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**NEWS AND COMMENTARY**

# Gene therapy approaches for prevention of retinal degeneration in Usher syndrome

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The accessible and immune-privileged environment of the eye makes it ideal for gene therapy. It has been 8 years since successful gene therapy was first reported for patients with an inherited form of progressive retinal degeneration (RD), Leber Congenital Amaurosis (LCA2), following treatment in one eye with AAV2-*RPE65*.[1–3] Now, Bennett *et al.*[4] have reported improved vision without adverse effects, such as immunogenicity, following treatment of the second eye with AAV2-*RPE65*, in children with LCA2. These results strongly support the use of *in situ* gene therapy in patients who can be treated prior to the onset of RD.

Paramount to this approach is the early identification of patients, before the retina has undergone any irreversible changes. While most forms of RD are detected only after significant retinal pathogenesis, Usher syndrome types 1 and 2 present congenital deafness that facilitates genetic identification of the disease in infancy; Usher 1 patients are born profoundly deaf and Usher 2 patients are insensitive to high frequencies.[5] Nowadays, the deafness can be treated with cochlear implants, and genetic testing of deaf infants is used to identify Usher syndrome and thus predict ensuing RD.

The first retinal gene therapy studies with Usher genes were carried out using lentiviral (LV) delivery of *MYO7A*, the gene responsible for Usher syndrome 1B (USH1B). MYO7A is present in both the photoreceptor and retinal pigment epithelial (RPE) cells[6] (Figure 1a). Injection of LV-*MYO7A* into the subretinal space of *Myo7a*-deficient mice was found to correct mutant phenotypes in both these cell types.[7] A phase I/II clinical trial, using LV-*MYO7A* to treat RD in USH1B, has been under way since 2012 (https://clinicaltrials.gov/ct2/show/NCT01505062).

Because the *MYO7A* coding sequence is 6.7 kb, it was thought that a viral vector such as LV was needed for delivery, since it features a larger carrying capacity than the reported maximum of 5 kb for adeno-associated virus (AAV). However, more recently, it was found that use of AAV, including AAV2, as used in the LCA2 treatments, mentioned above, resulted in WT levels of MYO7A and correction of retinal phenotypes in mutant mice.[8–10]

A significant body of research has now demonstrated that oversized AAV genomes can be packaged into high titer AAV as 5` truncated sense and anti-sense genomes, termed fragmented vectors (fAAV). The truncated genomes are efficiently reassembled, with high fidelity, into the full transgene product following transduction of target cells, as a result of recombination that is biased towards homologous recombination (HR) rather than non-homologous end joining (NHEJ)[11–13] (Figure 1b). Oversized gene replacement therapy, using fAAV expressing *MYO7A* cDNA, not only demonstrated reconstitution of the intact transgene product *in vivo* but also reported more reliable phenotypic correction of the underlying mutation than a dual AAV vector expression system.[9] Similar success was also reported for fAAV expressing a 7.5 kb dysferlin transgene.[14] Recently, an optimized dual vector system was shown to be comparable to fAAV vectors in a mouse model of Stargardt's RD.[15] There is an ongoing debate over which AAV system is the best for delivery of large transgenes.[10,14,16] However, it is likely that many factors, including the transgene sequence and the epigenetic and transcriptional state of the target tissue will influence the success of each approach.

Despite the promise of gene augmentation therapy for genetic RDs, this approach is not amenable for a gene whose functional cDNA is very large, or which expresses multiple essential isoforms. All the Usher genes have been reported to express multiple isoforms, although their relative importance in the human retina is unknown (Table 1). The expression of two major isoforms of *MYO7A* in the human retina[17] is a concern for the current USH1B clinical trial, which is using the single cDNA that was generated in the original mouse studies.[7] This isoform corrects mouse retinal phenotypes,[7,9] but the relative isoform expression may differ between mouse and human retinas.

Direct targeting of genetic mutations can overcome these limitations. Recently, antisense oligonucleotides (ASOs) were used to correct a splice-site mutation in *CEP290*, a large gene defective in another form of LCA, and whose protein, like most of the Usher proteins, functions in the photoreceptor cilium.[18] Similarly, ASOs were used to target a cryptic splice site in the orthologue of *USH1C*, thereby rescuing hearing and vestibular functions in a mouse model for USH1C.[19] ASOs are limited, however, to diseases amenable to repair by blocking translation or a specific splice site.

