recessive progressive retinochoroidal degenerative disease associated with mutations within the choroideremia (*CHM*) gene. It affects males in early life, causing night blindness, peripheral visual field loss, and in most cases, complete blindness within the first 30 years of onset.[113–117] Chorioretinal atrophy, RPE degeneration, and abnormal ERG responses of retina sensitivity are seen even before patients report visual loss.[116] Female carriers often present with altered ERGs and irregular areas of pigmentation on fundus. For females, this disease is generally asymptomatic in early

years, although some females can present with severe disease resulting from unequal inactivation of the X chromosome.[115,118,119]

The *CHM* gene encodes Rab escort protein 1 (REP1), which recognizes Rab proteins and delivers them to Rab geranylgeranyl transferase (RabGGTs), thereby participating in intracellular vesicular transport and in modification of Rab via addition of geranylgeranyl groups. This process, referred to as prenylation, allows Rabs to attach to lipid bilayers.[120,121] Additionally, REP1 escorts prenylated Rabs to their destination membrane.[117] Mutations in the CHM gene lead to truncation or absence of REP1, resulting in defects in delivery of opsin to photoreceptor outer segments, in phagocytosis of photoreceptor outer segments by the RPE, and in apical migration of RPE melanosomes.[122] As indicated by experimental evidence, the severity of the CHM phenotype correlates with the severity of defects in intracellular trafficking processes.[123]

Chm knockout mice models showed that disease pathogenesis is associated with independent Rab prenylation defects that trigger photoreceptor and RPE degeneration.[124] A study also found that regardless of whether *CHM* was knocked out in photoreceptors or the RPE, defects in the RPE accelerate degeneration of photoreceptors.[125]

In vitro and animal studies with *Chm* knockout models have been performed using a number of AAV serotypes. Restoration of REP1 expression and function have been observed in lymphocytes, fibroblasts, and induced pluripotent stem cells (iPSCs) derived from choroideremia patients using AAV vectors *in vitro*.[126,127] In 2013, AAV2-mediated *Chm* gene was used to achieve functional expression of REP1 in human cells *ex vivo* and $Chm^{Null/WT}$ female carriers *in vivo*.[128] AAV8 has also been demonstrated to reverse the biochemical defects both *in vitro* and in the conditional *Chm* knockout mice.[129]

There are several clinical studies ongoing evaluating efficacy and safety of subretinal injections of AAV2-hCHM (Table 1). A completed phase I/II clinical trial (ClinicalTrials.gov Identifier: NCT01461213) showed retina sensitivity, visual improvement, and treatment safety in patients treated with subfoveal injections of AAV-REP1.[130]

Wet age-related macular degeneration

Wet (exudative) age-related macular degeneration (AMD) is the leading cause of blindness for people over 65 years of age. Current treatments for AMD involve inhibition of vascular endothelial growth factor (VEGF) with antibodies, RNA aptamers, or soluble receptors.[131–133] VEGF is implicated in intraocular neovascularization associated with diabetic retinopathy and age-related macular degeneration, promoting damaging neovascularization in the choroidal vasculature.[134,135] The current VEGF antibody treatment used in practice requires long-term repetitive intravitreal injections, which places significant financial and psychological burden on the patient.

All VEGF isoforms bind to two type III receptor tyrosine kinases, FLT1 and KDR (also known as FLK1).[136,137] Several studies have shown that injecting AAV2 with full-length soluble FLT-1 (sFLT1) subretinally can safely inhibit ocular neovascularization for up to 8 and 17 months postinjection in mice and in monkeys, respectively.[138,139] A study using an AAV2 vector with a chimeric soluble protein (AAV2-sFLT01) revealed that the protein was consistently expressed and was effective at managing neovascularization in a rodent model with minimum toxicity.[138–141] In 2017, a phase I/II clinical trial to treat wet AMD was completed (ClinicalTrials.gov Identifier: NCT01494805), in which a single subretinal injection of rAAV.sFlt-1 into a patient's eyes was found to be safe, highly reproducible, and may reduce ranibizumab retreatments.[142,143] Intravitreal injection of AAV2-sFLT01 was also evaluated in another ongoing clinical trial (ClinicalTrials.gov Identifier: NCT01024998) and was found to be safe and well tolerated at all injected doses.[144]

