Gene therapy and genome surgery in the retina

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Precision medicine seeks to treat disease with molecular specificity. Advances in genome sequence analysis, gene delivery, and genome surgery have allowed clinician-scientists to treat genetic conditions at the level of their pathology. As a result, progress in treating retinal disease using genetic tools has advanced tremendously over the past several decades. Breakthroughs in gene delivery vectors, both viral and nonviral, have allowed the delivery of genetic payloads in preclinical models of retinal disorders and have paved the way for numerous successful clinical trials. Moreover, the adaptation of CRISPR-Cas systems for genome engineering have enabled the correction of both recessive and dominant pathogenic alleles, expanding the disease-modifying power of gene therapies. Here, we highlight the translational progress of gene therapy and genome editing of several retinal disorders, including RPE65-, CEP290-, and GUY2D-associated Leber congenital amaurosis, as well as choroideremia, achromatopsia, Mer tyrosine kinase– (MERTK–) and RPGR X-linked retinitis pigmentosa, Usher syndrome, neovascular age-related macular degeneration, X-linked retinoschisis, Stargardt disease, and Leber hereditary optic neuropathy.

Introduction

The field of gene therapy aims to correct genetic deficits by modifying pathology at the genetic level. The eye is a unique organ with several features that buoy the success of gene therapy. Its small anatomical size and subdivision into yet smaller compartments that are easily accessible by surgery and allow gene delivery vectors to be concentrated enable delivery of up to 1.0×10^{10} to 2.0×10^{10} copies of each vector within a volume of approximately 100 microliters. The eye also has a special relationship with the immune system, in part due to the retina-blood barrier, which alters the trafficking of immune cells from the systemic circulation to the eye (1, 2). Immunosuppressive cytokines and surface molecules displayed on ocular parenchymal cells, which interact with Tregs to dampen inflammatory responses, also contribute to the eye's immune-privileged state (1, 3). Additionally, the eye's duplicity as an organ allows for within-subject comparisons in animal models and clinical trials, allowing for one eye to be tested and the other to serve as a control. Many genetic therapies in ophthalmology have focused on the retina and its supporting cells. This Review seeks to provide an overview of the current state of gene therapy in the retina and discuss future directions.

Gene supplementation or genome surgery?

Current clinical gene therapy trials of gene supplementation in the eye involve the delivery of exogenous genetic material into cells with inherited genetic defects, while genome surgery focuses on the precise modification of endogenous genomes to correct mutant alleles. This delivery can occur via viral or nonviral vectors. Currently, adenoviruses, adeno-associated viruses (AAVs),

and lentiviral vectors represent the majority of viral vectors used for gene therapy (4, 5). Moreover, some groups have examined the precise correction of ocular genetic mutations using site-specific nucleases, allowing for genome modification with surgical precision (examples are depicted in Figure 1 and refs. 6–13). It should be noted that editing the genomes of postmitotic differentiated neurons by using homologous recombination remains challenging because of low to absent rates of recombination, and further work is needed to optimize these rates (14).

Adenoviruses. While adenoviruses are not frequently used for the transduction of eye cells, efficient transduction, episomal nature, and large genome size (~30–40 kb pairs) make them attractive for use in gene therapy of the eye (15). In 1996, an adenoviral vector was one of the first vectors used to study eye disease in an animal model when Bennett et al. used an adenoviral vector to deliver a cDNA copy of the phosphodiesterase β subunit to photoreceptors in the *rd1* mouse model, successfully delaying photoreceptor degeneration by six weeks (16). A downside to adenoviruses is their relatively high immunogenicity due to the high prevalence of certain serotypes, such as the Ad5 serotype, in the human population, resulting in most patients carrying circulating neutralizing antibodies to these viruses (17). Immunogenicity, however, has been found to be serotype and site-of-introduction dependent (with subretinal delivery eliciting a lower T cell–mediated response than occurs with intravitreal injections) (18, 19). Since their first use to transduce murine photoreceptors, adenoviruses have been used to dampen retinal and choroidal neovascularization in rat and rabbit models and inhibit retinoblastoma growth in a mouse model; however, they have not been used extensively for ocular gene therapy (20–22).

