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Gene Therapy and Stem Cell Transplantation in Retinal Disease: The New Frontier

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Abstract

Gene and cell therapies have the potential to prevent, halt, or reverse diseases of the retina in patients with currently incurable blinding conditions. Over the past 2 decades, major advances in our understanding of the pathobiologic basis of retinal diseases, coupled with growth of gene transfer and cell transplantation biotechnologies, have created optimism that previously blinding retinal conditions may be treatable. It is now possible to deliver cloned genes safely and stably to specific retinal cell types in humans. Preliminary results testing gene augmentation strategies in human recessive diseases suggest promising safety and efficacy profiles, including improved visual function outcomes over extended periods. Additional gene-based strategies under development include approaches to autosomal dominant disease (“gain of function”), attempts to deliver genes encoding therapeutic proteins with proven mechanisms of action interfering with specific disease pathways, and approaches that could be used to render retinal cells other than atrophied photoreceptors light sensitive. In the programs that are the furthest along—pivotal regulatory safety and efficacy trials studying individuals with retinal degeneration resulting from *RPE65* mutations—initial results reveal a robust safety profile and clinically significant improvements in visual function, thereby making this program a frontrunner for the first approved gene therapy product in the United States. Similar to gene therapy, progress in regenerative or stem cell-based transplantation strategies has been substantial. It is now possible to deliver safely

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stem cell–derived, terminally differentiated, biologically and genetically defined retinal pigment epithelium (RPE) to the diseased human eye. Although demonstration of clinical efficacy is still well behind the gene therapy field, multiple programs investigating regenerative strategies in RPE disease are beginning to enroll subjects, and initial results suggest possible signs of efficacy. Stem cells capable of becoming other retinal cell types, such as photoreceptors, are on the cusp of clinical trials. Stem cell–derived transplants can be delivered to precise target locations in the eye, and their ability to ameliorate, reverse, regenerate, or neuroprotect against disease processes can be assessed. Results from these studies will provide foundational knowledge that may lead to clinically significant therapies for currently untreatable retinal disease.

Photoreceptors are specialized neuronal cells that convert light energy into electrical signals. This activity requires a complex interaction of enzymes and substrates, nutrients, and energy sources, many of which are provided by retinal pigment epithelium (RPE) cells. Further activities occur in a highly oxygenated environment. Consequently, photoreceptors seem to be particularly susceptible to metabolic, environmental, or genetic alterations within the retina. Not surprisingly, therefore, photoreceptor compromise and loss is the most common end-stage cause of irreversible blindness in the developed world. This article briefly reviews current strategies under development using gene- and cell-based therapies aiming to treat diseases affecting photoreceptors, either by genetic correction, by introduction of modifier genes, or by cell augmentation or replacement.

Before examination of the scientific concepts, it is useful first to see how gene and cell therapy may be applied in different diseases, because there is considerable overlap in the 2 approaches. In age-related macular degeneration (AMD), for instance, it may be possible to alter the microenvironment using gene therapy either to block angiogenesis or to inhibit the alternative complement pathway. Similarly, early improvement of RPE function with stem cell–sourced transplantation may modify the microenvironment sufficiently to the point of altering disease activity. As soon as the outer retina has undergone degeneration, cell replacement likely would be needed to restore function. In the end stages of AMD, it may be necessary to replace or regenerate not just RPE cell line, but also the underlying choriocapillaris and overlying photoreceptors, because all 3 tissues are involved pathologically in the disease process. In contrast to multifactorial conditions like AMD, retinitis pigmentosa (RP) presents a relatively straightforward paradigm early in the course of disease because single gene replacement theoretically can prevent or stall retinal degeneration, and typically only 1 cell type, the photoreceptor, is affected, particularly in recessive diseases.¹ In a subset of inherited retinal degenerations that includes certain forms of RP, the deficient gene may be in the underlying RPE, such as occurs in some forms of Leber congenital amaurosis and in choroideremia (Fig 1). In these diseases, gene replacement to the RPE before the onset of photoreceptor loss may be the ideal approach. Given the interdependent nature of the photoreceptor, RPE, and choriocapillaris complex, virtually any late-stage retinal disease process, whether monogenic, cell specific, or multifactorial, ultimately leads to loss of all 3 tissues. Thus, cell replacement or regeneration may be necessary for patients in whom end-stage retinal degeneration has occurred.² Hence, gene and cell therapies should be considered as overlapping approaches that may or may not correlate to the early and late stages of the disease, respectively. Note also that approaches

combining gene and cell therapies, such as ex vivo strategies, make sense in certain circumstances and already are being studied for a number of nonocular conditions.³ These also may be applicable to retinal disease as expertise evolves.