Soluble VEGF receptors are not the only way to suppress angiogenesis in the eye. Pigment epithelium-derived factor (PEDF), produced during normal wound repair, endostatin, cleavage product of collagen VII, and angiostatin, cleavage product of plasminogen, are endogenous proteins that attenuate physiologic neovascularization.[145–147] Studies have shown that AAV-driven upregulation of PEDF, endostatin, or angiostatin, resulted in suppression of laser-induced choroidal neovascularization (CNV) in mice.[148–150] This led to the launch of a now completed phase I clinical trial (ClinicalTrials.gov Identifier: NCT01024998), in which a modified AAV-PEDF (AdGVPEDF.11D) was delivered intravitreally.[151] The high dose treatment group showed a slightly lower neovascular lesion size than the low dose group, but the effect was not lasting, and thus not viable for management of a chronic disease such as AMD.[152] Concurrently, a study reported that subretinal delivery of a EIAV lentivector encoding LacZ resulted in long-term expression of LacZ in the RPE for up to 1 year in mice. Subretinal injection of the same vector, but encoding for murine angiostatin and endostatin, resulted in suppression of laser CNV.[153] This resulted in a phase I clinical study completed in 2017, as well as a long-term follow-up cohort (ClinicalTrials.gov Identifiers: NCT01301443 and NCT01678872) in which EIAV expressing endostatin and angiostatin (RetinoStat) was used to treat late-stage AMD. At completion, the trial reported safety, tolerability, and long-term therapeutic gene expression (up to 4.5 years), showing promise as a platform for chronic disease treatment. However, the treatment was not reliable in eliminating sub- and intra-retinal fluid in severe wet AMD.[154]

Achromatopsia

Achromatopsia (ACHM) is characterized by poor central visual acuity (<20/200), photophobia, complete color

blindness, and reduced cone-mediated ERG response amplitudes, and has a prevalence of about 1 in 30 000.[155] It has recently been shown that cone degeneration begins early in childhood, with deterioration progressing at a moderate rate.[156] A combined 80% of all ACHM cases can be characterized by mutations in genes encoding the cone-specification channel, cyclic nucleotide gated channel α3 and β3 (*CNGA3* and *CNGB3*), while fewer than 5% of all cases combined are caused by mutations in the cone-specific α subunit of transducin (*GNAT2*), activating transcription factor 6 (*ATF6*), and α subunit of cone-specific phosphodiesterase (*PDE6C*).[157–159] The first gene therapy for ACHM was performed in a mouse carrying a recessive mutation in *Gnat2*, resulting in little to no cone-mediated ERG and poor visual acuity. Subretinal injection of AAV5 containing *Gnat2* driven by the human red cone opsin promoter was shown to restore cone-mediated ERG amplitudes and cone-mediated behavior responses to the levels of the age-matched wild-type mice.[160,161]

Delivery of CNGA3 and GNAT2 using AAV5/AAV8-based vectors has been shown to normalize protein expression and improve vision in murine models of *CNGA3* and *CNGB3* forms of ACHM.[162–164] Subretinal injection of AAV5-CNGB3 in ACHM-affected dogs, or AAV5-CNGA3 in diseased sheep both resulted in restoration of cone function and day vision, which lasted up to 33 months in dogs and up to 3 years in sheep.[165,166] To optimize the effectiveness, studies also showed that decreasing the length of promoter and using AAV2 with single tyrosine-to-phenylalanine (YF) mutations can increase the efficiency for CNGB3 expression.[167,168] These studies have resulted in initiation of several independent phase I/II clinical trials for both CNGA3- and CNGB3-linked ACHM launched in Europe by STZ eyetrial and MeiraGTx, and in the United States by AGTC (Table 1). All these approaches rely on modified rAAV2-based vectors delivered with a single subretinal injection to supplement the affected gene and aim to assess the safety and efficacy of the treatment.

X-linked juvenile retinoschisis

X-linked juvenile retinoschisis (XLRS) is the leading cause of monogenic macular dystrophy with between 1:5 000 and 1:25 000 males afflicted.[169] XLRS is typically classified by localized splitting in the retina (schisis) and an unusual electronegative ERG with a preserved a-wave and a diminished b-wave.[170–172] It starts with retinal presentation in early childhood, exacerbation of symptoms in teenage years, and then stabilizes during adulthood.[169,173]

XLRS is associated with mutations in the retinoschisin (*RS1*) gene, which encodes the RS1 protein (24 kDa) secreted from retinal photoreceptors with a discoidin domain that is likely to be involved in cell adhesion.[174–177] It was first reported that intravitreal delivery of an AAV2 vector containing murine *RS1* cDNA driven by cytomegalovirus promoter in the *Rs1h* knockout mice at 13 weeks of age led to visual improvements as tracked by ERG and schisis cavities out to 6 months of age.[178] Subretinal delivery of AAV5 vector expressing murine opsin promoter driving human *RS1* cDNA or intravitreal delivery of AAV8 vector expressing human retinoschisin promoter driving human *RS1* cDNA into Rs1h-KO mice at young stage (P14 to 2 month) both showed improved retinal structure and function.[179,180] However, treatment at 7 months of age improved only retinal structure and not ERG function,[181] indicating a critical window of treatment. Currently, a phase I/II clinical study is being conducted to evaluate the safety and efficacy of a rAAV vector expressing retinoschisin (rAAV2tYF-CB-hRS1) delivered intravitreally in XLRS patients (ClinicalTrials.gov Identifier: NCT02416622).