AAVs. AAVs have high transduction efficiency and serotype-dependent, cell-type specificity as well as low immunogenicity, making them attractive tools for gene therapy. While these viruses can integrate into host genomes, removal of the *rep* ORF from their
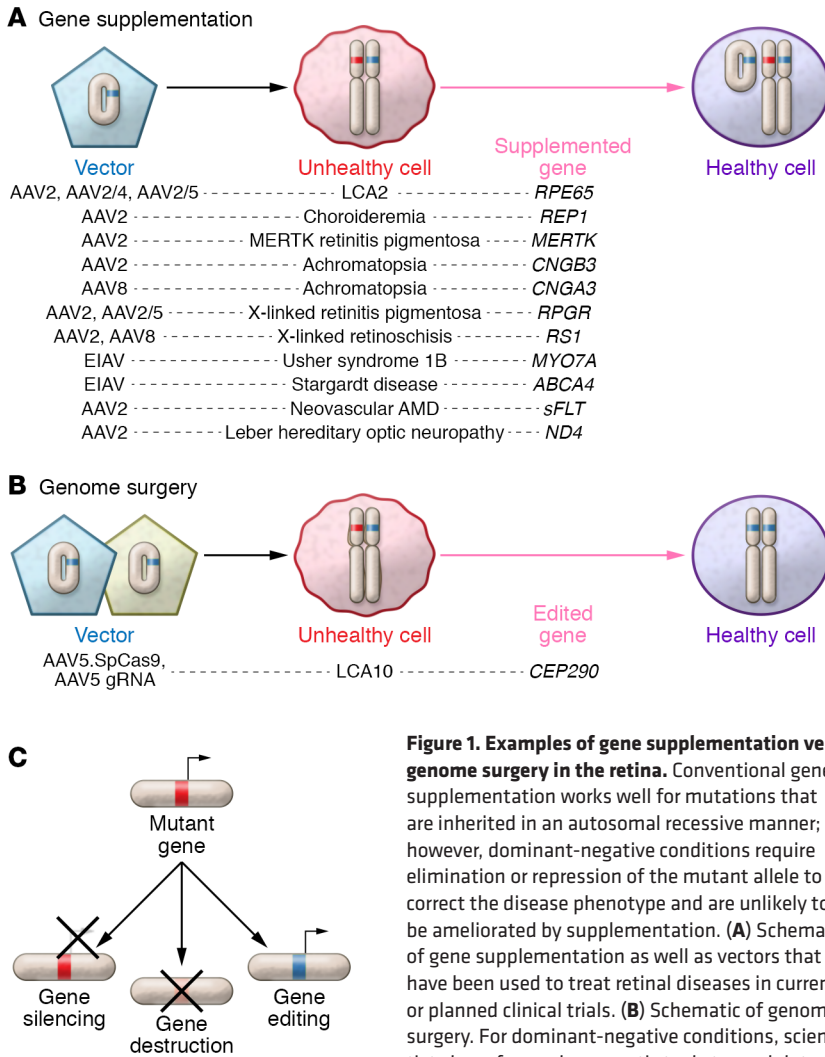
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Figure 1. Examples of gene supplementation versus genome surgery in the retina. Conventional gene supplementation works well for mutations that are inherited in an autosomal recessive manner; however, dominant-negative conditions require elimination or repression of the mutant allele to correct the disease phenotype and are unlikely to be ameliorated by supplementation. (**A**) Schematic of gene supplementation as well as vectors that have been used to treat retinal diseases in current or planned clinical trials. (**B**) Schematic of genome surgery. For dominant-negative conditions, scientists have focused on genetic tools to modulate gene expression, such as RNAi, or tools that modify the patient's genome to mutate the pathogenic allele, such as site-specific nucleases like CRISPR-Cas. (**C**) Description of different approaches used to affect gene expression.

mented AAV, or fAAVs) (27, 28). Scientists have also attempted to split transgenes between AAV vectors or generate functional truncated genes, also known as minigenes, to overcome the size limitations of AAVs (29).

Lentiviral vectors. Belonging to the family of viruses known as *Retroviridae*, lentiviruses are RNA viruses that integrate into host genomes using genome-encoded reverse transcriptase and integrase (30). The lentivirus genus includes HIV and other retroviruses that are capable of integrating into dividing and nondividing cells, depending on the serogroup (30, 31). The first lentivirus applied in human clinical trials was the nonpathogenic equine infectious anemia virus (EIAV), which was shown to be effective and safe for application in human photoreceptor gene delivery (32–35). Lentiviral vector gene–carrying capacity is between that of adenoviruses and AAVs, with a maximum payload of approximately 8 to 9 kb, a size that somewhat reflects that of a sweet spot for many human genes (30).

Nucleic acid therapies and nonviral delivery. The majority of gene therapies for ocular diseases have focused on the delivery of therapeutic DNA or precise editing of pathogenic alleles, but the therapeutic promise of catalytic RNA and RNAi has remained an active area of interest since their discovery (36–38). A recent example of these tools includes the application of antisense oligonucleotides (AONs) targeting a de novo cryptic splice donor of the dominant-negative LCA CEP290 (c.2991 + 1655A > G) mutation. AAV delivery of AONs to patient-derived fibroblasts in vitro enhanced CEP290 protein levels and improved the splicing profile of *CEP290* mRNA in a humanized mouse model of CEP290 LCA (39).

Several groups have examined delivery methods for therapeutic nucleotides beyond viral vectors. Lipid-based delivery of miR-184 was shown to modulate the development of ischemia-induced neovascularization in mice (40). Additionally, in vivo delivery of glycol-substituted lysine peptide–compacted (CK30PEG-compacted) DNA nanoparticles showed effective transgene delivery and expression in mouse retinae (41).

Genome surgery with programmable nucleases. Strategies to correct pathogenic alleles by editing endogenous loci have largely relied on the development of designable site-specific endonucleases (5). In the three decades since the first use of site-specific meganucleases for in vivo eukaryotic genome modification, the field of designable endonucleases has exploded (5, 42, 43).

Recently, clustered regularly interspaced short palindromic repeats (CRISPR) and CRISPR-associated (Cas) proteins, both derived from prokaryotic immune systems, have been used to mod-

genome results in a vector that is more likely to exist as an episome rather than integrate (23). Moreover, they have proven their potential for long-term gene delivery, with effects persisting for up to 11 years in canine models and 6 years in Leber congenital amaurosis (LCA) patients treated with an AAV2-delivered *RPE65* cDNA construct (described in more detail below) (24, 25). Several modifications to these vectors, such as removal of their endogenous Rep protein, as well as encoding two self-complementary copies of their single-stranded DNA viral genome (scAAVs), have drastically decreased the integrating/mutagenic capability of AAVs and increased their transduction efficiencies by 140-fold, respectively (23, 26). An ongoing struggle of AAV-based therapies is their small genome, which limits genetic payloads to no more than 4 to 5 kb pairs. While groups have observed larger gene delivery using AAVs, such as the case of a 8.9-kb *ABCA4* expression cassette delivery in a mouse model of Stargardt disease, this is now believed to be the result of recombination of viral packaged gene fragments (frag-

ify mammalian genomes. CRISPR-Cas provides a foundational advance for the simple design of novel site-specific endonucleases (44). The *Streptococcus pyogenes* CRISPR-Cas9 system was the first in which the uncovered molecular mechanisms allowed adaptation for genome engineering (45–47). This system uses a Cas9 endonuclease (SpCas9) guided to the site of cleavage by so-called guide RNA molecules (gRNAs) originally derived from CRISPR elements of the immune system. Numerous CRISPR-Cas systems have been used for genome engineering since, including smaller Cas9 proteins such as those from *Campylobacter jejuni* (CjCas9) or *Staphylococcus aureus* (SaCas9) (48, 49). While off-target effects of CRISPR-Cas systems are a concern to scientists and clinicians, higher-fidelity CRISPR systems have been developed, along with anti-CRISPR systems to modulate cleavage activity (50, 51). Moreover, CRISPR-Cas systems devoid of cutting activity have been utilized for transcriptional and epigenetic control of DNA expression (5).