Recent transformative breakthroughs have yielded the capacity to modify viral vectors and transgene promoters such that gene therapies selectively target specific retinal cell types, such as rods, cones, RPE, ganglion cells, and others.^{4,5} Current studies use a variety of delivery strategies such as submacular delivery of therapeutic material, placing the vector in direct contact with the target cell layers. Future studies may develop next-generation vectors evolved with precise tropism to target specific cell types and the ability to cross the internal limiting membrane and to penetrate the neurosensory retina.⁶ Similarly, novel promoters may yield improved expression efficiency in specific cell types. Alternatively, it may be possible to regulate promoter activity externally.

Regenerative or cellular therapies face similar delivery challenges as well as additional hurdles to success. Stem cells or stem cell–derived differentiated tissue must survive surgical transplantation and achieve biologically viable anatomic characteristics to replace diseased tissue effectively. For example, RPE in suspension has been transplanted safely into the subretinal space,⁷ but this tissue must survive surgical delivery, engraft onto Bruch's membrane, integrate into the native RPE layer, polarize, and avoid immune rejection as prerequisites of function (Fig 2). Alternatively, sheets of RPE grown on a variety of artificial Bruch's membrane substrates may obviate the challenge of polarity, but also may increase the risk of surgical delivery via a larger macular retinotomy. Stem cell–derived photoreceptors or neurosensory cells face the additional challenge of achieving viable intercellular connectivity or synapses. Stem cells transplanted to alter the microenvironment via secretory factors may face challenges related to proliferative vitreoretinopathy or uveitis, and thus may require very selective surgical delivery into the subretinal, sub-RPE, or suprachoroidal space as well as the need for immunosuppression. Studies addressing each of these issues are planned or currently are enrolling patients.

Gene Replacement Therapy

Early gene replacement studies to treat single gene retinal degeneration in humans began using an adeno-associated viral vector serotype 2 (AAV2) to target the RPE in the treatment of Leber congenital amaurosis and other retinal dystrophies caused by recessive mutations in the *RPE65* gene (Fig 1). Several studies using adeno-associated virus (AAV) vectors that contained different versions of the ubiquitous chicken β actin promoter have shown a high degree of safety and sustained, significant improvements of retinal function and vision in many cases.^{8–10} One group reported diminution in efficacy after 3 years, although levels of visual function remained significantly higher than at baseline.¹¹ Numerous variables differed between the various trials, including differences in vector design, doses and volumes of vector, excipient injection devices, surgical techniques, and different retinal locations, making identification of the variables affecting the durability of benefit difficult. Further study likely will help to delineate the relative impact these variables have on durability of effect. One of the programs in progress is the first randomized controlled phase 3 or pivotal regulatory gene therapy trial for any human eye disease. The first set of results from the

phase 3 studies reveals robust improvements in functional vision (in this case, the ability to navigate accurately and quickly in dim light; see Fig 3 for example from the preceding phase 1/2 study) as well as a high degree of safety, thereby placing this program as the frontrunner for the first approved gene therapy drug in the United States.¹²

More recently, a clinical trial using a similar approach with an AAV2 vector has reported promising results in the treatment of choroideremia.¹³ This trial also uses the chicken β actin promoter in the AAV2 vector and also has shown excellent safety with subretinal surgical delivery of transgene via AAV and sustained long-term improvements in visual acuity in an otherwise blinding condition.¹⁴ Another clinical trial involving AAV2 and a cell-specific promoter, VMD2, tested intervention in RP resulting from *MerTK* mutations. Ghazi et al¹⁵ reported a high degree of safety and visual acuity improvement in a subset of subjects. Other clinical trials that have recruited patients, but have not yet reported results, include AAV gene therapy for X-linked retinoschisis (clinicaltrials.gov identifiers, NCT02317887 and NCT02416622) and achromatopsia (clinicaltrials.gov identifier, NCT02610582). Additional trials use AAV vectors to test treatment for a mitochondrial disorder, Leber hereditary optic neuropathy (clinicaltrials.gov identifiers, NCT02161380, NCT01267422, and NCT02064569). Patients and families affected by these diseases and physicians caring for them should be made aware of the ongoing clinical trials.