Other mutations can potentially be repaired by gene editing strategies, including the clustered, regularly interspaced, palindromic repeats (CRISPR)-associated (Cas) system, which has revolutionized the field of genomic engineering since its introduction.[20] Adapted from the microbial immune system, this technology uses a short guide RNA to target the Cas endonuclease to a specific locus in the genome. The Cas endonuclease can then generate double-stranded breaks in the DNA, which can be repaired by one of the two mechanisms: (1) NHEJ, an error-prone process that often results in insertions or deletions, or (2) homology-directed repair (HDR), which requires a repair template to introduce modifications to the targeted genetic locus.[21] NHEJ occurs at a higher frequency, while HDR is more suitable for repairing mutations. Recent RD studies

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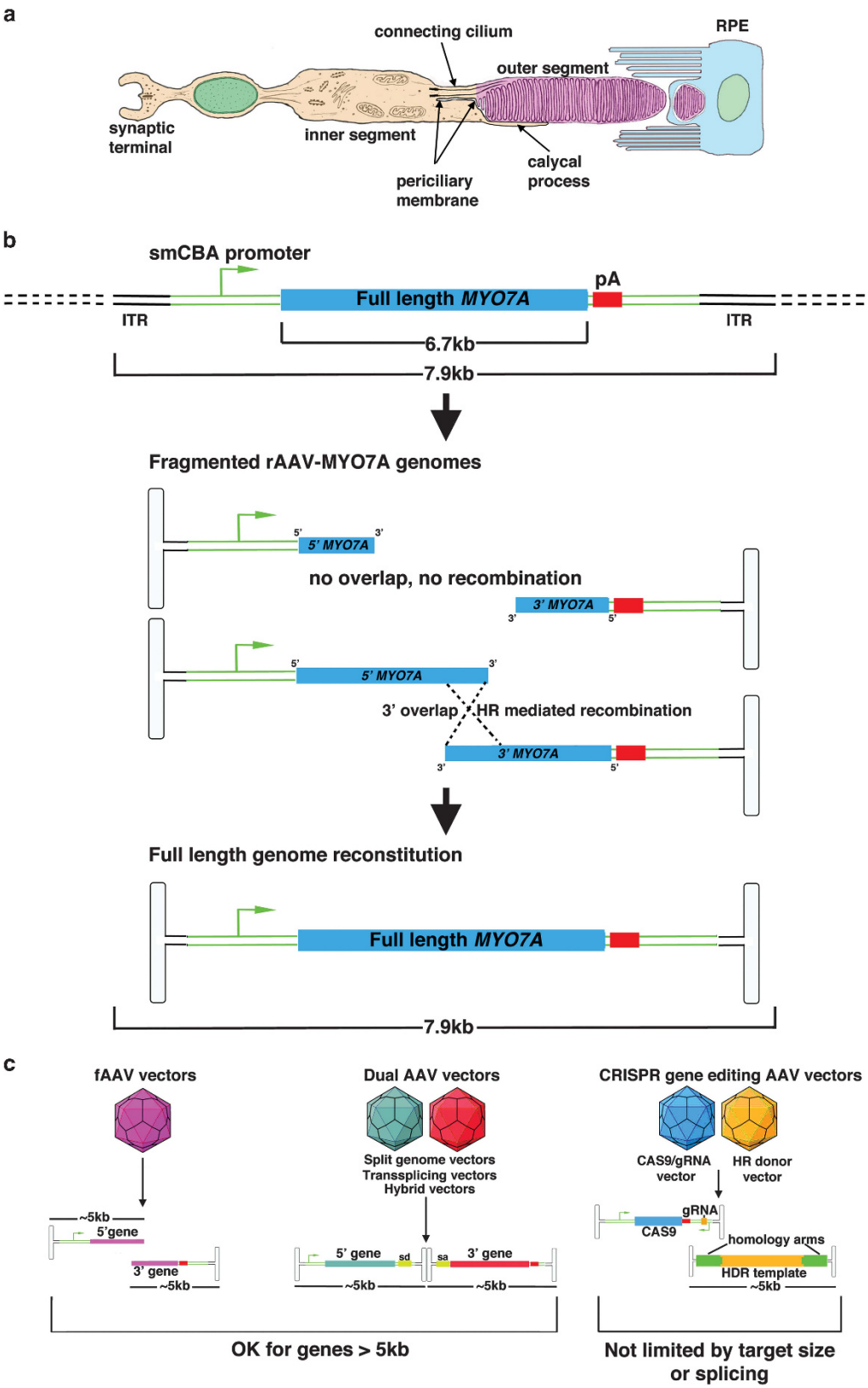


**Figure 1.** Retinal gene therapy strategies for genes defective in Usher syndrome. (**a**) Diagram of a photoreceptor cell and an RPE cell. Most Usher proteins are associated with the connecting cilium or periciliary membrane. Usher 1 proteins are also present in calycal processes. However, the Usher 1B protein, MYO7A, is most abundant in the RPE. (**b**) Schematic illustrating an overview of large genome fragmentation and subsequent full-length genome reconstitution for the AAV2-*MYO7A* fAAV vector. (**c**) Examples of AAV vector-mediated approaches for gene therapy, resulting from defects in large, very large or alternatively spliced genes (ITR, inverted terminal repeat; pA, polyadenylation signal; smCBA, small chicken beta-actin promoter; SD, splice donor; SA, splice acceptor).