In ophthalmology, CRISPR-Cas systems have been used to modify a plethora of disease models, highlighting their potential for therapeutic application (6, 8). While precise modifications can be generated if repair template DNA is supplied, knockouts of autosomal dominant-negative alleles have also been effective in animal models as discussed in Figure 1. Bakondi et al. showed that the *Rho*[S334] dominant-negative allele of the rhodopsin gene in a rat model of retinitis pigmentosa (RP) could be mutated in rats in vivo, resulting in reduced expression and 53% visual improvement (8). Such an approach could be useful for several diseases in humans and relies on the generation of a de novo mutation producing a CRISPR-Cas–targetable site. This approach is powerful, but not without challenges, as Christie et al. demonstrated when attempting to target pathogenic mutations in *TGFBI* corneal dystrophy, finding it difficult to design gRNAs that were specific enough to the targeted mutation to avoid WT locus cleavage (52).

Progress of disease-specific gene delivery

In the following sections, we aim to discuss the progress of gene therapy in a disease-specific manner. We have organized these diseases on the basis of their genetic and cellular characteristics including retinal pigment epithelium (RPE) disorders, photoreceptor disorders, inner retinal disorders, and oligogenic disorders. RPE disorders are listed first in Table 1, as mutations affecting the RPE have long been considered low-hanging fruits for gene supplementation. Moreover, achromatopsia and X-linked retinoschisis, which feature relative preservation of photoreceptors but severe loss of retinal function, are included in the photoreceptor disorder section.

RPE disorders

RPE65-associated LCA2. Early-onset retinal dystrophy, also known as LCA, is characterized by poor vision, extinguished electroretinography responses, nystagmus, and abnormal pupillary light reflexes, usually resulting in severe visual impairment in the first year of life due to mutations in the RPE (*RPE65*) gene, which encodes a retinoid isomerase (refs. 53–55 and Figure 2).

In 1999, Veske et al. identified a retinal dystrophy linked to a *RPE65* mutation in the Briard breed of dog, which became an important model for LCA2 (56). As mentioned previously,

AAV2-delivered *RPE65* cDNA produced rapid improvement in visual function in Briard dogs, which was sustained in some of the treated animals 11 years later (57). Sustained visual improvement was only noted in treated dogs that exhibited retinal dysfunction without degeneration, indicating that the timing of therapy was crucial for visual restoration (57). Studies in the *rd12* mouse model of LCA2 (expressing a nonsense mutation in *Rpe65*) also showed that AAV-delivered and adenovirus-delivered *RPE65* restored vision-dependent behavior in visually impaired animals (58, 59). Beginning in 2007, multiple phase I/IIa trials of subretinal delivery of *RPE65* cDNA using AAV2 resulted in no serious adverse events and showed improvements in visual acuity, pupillary reflexes, and mobility in some treated patients (25, 60, 61). Long-term follow-up showed that, while some of these patients maintained visual improvement, retinal degeneration progressed in other patients (24, 57).

A landmark study carried out by the Children's Hospital of Philadelphia showed that readministering an AAV2.*RPE65* vector to the contralateral eye of patients previously treated with AAV2.*RPE65* was safe and resulted in improvement in full-field light sensitivity and mean mobility (62). This trial was important, as it showed that immune responses from previously administered vectors would not affect later therapy in the untreated eye. Encouraged by previous successes, a phase III trial using bilateral subretinal delivery of AAV2.h*RPE65*v2 was initiated in 2013. In 2015, this trial showed improved mobility and light sensitivity in treated patients, without changes in visual acuity (63). An FDA advisory panel unanimously lauded this therapy as effective in October 2017, and the FDA approved the therapy in January 2018 (64).

MERTK RP. The Mer tyrosine kinase (MERTK) is a crucial receptor in the phagocytosis of light-sensitive photoreceptor outer segments in the RPE apical membrane, enabling turnover (65). Mutations in *MERTK* interrupt the recycling of these light-sensitive membrane segments, resulting in photoreceptor degeneration and loss (66). Large-scale sequencing approaches have shown that approximately 3% of retinal dystrophies may result from mutations in *MERTK* in an autosomal recessive pattern (67, 68). *MERTK* mutations are associated with a retinal dystrophy phenotype characterized by childhood rod and cone dysfunction and atrophy of the macula (69, 70). Several groups have shown success in MERTK supplementation using adenovirus and AAV vectors in the Royal College of Surgeons (RCS) rat model of retinal dystrophy, noting improvement in photoreceptor lifespan weeks after subretinal injection (71–73). Spurred on by successes in animal models, the King Khaled Eye Specialist Hospital sponsored a phase I trial that began in 2011 and is currently recruiting patients to examine the safety of subretinal injection of a recombinant AAV2 vector expressing a human *MERTK* gene in patients with MERTK RP (see Table 1). Other approaches have shown that RPE cells overexpressing the *OTX2* gene (a *Drosophila* homolog important in RPE development) can rescue photoreceptor degeneration in the RCS rat, a model of inherited retinal dystrophy, highlighting such an approach for therapeutics for MERTK RP (74, 75).