Dominantly inherited diseases may pose more complex challenges for gene therapy approaches. In some cases, it may be necessary to reduce expression of the mutant allele, now theoretically possible with gene editing strategies such as using a micro-RNA silencing sequence targeting mRNA from both mutant and wild-type rhodopsin alleles, while simultaneously introducing a codon optimized rhodopsin mRNA coding sequence in the AAV vector.¹⁶ Because each amino acid may have up to 4 or even 6 different codons for the transfer RNA, codon optimization (changing the codon sequences, but retaining the same amino acid) can be used to alter the mRNA without affecting the canonical protein sequence. In other instances, simply overexpressing the wild-type gene to a sufficiently high level may have a beneficial effect in treating dominantly inherited mutations.¹⁷ For example, certain retinal diseases in which a dominantly inherited gene is operating through a mechanism of haploinsufficiency may be amenable to gene augmentation ameliorating pathologic features. In particular, haploinsufficiency may be the mechanism of RP caused by mutations in splicing factors,^{18,19} raising the possibility of near-term gene replacement for these conditions.

Another challenge relates to delivering genes that are larger than the payload capacity of AAV vectors. Currently, the maximum transgene size is approximately 5 kb in the most optimal scenario, but gene replacement also requires including additional elements such as a promoter and polyA signal to regulate gene expression, thus reducing the space for therapeutic transgene to approximately 3 kb. Alternative approaches may include using 2 AAV vectors encoding for different parts of large transgene in a given ratio as part of a combination product that would depend on the parts recombining to form the large target protein after infection. Reduced efficiency is one of many challenges facing this approach.²⁰ Lentivirus, a different viral vector, can deliver genes up to 9 to 10 kb.²¹ Both AAV-mediated recombination or lentiviral delivery approaches may allow replacement of a large transgene

for Stargardt disease (ABCA4, 7.3 kb) or Usher syndrome type 1B (MYO7A, 7.4 kb). However, lentiviral vectors raise additional safety, regulatory, and efficacy challenges. Recombinant lentivirus integrates into the human genome, and therefore, its use is accompanied by safety concerns and higher regulatory hurdles. In contrast, recombinant AAVs do not integrate into the genome, but rather form high-molecular-weight concatemers in the cytoplasm of the infected cell. This, together with the large and growing body of human safety data with respect to AAV, obviates concerns about germ-line integration and mutagenicity. Furthermore, successful transduction of adult rodent photoreceptors with lentiviral vectors is poor, which has hindered demonstration of potential efficacy in laboratory studies to date, although there is evidence of improved efficacy in primates.²² However, for RP caused by mutations in genes associated with even longer mRNA transcripts, such as EYS (10.5 kb) and USH2A (18.9 kb), the option for viral vector-based gene therapy is limited at present, although there may be scope using nonviral gene therapy approaches.²³ Recent development of RNA-guided genome editing methods used to carry out genome editing in vivo may make correction of specific mutations in otherwise untreatable genetic conditions possible through recombinant viral vector-mediated delivery of the relevant targeting and editing molecules.²⁴

Therapeutic Gene Expression

In addition to replacing deficiencies in wild-type genes through gene augmentation, gene therapy also may be used for the sustained expression of genes encoding therapeutic proteins. Potential benefits of this strategy as compared with monthly anti-vascular endothelial growth factor (VEGF) injections include steady-state pharmacokinetics. This theoretically avoids systemic safety issues experienced at peak levels when systemic exposure is most likely and limits disease progression at trough levels because the bolus of drug decays to less than therapeutic levels toward the end of dosing intervals. Further advantages of gene therapy for this purpose may include avoiding the pitfalls of compliance and treatment burden in that the treatment may need to be administered on only 1 occasion or very infrequently. Potential disadvantages include the variability of gene therapy in terms of transgene expression, secretion, and diffusion such that dosing and delivery location must be studied carefully. Future developments with inducible promoters may help to regulate transgene expression more precisely and could be relevant in the case of AMD or diabetic retinopathy, when the requirement for VEGF blockade may decline over time. A recent AAV gene therapy clinical trial has shown robust safety and possible signs of efficacy in exudative or wet AMD through the subretinal expression of s-flt1, which encodes a naturally occurring, soluble protein that binds to VEGF with the same mechanism as aflibercept (Rakoczy et al., 2015).²⁵

For the treatment of RP, ex vivo gene therapy has been used to deliver ciliary neurotrophic factor from genetically modified cells in an encapsulated cell-based device in a clinical trial that showed mixed early results.²⁶ Nevertheless, potentially lifelong prevention of RP with preservation of visual function may be achievable if the correct dose can be identified.²⁷ A broad neuroprotection strategy potentially would be of use in any retinal degeneration that cannot be treated by gene replacement, potentially even in cases in which the causative gene cannot be identified or the inciting event is not genetically based.^{28,29} Strategies with which

to provide metabolic support to the diseased photoreceptors³⁰ also may be useful in prolonging visual function in a diverse set of diseases.