CRISPR/Cas9-mediated gene and mutation-independent therapy

Although current gene therapy offers many promising treatments for various human diseases, its application is often limited to a narrow spectrum of diseases and patient population, because it can only be directed to a single gene. Similarly, in current regenerative medicine, the application of endogenous stem cells in tissue repair/regeneration represents an important method in treatment of many diseases. Promising results have been demonstrated in mouse liver, zebrafish heart, and human lens.[182–184] However, as in gene therapy, endogenous stem cell treatment can be applied to only a very narrow spectrum of disease. The major challenge is that normal genetic makeup and function are required in the starting cells for tissue regeneration; if the starting cell type harbors a causal genetic mutation which renders subsequently generated cells susceptible to the same disease etiology, then regenerated cells will assume the previous cell fate.

One approach to overcome the above drawbacks is to combine the advantages of both gene therapy and regenerative medicine. The resulting method is called therapeutic cellular reprogramming. Using CRISPR/Cas9-based gene editing, this strategy switches a cell type sensitive to a mutation to a cell type that is resistant to the same mutation, with related function. Therefore, this strategy eliminates the occurrence of underlying mutation, while preserving tissue structure and function. As a result, distantly related cells can be directly converted *in vivo* by appropriate combinations of developmentally relevant transcription factors,[185] expanding the application of conventional regenerative medicine in both disease spectrum and patient population.

The potential of therapeutic cellular reprogramming was first examined on RP. As RP is caused by mutations in over 200 genes, the therapeutic impact of conventional gene therapy is limited. Acute gene knockout of either rod determinant *Nrl* or its downstream transcriptional factor *Nr2e3* showed successful rod to cone reprogramming in adult rod photoreceptors.[186,187] The resulting

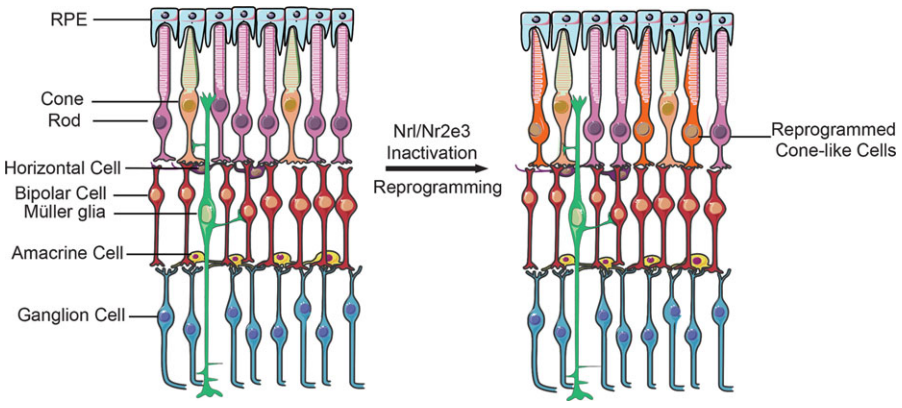

Figure 5. Conversion between two cell types from the same lineages: reprogramming rod photoreceptors to cone photoreceptors.[126]

cone photoreceptors demonstrated resistance to mutations in RP-specific genes on rod photoreceptors, which consequently prevented secondary cone loss. More importantly, by combining an AAV-based delivery system with CRISPR/Cas9-mediated targeted inactivation of *Nrl* or *Nr3e3*, successfully *in vivo* reprogramming of rod photoreceptors into cone photoreceptors with consequent retinal photoreceptor preservation and visual rescue was achieved (Fig. 5).[188,189] These results indicate that therapeutic cellular reprogramming can serve as a novel treatment approach that is gene- and mutation-independent, broadening implications for genetic disease therapy.

Retinal gene therapy has always been at the forefront of human gene therapy and much progress has been made in retinal gene therapy. The successful approval of the first retinal gene therapy for LCA2 caused by RPE65 mutations has ushered in a new era in human gene therapy. The application of CRISPR/Cas9-mediated gene editing technology is transforming how the gene therapy is administered. We anticipate great progress and further approvals of retinal gene therapy products in the near future.

Conflict of interest statement

The authors declare no conflict of interest.

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