Choroideremia. Choroideremia is an X-linked degenerative disorder of the RPE, photoreceptors, and choroid caused by loss of the Rab escort protein 1 (REP1), which is encoded by *CHM*
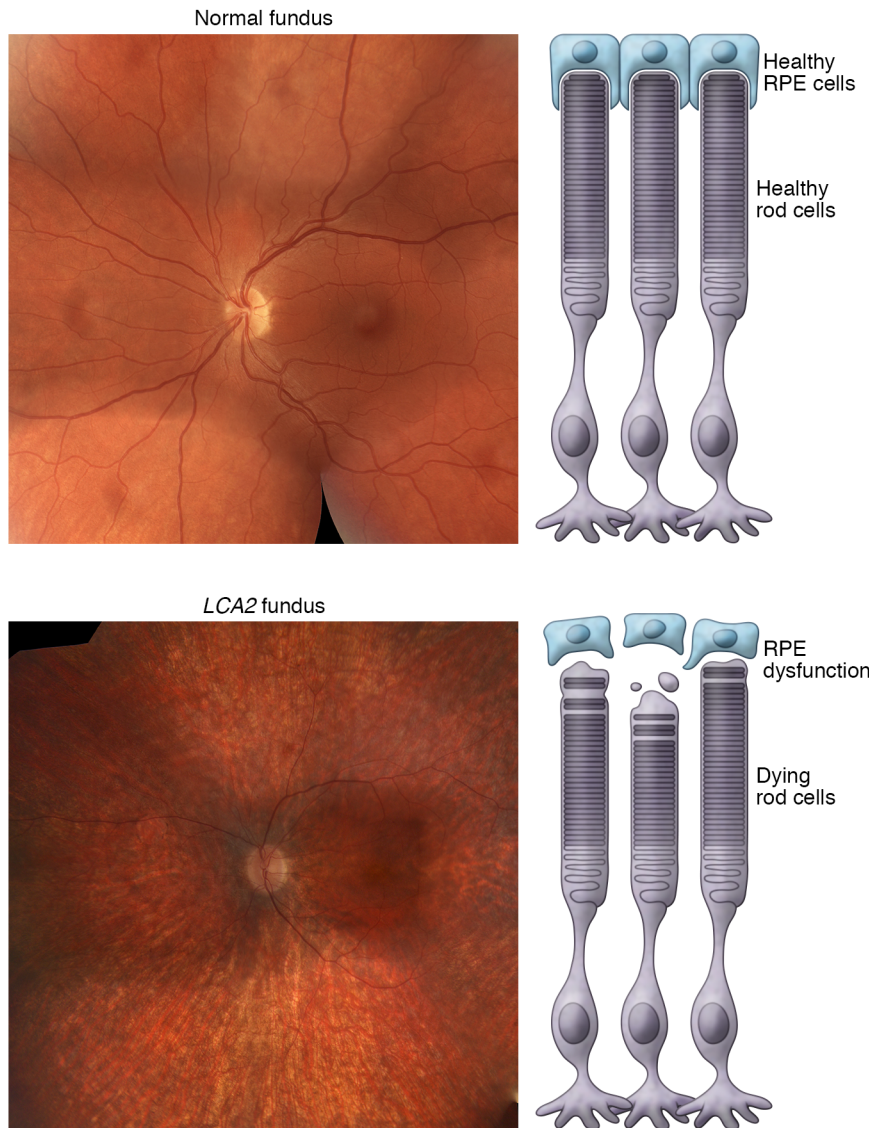
Figure 2. RPE65-associated LCA2. Mutations in the gene encoding *RPE65* isomerase results in autosomal recessive LCA. This gene is largely active in the RPE and is responsible for the isomerization of all-*trans*-retinyl esters to 11-*cis*-retinyl esters, the rate-limiting step in the retinal visual cycle, and mutations in *RPE65* result in RPE degeneration and photoreceptor death. Salt and pepper retinopathy and arteriole attenuation seen in a 28-year-old man with compound heterozygote mutations (c.11 + 5G > A) and (c.715T > G) in *RPE65*. Fundoscopy revealed optic disc pallor and a relatively spared macula. Note the absence of bone spicule–like pigmentation in the periphery.

icant decrease relative to baseline vision in the treated eye, resulting in a decrease in the ability to read letters from 29 to 18 letters (82). Additionally, a prospective study by Simunovic et al. showed that subretinal delivery of AAV.REP1 in 5 patients was well tolerated, with structural resolution of iatrogenic retinal detachment occurring by 1 week after treatment and a single patient reporting a subtle decrease in color perception (83). A phase III trial comparing high and low single doses of recombinant AAV2.REP1 (rAAV2.REP1) is currently underway, with several other trials occurring in parallel for AAV-delivered REP1.

Photoreceptor disorders

Achromatopsia. Affecting approximately 10,000 Americans (1 in 30,000 live births), achromatopsia (rod monochromatism) is an autosomal recessive condition characterized by pendular nystagmus, photophobia, and poor visual acuity, along with colorblindness (garnering achromatopsia the moniker "rod monochromatism") (84). Currently, the only treatment for achromatopsia is supportive care that involves the use of filtered Corning Glare control lenses, tinted contact lenses, or glasses to reduce the severity of photophobia, as well as occupational aids for individuals with severely reduced visual acuity, which varies widely in severity (84, 85). Mutations in genes crucial for cone cell phototransduction are the main cause of achromatopsia, with approximately 50% of causative mutations in cyclic nucleotide gate ion channel β 3 (*CNGB3*), encoding a cyclic guanosine monophosphate (cGMP) concentration–dependent ion channel important for cone cell signal transduction (86). Additionally, other mutations have been identified in *CNGA3*, two phosphodiesterase genes (*PDE6C* and *PDE6H*), and guanine nucleotide–binding protein α-transducing activity polypeptide 2 (*GNAT2*), as well as in the unfolded protein response regulator *ATF6* (86, 87). Numerous animal models of achromatopsia have shown that gene replacement can improve cone cell function, with particular success in mouse models of *CNGB3*, *CNGA3*, and *GNAT2* mutations, a canine model of *CNGB3* mutation, and a sheep model of *CNGA3* mutation (88–94). Encouraged by successes in gene delivery for achromatopsia in animals and the lack of current treatments, a phase I/II trial that began in 2015 is currently recruiting patients with CNGB3 achromatopsia to study the efficacy and safety of a rAAV2 vector delivering *CNGB3*. Additionally, a separate trial is investigating the safety and efficacy of AAV delivery of *CNGA3* to patients with CNGA3 achromatopsia (see Table 1 and ref. 95).