Gene therapy also is being explored for its ability to render secondary neurons in the retina light sensitive after photoreceptors have degenerated. The strategy for optogenetic therapy is to deliver genes encoding light-sensitive channels to remaining cells in the retina.³¹ One study, delivering the channelrhodopsin-2 gene to ganglion cells, is in an early phase clinical trial (clinicaltrials.gov identifier, NCT02556736). Such an approach would not be expected to lead to high-resolution vision, but hypothetically could be sufficient to allow independent navigation.

Cell-Based Therapies for Retinal Pigment Epithelium Replacement

When considering cell-based therapies for the treatment of retinal disease, it is important to compare the different approaches of replacing the RPE or photoreceptors. Although the functions of the RPE are myriad and include maintenance of the outer blood–retinal barrier, participation in the visual cycle, and support of surrounding tissue, RPE replacement has the advantage of not requiring reconnection of functional synapses. Retinal pigment epithelium transplantation arguably is one of the most well-studied cell therapies in terms of clinical trials to date. Early studies have used autologous cells harvested from patients (not stem cell derived), either as cell suspensions³² or as full-thickness grafts, including the underlying choriocapillaris, that in some cases restored vision up to 20/30.^{33,34}

These early trials were pioneering, but took place in an age when there were no anti-VEGF treatments available for wet AMD. Now it would be difficult to justify intervening to replace RPE when relatively safe and effective approved treatments exist for these patients. Hence, surgical research into autologous RPE transplantation for wet AMD has slowed, but still may be applicable in cases that are known not to respond to anti-VEGF, for instance in the presence of massive subretinal hemorrhage or RPE tears or in atrophic AMD.

Atrophic or dry AMD remains untreatable to date, and the hypothesis that stem cell–derived RPE transplants may be a viable approach has been explored in humans. The first successfully safe stem cell–derived transplant in humans for retinal disease was reported in the *Lancet*.⁷ In this study, patients with advanced atrophic AMD and advanced Stargardt disease (*ABCA4* mutation) were immunosuppressed systemically for 1 week before and 3 months after surgery. A suspension of differentiated but immature RPE cells derived from non-abortion-sourced human embryonic stem cells was delivered surgically into the subretinal space in a 150-ml bleb. The bleb straddled a transition zone comprising the outer border of the atrophic lesion, compromised of viable tissue adjacent to the atrophic lesion, and more peripheral normal tissue with the hope that treatment of the peripheral area would prevent recapitulation of the disease first presenting in the central macula. Ocular safety was excellent. Systemic safety issues observed were largely the result of immunosuppression. Importantly, neither tumor-associated proliferation nor significant rejection-related inflammation were observed. Potential indicators of functional improvement were observed in a number of patients; however, the trial was not placebo controlled and a direct anatomic correlate of the functional gains is still being sought. Nonetheless, the first safe human stem

cell-derived transplantation of any retinal tissue has initiated a study that it is hoped will lead to a cascade of safe and effective treatments applying regenerative medicine to ophthalmology.

With regard to the role of Bruch's membrane, Stargardt disease also was included as a target condition in these trials to answer questions about the ability of senescent Bruch's membrane to accept and support transplanted RPE. The genetic basis for Stargardt disease resides in the photoreceptor, but the RPE becomes diseased through a secondary process. As a result, Bruch's membrane is thought to be less senescent in Stargardt disease than in atrophic AMD. Thus, key knowledge may be gained by studying both Stargardt disease and atrophic AMD using the same suspension of stem cell-derived RPE transplants. One question that remains is whether Bruch's membrane needs to be augmented biologically for RPE transplantation to be successful. Another approach to this issue is addressed by an ongoing clinical trial that includes implanting a synthetic polymer substrate for Bruch's membrane in addition to the RPE cell monolayer ([clinicaltrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT01691261) identifier, NCT01691261). This strategy may have other advantages such as guaranteed polarity and delivery of more mature and confluent RPE. However, surgical delivery is more challenging because of a need for a larger retinotomy and the potential safety issues surrounding the polymer.