**Table 1.** Usher syndrome genes that are large or alternatively spliced

| *USH Subtype* | *Gene* | *Number of isoforms* | *Length (amino acids)* | *Reference* |
|---|---|---|---|---|
| USH1B | *MYO7A* | 2[a] | 2175, 2215 | 17 |
| USH1C | *USH1C* | 3 classes[a,b] | Up to 899 | 32 |
| USH1D | *CDH23* | 3 classes[c,b] | Up to 3354 | 33 |
| USH1F | *PCDH15* | 3 classes[a,b] | Up to 1955 | 34 |
| USH1G | *SANS* | 2[c] | 358, 461 | |
| USH1J | *CIB2* | 3[c] | Up to 210 | 35 |
| USH2A | *USH2A* | 2[a] | Up to 5202 | 36 |
| USH2C | *GPR98* | 3[a] | Up to 6306 | 37 |
| USH2D | *WHRN* | 3[c] | Up to 907 | 38 |
| USH3A | *CLRN1* | 11[a] | Up to 245 | 39 |

[a]Expressed in the human retina. [b]Each class represents multiple isoforms. [c]Total number expressed in human; the number in the retina is not known.

demonstrated correction of *rd1* via CRISPR-mediated HDR of mutant mouse zygotes,[22] and allele-specific ablation of a dominant mutant allele of *Rho* by CRISPR-mediated NHEJ with neonatal mice.[23]

Viral delivery of CRISPR-Cas components offers high transduction efficiency, limited only by the size of the donor HDR template with respect to viral capacity (see above). HDR is currently also limited by its low efficiency, especially in post-mitotic cells.[24] Nonetheless, the observation that an oversized AAV genome is regenerated *in vivo* by photoreceptor and RPE cells[9,10] indicates the presence of HDR in these cell types,[11,13,25] and corroborates previous work, demonstrating HDR in developed adult photoreceptors.[26]

One strategy to help overcome limitations of HDR is to take advantage of different classes of CRISPR-Cas systems. For example, unlike the commonly used Cas9, the endonuclease Cpf1 generates staggered cuts with 5` overhangs.[27] The resulting cleavage could mediate the insertion of a DNA fragment to correct a mutation by NHEJ, the more dominant repair mechanism. Another strategy is to use homology-independent targeted integration (HITI), whereby the nuclease cuts both donor and genomic DNA, resulting in ligation of the donor fragment into a genomic locus using NHEJ. This approach has been demonstrated very recently in a study that included *in vivo* gene editing of the Mertk gene, which is expressed in the RPE and is essential for ingestion of the photoreceptor outer segment disks by the RPE. The HITI-edited gene resulted in morphological and physiological repair that was more significant than that obtained using an HDR-based approach.[28]

Novel innovations in the field of gene editing will provide opportunities to optimize gene repair for RDs, however, a genetic model with well-characterized cellular phenotypes would be useful to test and optimize the efficiency of gene editing. Among RDs, the cellular phenotypes resulting from loss of MYO7A in mutant mouse retinas have been particularly well characterized. These phenotypes, such as melanosome localization in the RPE[29] and opsin concentration in the proximal photoreceptor cilium,[30] can be scored on a cell-by-cell basis,[9,31] thus giving a direct readout of efficiency, making them potentially useful in optimization studies.

In conclusion, because patients with Usher syndrome are typically identified before RD begins, they are particularly suitable for gene therapy approaches. Preclinical tests are needed to determine if only one isoform is essential to prevent RD; in this case, all but 2 or 3 of the largest genes would appear suitable for augmentation by AAV, fAAV or dual AAV vector delivery of a single cDNA. Subtypes that are associated with a very large gene (USH2A and 2C) or more than one essential retinal isoform represent appropriate candidates for testing AAV vectors in the context of new gene-editing strategies (Table 1; Figure 1c).

## CONFLICT OF INTEREST

The authors declare no conflict of interest.

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