(76, 77). Classically, choroideremia is characterized by atrophy of the choroid, resulting in pallor in the outer retina with progressive vision loss and night blindness (78). Zebrafish, mouse, and human induced pluripotent stem cell (iPSC) models have been used to study therapies for *CHM* mutations (78–80). Success with these models led to a phase I/II trial of a subretinal injection of the AAV2-delivered native *CHM* gene. Initial results showed improved rod and cone function and a mean gain of 3.8 letters read (a metric of visual acuity), negating the detrimental effect of retinal detachment caused by the subfoveal detachment (81). At present, a 3.5-year follow-up of a clinical trial using this vector shows sustained visual improvement in 2 of 6 patients, with progressive degeneration and visual loss in the untreated eye controls (82). In the same follow-up period, one patient reported a signif-

GUCY2D photoreceptor-related LCA1. Of the 18 genes involved in LCA, *GUCY2D* was the first identified, hence the designation LCA1 (96). *GUCY2D* encodes the enzyme guanylate cyclase 1 (GC1), located predominantly in cone photoreceptor neurons of the retina. GC1 is responsible for sensing low levels of intracellular calcium and producing cGMP, thus causing cGMP gate channels to open and allowing an influx of calcium to return photoreceptors to their preexcitation state (97, 98). In patients, mutations in *GUCY2D* cause photoreceptor dysfunction resulting in decreased visual acuity, nystagmus, and extinguished electroretinographic (ERG) recording abnormalities (53, 99).

Recently, Sharon et al. correlated the genotype with phenotypes of known *GUCY2D* mutations and found that the type of mutation and its genetic location correlate with the pattern of inheritance of LCA1 (100). Subretinal delivery of rAAV2/8 carrying human and mouse *GUCY2D* genes in *Gucy2e⁻/⁻* mice produced improvements in visual behavior and cone preservation 6 months after delivery (101). Moreover, subretinal delivery of an AAV5 vector containing native human *GUCY2D* in *Nrl⁻/⁻ Gucy2e⁻/⁻* mice (an all-cone mouse model of LCA1) improved retinal function for at least 6 months (102). While there is strong preclinical evidence for AAV-based LCA1 treatment, no clinical trials for LCA1 have been initiated.

RPGR X-linked RP. Caused by mutations in the RP GTPase regulator (RPGR), RPGR X-linked RP affects approximately 1 in 3,500 people, resulting in night blindness and progressive loss of visual fields due to dysfunctional protein trafficking that is normally governed by native RPGR and its interacting partner, the Δ subunit of rod cGMP phosphodiesterase (103–106). There are several mouse models of RPGR X-linked RP and two canine models, with the canine models recapitulating distinct RPGR X-linked RP phenotypes (107–110). Subretinal injection of a AAV2/5 vector carrying human *RPGR* to the XLRPA2 canine model, which harbors a microdeletion in canine *RPGR* ORF15, showed preserved photoreceptor nuclei in treated regions and correction of opsin protein translocation (111). Currently, there are two phase I/II trials examining the efficacy and safety of AAV2/5 vector delivery of a native *RPGR* gene to affected individuals (see Table 1). Additionally, Applied Genetic Technologies Corporation (AGTC) has announced a similar phase I/II trial for their rAAV2tYF-GRK1-RPGR vector (see Table 1).

Stargardt disease. Stargardt disease is the most common type of autosomal recessive macular degeneration, with a varying age of onset and a carrier frequency of 1 in 30. It is caused by mutations in the ATP-binding cassette transporter gene *ABCA4*, which acts as a retinal transporter (112, 113). While the phenotype can be variable, it is generally characterized by central visual loss due to accumulation of bis-retinoids, which are cytotoxic lipofuscin-rich lysosome residues that accrue as a result of impaired retinoid transport and deposit in the RPE (114). Human *ABCA4* cDNA is approximately 7 kb in size, exceeding the cargo limit for AAV packaging, which spurred the development of an EIAV vector that was shown to reduce lipofuscin accumulation in photoreceptors after subretinal injections in the *Abca4⁻/⁻* Stargardt mouse model (115). Currently, Sanofi is overseeing a 46-patient phase I/IIa trial examining a native *ABCA4*-carrying EIAV vector to supplement the defective copy that is scheduled to be completed in 2019 (116, 117).

Several groups have shown a reduction in lipofuscin accumulation in mouse models of Stargardt disease after delivery of full-length *ABCA4* in dual-AAV vectors (118–120).