Induced pluripotent stem cells (iPSCs) are similar to embryonic stem cells, but are derived by dedifferentiating fully differentiated adult somatic cells, such as from skin or blood, into stem cells and then redifferentiating them into target cell lines.³⁵ This has theoretical ethical advantages because there is no need for a human embryonic cell donor. Furthermore, transforming the iPSCs is not without risk, and the biology of transformation is not well understood. The transcription factors used may induce cells with malignant features.³⁶ A clinical trial transplanting autologous RPE cells generated from a patient through iPSCs started in 2014,³⁷ but was put on hold in early 2015. Although not directly related to stopping the trial, risks of malignant transformation had been identified in control cells from the second patient.

Other issues, such as purity, unwanted differentiation, and the fact that iPSC tissue isolated from each patient would still carry the donor's disease-causing mutations, are still being worked out. Begging the questions of safety and efficacy, the notion that major diseases like atrophic AMD may be addressable by iPSC-sourced tissue from individual patients also raises the issue of scalability. Generating a cell line that is edited genetically such that the disease-carrying mutations are removed and then scaling that personalized cell line up for therapeutic use in an individual patient may be beyond current commercial and regulatory capacities. Nevertheless, some form of cell replacement arguably would be needed for retinal degenerations in which all photoreceptors, RPE cells, or both are lost, such as end-stage RP and AMD. Here, a regenerative medicine approach to reconstruct the outer retina, for instance, using iPSC-derived retinal cells, may be more appropriate.

Cell-Based Therapies for Photoreceptor Replacement

Photoreceptor transplantation is not only an important potential strategy that may be useful across broad-spectrum blinding retinal conditions, but also, if successful, may represent the first transplanted stem cell-derived neuron. As a neuron, the photoreceptor does not divide. In contrast to most other neurons, however, the photoreceptor makes only 1 synaptic connection. Its position in the subretinal space connects it directly to the appropriate bipolar or horizontal cell that normally would receive and transmit stimuli into the retinal circuitry. Hence, there is no requirement for lengthy navigation of axon terminals to make appropriate connections. Furthermore, the retina is relatively devoid of myelin proteins, known inhibitors of neuronal regeneration elsewhere in the central nervous system. As mentioned previously, the subretinal space is accessible surgically for the transplantation of cells, often with minimal trauma to the surrounding tissues. Although the concept of photoreceptor transplantation is daunting, the biological barriers that need to be overcome are considerably lower than would be the case for transplantation of possibly any other neuron.

Initial investigations examined transplanting photoreceptor sheets or dissociated cells into the subretinal space of *rd* mice, which have a retinal degeneration similar to human RP.^{38,39} Subsequent clinical trials also explored the use of whole retinal explants taken from embryonic tissue in RP patients.⁴⁰ Photoreceptor transplantation also has been used as a cell suspension to regenerate a functional outer nuclear layer in *rd* mice.⁴¹ Since that time, major advances in surgery, stem cell biology, and photoreceptor biology have occurred. To date, however, stem cell-sourced photoreceptor transplantation in humans has not yet been reported, although clinical trials are underway. Encouraging evidence from electronic retinal implants placed in the subretinal space suggests that patients with no light perception and end-stage RP can regain the capacity to see with the appropriate light-sensing stimulus applied to the residual retinal circuitry. In certain conditions, such as RP, isolated photoreceptor loss and minimal trans-synaptic degeneration render these eyes attractive targets for stem cell-derived photoreceptor transplantation. Whether mature photoreceptors or terminally differentiated progenitors are transplanted, and whether these transplants are performed as cellular suspensions or tissue sheets or with some form of biocompatible support matrix, is as yet unknown. Also unknown are details of many other variables important for targeting different disease stages and states and also variables that could affect success (such as immunosuppression). What is apparent, however, is that we have reached an era of biologic plausibility. Thus, it seems likely that we will achieve safe and effective regenerative stem cell-based therapies for neurodegenerative photoreceptor diseases. It is just a question of time.