Usher syndrome. Usher syndrome (types 1, 2, and 3) refers to a group of three autosomal recessive and clinically separate deafness-blindness syndromes caused by mutations in one of nine genes. It is responsible for at least 50% of congenital deafness-blindness cases (121, 122). Usher type 1 is caused by mutations in *PCDH15*, *MYO7A*, *USH1C*, *USH1G*, or *CDH23* (121). Sensorineural hearing loss, RP, and vestibular pathology are the hallmarks of Usher syndrome type 1B, which is caused by mutations in *MYO7A*. This gene encodes a myosin involved in organelle trafficking within the RPE that was first identified as a causative gene in *Myo7a*-deficient shaker-1 mice, which exhibit a shaking and head-tossing phenotype due to cochlear and vestibular deficits (123–126). The entire *MYO7A* gene is approximately 100 kb in size, with a coding sequence of approximately 7 kb, making AAV delivery difficult and resulting in a focus on lentiviral delivery of MYO7A. As mentioned previously, dual AAV vector delivery and fAAV delivery can deliver genes larger than the carrying capacity of AAVs alone. While Tripani et al. and Dyka et al. have expressed *MYO7A* in shaker-1 mice via the use of dual AAV/fAAV approaches, clinical efforts have focused on lentiviral delivery (127–130). Additionally, both isoforms of MYO7A have been shown to rescue the Usher syndrome phenotype in animal models (127, 128, 131, 132). Zallocchi et al. showed that subretinal delivery of native *MYO7A* within the EIAV vector in shaker-1 mice significantly reduced photoreceptor loss and improved intracellular G protein transport (133, 134). The same study found the injection to be safe in macaques, prompting the launch of a phase I/IIa trial, scheduled to end in April 2019, that involves subretinal monocular injection of the EIAV-*MYO7A* vector (UshStat) in patients with Usher syndrome 1B (see Table 1).

Inner retinal disorders

X-linked retinoschisis. Hereditary X-linked retinoschisis is a common form of rod-cone dystrophy featuring early juvenile macular degeneration in males, with a prevalence of 1 in 5,000 to 25,000 in the general population (135, 136). Schisis refers to the separation of retinal layers, which, together with macular cysts, is the cause of vision loss in this disease (135). Patients with X-linked retinoschisis, during EEG, have a distinctive phenotype resulting from decreased synaptic communication at the photoreceptor-bipolar cell synapse that generates a characteristic "electronegative" waveform, i.e., a decreased b-wave with a preserved a-wave (135). X-linked retinoschisis is caused by a mutation in retinoschisin (*RS1*), which encodes a protein that binds plasma membrane proteins of multiple layers of the retina and is involved in cell signaling within retinal cells (137–139).

Many methods have focused on replacement of RS1 protein in affected cells. One approach by Bashar et al. injected RS1-producing mesenchymal stem cells into the vitreous of *Rs1*-deficient mice (XLRS mice) and observed a 78% reduction in the schisis cavities and significant improvement in b-wave/a-wave ratios on ERG (140). Most other methods focus on genetic replacement of *RS1*. Interestingly, targeting murine photoreceptors with AAV vectors containing native *Rs1* rescued degeneration and restored ERG signaling more effectively than
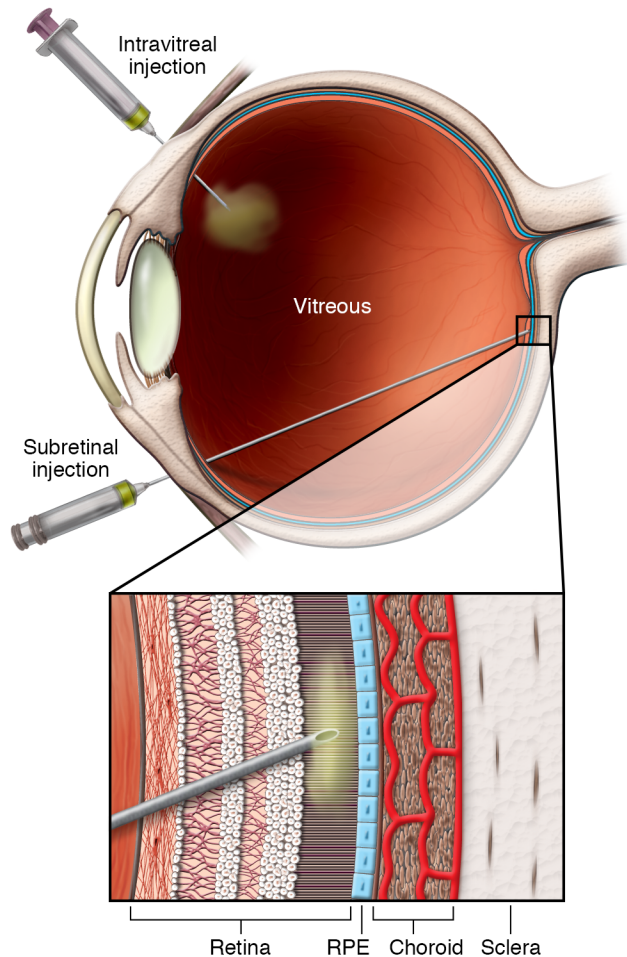
Figure 3. Intravitreal versus subretinal delivery. Intravitreal delivery is less technical and has fewer risks associated with structural damage to ocular tissues, however, concentrated delivery of vector to the disease tissue can be problematic because of the diffusional volume of the vitreous. Subretinal delivery is more technically challenging and causes transient retinal detachment, which poses a risk for permanent retinal damage, however, the gene therapy concentration is much higher and the transduction to nearby tissue is often significantly greater.

did targeting Muller glia (138). A rAAV2 and an AAV8 vector have been shown to restore ERG recordings in *Rs1*-deficient mice after intravitreal injection, inspiring separate clinical trials to examine their efficacy in humans (refs. 141, 142, and Figure 3). Safety studies of the rAAV2 vector in mice showed high biodistribution in treated eye tissue in both mice and macaques, but the macaque studies showed mild-to-moderate inflammatory cell recruitment in half of the treated eyes (143, 144). Currently, two phase I/II trials are examining AAV delivery of RS1 (see Table 1).

Leber hereditary optic neuropathy. Leber hereditary optic neuropathy (LHON) is caused by a mutation in mitochondria-encoded genes for complex I of the electron transport chain, usually *ND1*, *ND4*, or *ND6*. It is characterized by atrophy of the retinal ganglion cells, which results in severe and bilateral visual loss (145–147). The addition of a mitochondria-targeting sequence to a human mutant *ND4* gene allowed Qi et

al. to show that allotopic expression as well as nuclear expression with mitochondrial targeting modeled LHON in mice (148).

Successes in animal models led to a phase I/II trial by the University of Miami examining the safety of intravitreal-injected, AAV2-delivered, allotopically expressed *ND4* in patients with LHON, and is scheduled for completion in 2020 (149). Preliminary results showed the therapy's safety, with minimal adverse effects and quantitative improvement in vision in two of five patients, though it is unclear whether this was due to the success of the vector or the nature of LHON, which has low rates of spontaneous recovery. Larger sample sizes will be required to determine the significance of improvements in the trial (150, 151). A previous clinical trial for an intravitreally delivered AAV2.ND4 vector resulted in no adverse effects for the trial's nine patients, with a significant increase in visual acuity in six patients, without a change in retinal nerve fiber thickness (152, 153).

Oligogenic disorders

Neovascular age-related macular degeneration. Wet (or neovascular or exudative) age-related macular degeneration (AMD) is a significant cause of legal blindness in the United States. As of 2004, wet AMD along with atrophic AMD affected 30% of Americans over the age of 75, and this proportion is expected to increase by 50% by 2020 (154). Many factors stimulate neovascularization in AMD, a result of pathologic choroid blood vessel proliferation that causes macular dysfunction. While the causes of wet AMD can be multifactorial, with both genetic and environmental influences, VEGF inhibitor injections are widely effective (though short-lived) treatments (155). Gene therapies to ameliorate neovascular AMD have focused on genetic expression of VEGF inhibitors to reduce the need for recurrent anti-VEGF injections. Multiple phase I and II trials have focused either on expressing the soluble fms-like tyrosine kinase 1 (sFLT-1) to reduce VEGF-stimulated vessel proliferation or expressing VEGF-targeting antibody fragments (20, 156). Oxford BioMedica recently published results from their phase I trial of a lentiviral EIAV vector called RetinoStat (a combination of angiostatin and endostatin proteins expressed by a single vector) and found that the vector was well tolerated, without vector-associated side effects. Retinostat treatment resulted in sustained expression of endostatin and angiostatin after long-term follow up (>4 years in 2 patients) (157). A study expected to end in 2027 will examine the long-term safety of RetinoStat (see Table 1).

Progress of disease-specific genome surgery

Currently, the most extensive work on genome surgery, or precise manipulation of an endogenous genetic locus, in the retina has been in CEP290 LCA or LCA10, as described below.

CEP290 LCA10. Characterized by poor visual function within the first year of life, extinguished ERG, and nystagmus, LCA10 is inherited in an autosomal recessive pattern as a result of mutations in the *CEP290* gene (158, 159). Mutations in *CEP290*, encoding a protein crucial for centrosome and cilia function, are present in 30% of all patients with LCA (158). Because of its large size (~8 kb), *CEP290* is beyond the packaging capability of AAV vectors, so gene replacement studies have

Table 1. Current gene therapies for retinal disorders

Disease	Intervention	NCT ID	Number of participants	End date	Sponsor
LCA2	Subretinal AAV2-hRPE65v2	NCT00516477[I]	12	2024	Spark Therapeutics
		NCT01208389[I,II]	12	2026	
		NCT00999609[III]	31	2029	
	Subretinal rAAV2/2.hRPE65	NCT00643747[I,II]	12	2014	University College, London
	Subretinal rAAV2-CB-hRPE65	NCT00749957[I,II]	12	2017	Applied Genetic Technologies Corp.
	Subretinal rAAV2/4.hRPE65	NCT01496040[I,II]	9	2014	Nantes University Hospital
	Subretinal AAV2/5 OPTIRPE65	NCT02781480[I]	27	2018	MeiraGTx UK II Ltd.
		NCT02946879[I,II]	27	2023	
	Subretinal rAAV2-CBSB-hRPE65	NCT00481546[I]	15	2026	University of Pennsylvania
Choroideremia	Subretinal rAAV2.REP1	NCT02077361[I,II]	6	2017	Ian M. MacDonald
	Subretinal AAV2-REP1	NCT02553135[II]	6	2021	Byron Lam
	Subretinal AAV2.REP1	NCT01461213[I,II]	14	2017	University of Oxford
		NCT02407678[II]	30	2021	
	Subretinal rAAV2.REP1	NCT02671539[II]	6	2018	STZ eyetrial
	Subretinal AAV2-hCHM	NCT02341807[I,II]	15	2019	Spark Therapeutics
Achromatopsia	Subretinal rAAV2tYF-PR1.7-hCNGB3	NCT02599922[I,II]	24	2022	Applied Genetic Technologies Corp.
	Subretinal rAAV.hCNGA3	NCT02610582[I,II]	9	2017	STZ eyetrial
	Subretinal AAV2/8-hCARp.hCNGB3	NCT03001310[I,II]	18	2019	MeiraGTx UK II Ltd
X-linked retinoschisis	Intravitreal AAV8-scRS/IRBPhRS	NCT02317887[I,II]	24	2021	National Eye Institute
	Intravitreal rAAV2tYF-CB-hRS1	NCT02416622[I,II]	27	2022	Applied Genetic Technologies Corp.
RP	Intravitreal RST-001	NCT02556736[I,II]	21	2033	Allergan
	Subretinal rAAV2-VMD2-hMERTK	NCT01482195[I]	6	2023	Fowzan Alkuraya
	Subretinal rAAV2tYF-GRK1-RPGR	NCT03316560[I,II]	15	2024	Applied Genetic Technologies Corp.
	Subretinal AAV2/5-hPDE6B	NCT03328130[I,II]	12	2022	Horama S.A.
	Subretinal AAV2/5-hRKp.RPGR	NCT03252847[I,II]	36	2020	MeiraGTx UK II Ltd.
	Subretinal AAV-RPGR	NCT03116113[I,II]	24	2019	Nightstar Therapeutics
Usher syndrome	Subretinal EIAV-CMV-MYO7A (UshStat)	NCT01505062[I,II]	18	2020	Sanofi
		NCT02065011[I,II]	18	2035	
Stargardt disease	Subretinal SAR422459	NCT01736592[I,II]	46	2034	Sanofi
		NCT01367444[I,II]	46	2019	
Neovascular AMD	Subretinal rAAV.sFlt-1	NCT01494805[I,II]	40	2017	Lions Eye Institute
	Intravitreal AAV2-sFLT0	NCT01024998[I]	19	2018	Genzyme
	Subretinal RetinoStat	NCT01301443[I]	21	2015	Oxford BioMedica
		NCT01678872[I]	21	2027	
LHON	Intravitreal GS010, sham-control	NCT02652767[III]	26	2019	GenSight Biologics
		NCT03293524[III]	90	2021	
		NCT02652780[III]	36	2019	
	Intravitreal rAAV2-ND4	NCT01267422[I]	9	2015	Bin Li, Huazhong University of Science and Technology
		NCT03153293[II,III]	40	2019	
	Intravitreal scAAV2-P1ND4v2	NCT02161380[I]	27	2019	John Guy