Summary

Translating fundamental biologic knowledge into viable, safe, and effective treatments represents a substantial amount of work. Each gene and cell therapy approach discussed herein rests on an enormous foundation of basic and translational research contributions from a large number of investigators, institutions, and now, patients. Together, the technological advances in gene and cell therapy, the robust safety and hopeful efficacy data from early phase human studies, and promising pivotal registration data from phase 3 trials

suggest that some of these approaches, which seemed like science fiction just 2 decades ago, are a foreseeable, near-term reality. The first approved gene and cell therapies will herald efforts applying the early successes and evolving strategies to treat other diseases. Major as-yet unanswered questions include issues such as ethical, social, and economic considerations; continued basic science and its translation; clinical optimization of treatment strategies; and the combination of gene and cell therapies. Clearly, many challenges remain to be negotiated, but there has never been as much promise for the development of new strategies to prevent, treat, reverse, and dare we say, cure some forms of retinal blindness.

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Abbreviations and Acronyms

AAV	adeno-associated virus
AAV2	adeno-associated viral vector serotype 2
AMD	age-related macular degeneration
ipsc	induced pluripotent stem cell
RP	retinitis pigmentosa
RPE	retinal pigment epithelium
VEGF	vascular endothelial growth factor.

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Figure 1.

Different gene therapy strategies and imaging parameters in Leber congenital amaurosis resulting from *RPE65* mutations and choroideremia. **A**, Fundus photograph showing a young patient with recessive *RPE65* disease who has had poor vision since birth. **B**, Autofluorescence (AF) image showing a lack of results, which is a hallmark of the disease and is presumed to be the result of the slowing of the visual cycle. **C**, Fundus photograph showing the typical appearance in choroideremia (CHM) with atrophy of the choroid peripherally and only a small area of central retina remaining (*white arrows*) in a patient who had excellent visual acuity earlier in life. **D**, Autofluorescence (AF) image in CHM delineating the corresponding central viable zone of retinal pigment epithelium (*white arrows*). In *RPE65* disease, there is often peripheral retina remaining that has poor function; the visual acuity is limited by cortical amblyopia. Hence, successful gene therapy may be manifest as improved visual field, but not necessarily improved visual acuity. In CHM, anatomic degeneration means that the peripheral field cannot be restored because there is no retina remaining. However, gene therapy may result in improved function on the fluorescent island, which includes visual acuity in cases where it has been compromised.

Figure 2.

Fundus images of eyes with pigmentation after transplantation with human embryonic stem cell (hESC) retinal pigment epithelium (RPE). **A–C**, Color fundus photographs and spectral-domain (SD) optical coherence tomography (OCT) images of an eye from a patient with age-related macular degeneration (*dotted circle* shows an outline of the transplanted area) obtained at **(A)** baseline, **(B)** 3 months, and **(C)** 6 months. Note the presence of a pigmented patch of transplanted cells (**B** and **C**, *arrows*) that becomes larger and more pigmented by 6 months. Optical coherence tomography images (inset) show the presence of cells on the

inner aspects of Bruch's membrane at 6 months compared with baseline. **D–F**, Color fundus photographs and SD OCT images of an eye from a patient with Stargardt macular dystrophy (dotted circle shows an outline of the transplanted area) obtained at (**D**) baseline, (**E**) 6 months, and (**F**) 1 year. Note the absence of pigment in the preoperative photograph (**D**). Patches of pigmented cells are evident around the border of baseline atrophy in RPE (**E**) that become more prominent at 1 year (**F**, *arrows*). Spectral-domain OCT images obtained at (**D**) baseline and (**E**) 6 months show increasing pigmentation is at the level of the RPE, normal monolayer RPE engraftment, and survival at 6 months (**E**, *arrows*) adjacent to a region of bare Bruch's membrane devoid of native RPE. **G–I**, Color fundus photographs of a patient with Stargardt macular dystrophy (*dotted circle* shows an outline of the transplanted area). **G**, A large central area of atrophy is visible on the preoperative photograph. An area of transplanted RPE cells is visible at the superior half of the atrophic lesion at (**H**) 6 months that becomes larger and more pigmented at (**I**) 15 months.

Figure 3.

Photographs documenting the navigational abilities of one subject before and after gene therapy in a phase 1/2 follow-on gene therapy clinical trial for Leber congenital amaurosis resulting from *RPE65* mutations. Frames from videos of the subject navigating an obstacle course under dim light are shown. The performance is graded on the accuracy and speed with which the subject traverses the course under different levels of illumination. Shown are examples of the subject's performance using the same level of dim (1 lux) illumination (**A–D**) before and (**E–H**) 3 years after gene delivery.⁴² Before intervention, the subject collides with obstacles (**A, B**) and goes off course repeatedly (**C, D**), whereas after intervention, the subject avoids collisions, steps over obstacles in his path (**F**), and finds the end of the course (the door, **H**) quickly and accurately.