NCT ID, National Clinical Trial identifier. The trial phase is indicated by superscript I, II, II, and/or III.

focused on lentiviral delivery of native genes, with success in in vitro and in vivo models of LCA10 (160). Novel approaches using a functional truncated version small enough for an AAV2/8 vector, a so-called miniCEP290, proved effective at significantly improving photoreceptor survival after subretinal injection in the *Cep290rd16* mouse model (which harbors a deletion of exons 35 to 39 in the *Cep290* gene) of human LCA10 (161).

With LCA10, many scientists have turned their attention toward CRISPR-Cas systems to precisely edit the most frequent *CEP290* mutation, also called the IVS26 mutation, which creates a de novo splice donor site (c.2991 + 1655A > G) (162). This splice

site can be removed using SpCas9 and a pair of gRNAs flanking the novel splice site. After nonhomologous end-joining repair of the cleavage site, this cryptic splice donor can be deleted and normal mRNA processing restored (162). Ruan et al. showed that genome surgery, as described above, was effective in the mouse retina, in addition to demonstrating a method for limiting SpCas9 expression to decrease the propensity for an immune response to the bacterial protein, involving cleavage of the SpCas9 plasmid itself (162). Editas Medicine, a CRISPR-Cas–focused biotechnology company in Cambridge, Massachusetts, has announced a similar genome surgery approach for LCA10 called EDIT-101, an

AAV5 vector encoding SaCas9 with two gRNAs flanking the IVS26 mutation, allowing it to be excised (163).

Conclusions and future perspectives

Current developments in gene therapy have been compared to monoclonal antibody development two decades ago, with the basic science and preclinical successes preceding a barrage of clinical trials and inevitably powerful therapies. Precision medicine for genetic disorders will continue to improve as we develop the ability to target patient-specific mutations more precisely, with improved directed gene delivery and more exacting genome surgeries. One active area of research is the development of viral vectors with more precise cell-type–targeting capabilities via the directed evolution of AAV capsids. Several groups have generated libraries of AAV capsid proteins, applied them to animal models, and examined their transduction in a cell-type–specific manner (164, 165). Deverman et al. used such a method to develop an intravenously injected AAV vector capable of transducing the mouse brain 40-fold better than could be achieved with standard vectors (166). Directed evolution is most powerfully replicated in the animal or system in which the selection was applied; hence, for AAV vectors with human retinal cell-type–specific tropism, a closer recapitulation of the human eye may be required, such as that afforded by primate models or human iPSC–derived optic cups (167).

The importance of selecting appropriate transgene promoters was observed early on in canine retinal gene delivery experiments when scientists observed species-specific rod-cone promoter expression (168). Promoter selection has prompted groups to design cell-specific promoters appropriate for AAV vectors, such the development by Ye et al. of a shorter, more primate-specific L-opsin promoter to drive *CNGB3* expression for achromatopsia (169) In addition to complementation of genetic defects, the delivery of therapeutics to change the transcriptional and metabolic state of cells has been shown to prevent disease-related degeneration. Zhang et al. showed that a small hairpin RNA downregulating the histone deacetylase repressor of glycolysis *Sirt6* could rescue rod cells in *PDE6* RP (170). Using RNA-silencing or transcriptional repression approaches to modulate metabolic flux could be a useful adjunct therapy or monotherapy in genetic mutations recalcitrant to gene therapy. In fact, strategies combining AAV gene delivery to edit or complement mutated genes with agents to alter metabolic and transcriptional activity could provide synergistic effects to inhibit apoptotic pathways and stall retinal cell degeneration.

The timing of intervention is a crucial consideration for gene therapy in the retina, as many conditions result in progressive and irreparable destruction of the retinal architecture. For patients with late-stage disease, it is possible that replacing or editing native genes may not rescue vision, and more aggressive approaches may be required, such as the use of optogenetic tools or the delivery of light-sensitive proteins (171). While many optogenetic approaches have focused on microbial channelrhodopsins and halorhodopsins, the use of mammalian rhodopsin and melanopsins have restored visual behavior in blind *rd1* mice (172, 173). Moreover, Allergan is sponsoring a phase I/II trial of an intravitreally delivered channelrhodospin-2–based optogenetic therapy, RST-100, in patients with advanced RP (174). Further developments in clinical application for other causative mutation retinal dystrophies are highly anticipated, as patients suffering from blinding inherited eye disease may gain options for previously untreatable conditions (175–179).

Acknowledgments

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