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Review Article

Recent preclinical and clinical advances in gene therapy for hereditary hearing loss

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ABSTRACT

Hereditary hearing loss is a genetically heterogeneous condition that affects millions of people worldwide and has limited curative treatment options. Recent advancements in gene therapy have opened promising avenues for correcting the underlying genetic defects in the inner ear. This review summarizes the key developments in vector platforms, delivery strategies, target genes, preclinical models, and clinical trials relevant to both gene supplementation and gene editing approaches, as well as future directions. Adeno-associated virus vectors have emerged as the leading platform for inner ear gene transfer, owing to their safety and efficacy. Clinical programs, such as those targeting *OTOF* variants, are currently underway and are supported by robust preclinical data. Additionally, genome editing technologies, including CRISPR/Cas9-mediated nonhomologous end joining, base editing, and prime editing, offer variant-specific therapeutic potential. Despite these advances, challenges remain in expanding the therapeutic window, ensuring long-term safety, and establishing ethical and regulatory frameworks for their use.

INTRODUCTION

According to the World Health Organization, hearing loss is one of the most prevalent sensory disorders worldwide, affecting approximately 466 million people, including 34 million children. Among the various etiologies, genetic factors account for more than 50% of congenital cases and 30% to 40% of postlingual cases of hearing loss (Jang et al., 2024). These inherited forms of deafness can be syndromic or nonsyndromic, and over 150 genes have been implicated in the pathogenesis of auditory dysfunction (hereditary hearing loss homepage: https://hereditaryhearingloss.org/). Despite the heterogeneity of causative variants, most forms of sensorineural hearing loss result from irreversible damage or dysfunction of cochlear hair cells or associated structures in the scala media.

Currently available treatments, including hearing aids and cochlear implants, provide notable auditory (re)habilitation, but do not restore native cochlear function or prevent progressive degeneration. Therefore, curative approaches are urgently needed to address these underlying genetic defects. In this context, gene therapy, which involves the delivery of functional genetic material to correct or compensate for mutant alleles, has emerged as a promising strategy for the treatment of hereditary hearing loss.

Recent advances in viral vector development, inner ear delivery techniques, and animal modeling have enabled successful proof-of-concept studies targeting various deafness genes such as *Otof*, *Gjb2*, and *Kcnq4* (Akil et al., 2019; Noh et al., 2022; Sun et al., 2025). In particular, adeno-associated virus (AAV) vectors have demonstrated robust transduction efficiency and safety in cochlear hair cells, making them suitable candidates for clinical translation (Landegger et al., 2017). Notably, early-phase clinical trials have begun to evaluate gene therapy in patients with autosomal-recessive forms of hearing loss, marking a milestone in this field (Lv et al., 2024; Qi et al., 2025; Wang et al., 2024).

In this review, we briefly outline general strategies for inner ear gene therapy and then focus on vector platforms, engineering, and delivery strategies applied to inner ear gene therapy. We also discuss recent advances in preclinical and clinical gene therapy for hereditary hearing loss and the associated translational challenges of these strategies, while

highlighting emerging genome editing technologies and their potential for personalized variant-specific interventions.

MECHANISMS OF HEREDITARY HEARING LOSS AND GENERAL STRATEGIES FOR INNER EAR GENE THERAPY

The cochlea is a complex sensory organ composed of diverse and heterogeneous cell types, each playing a distinct role in maintaining normal hearing. These cells are involved in a wide range of biological functions, including mechanical support, ion transport and recycling, mechano-electrical transduction, synaptic transmission, and innate immune defense. Based on their functional characteristics, cochlear cells implicated in hereditary hearing loss can be broadly categorized into 4 groups: sensory epithelium, nonsensory epithelium, extracellular matrix–associated cells, and neuronal structures (Fig. 1) (Jang et al., 2024; Jiang et al., 2023; Petit et al., 2023). Understanding the expression patterns of deafness-associated genes is critical for the success of inner ear gene therapy, as these patterns inform the selection of the most appropriate delivery strategies.

SENSORY EPITHELIUM

The sensory epithelium is the primary site of mechano-electrical transduction, where sound waves are converted into electrical signals. It is mainly composed of hair cells and supporting cells. Hair cells are further categorized into either inner hair cells or outer hair cells. Inner hair cells transmit auditory signals to type I spiral ganglion neurons via ribbon synapses, while outer hair cells amplify mechanical sound waves through electromotility, serving as active frequency filters. Supporting cells, which comprise a heterogeneous population including inner phalangeal/border cells, pillar cells, Deiter's cells, and Hensen's cells, provide structural support to the sensory epithelium and contribute to potassium recycling within cochlear microenvironment. Numerous genes associated with hereditary hearing loss are predominantly expressed in sensory epithelial cells. Representative genes include *PCDH15*, *CDH23*, *USH1C*, *USH2A*, *MYO7A*, *MYO15A*, *WHRN*, *TMPRSS3*, *MYO6*, and *TMC1* in hair cells; *OTOF* and *SLC17A8* in inner hair cells; *KCNQ4*, *STRC*, and *SLC26A5* in outer hair cells; *GJB2*, *GJB6*, *MPZL2*, and *TMPRSS3* in supporting cells.

NONSENSORY EPITHELIUM

Nonsensory epithelial cells play a critical role in potassium recycling, which is essential for maintaining the endocochlear potential necessary for auditory transduction. Additionally, they contribute to the structural integrity of the cochlear epithelium. The main cell types within the nonsensory epithelium include inner sulcus cells, outer sulcus cells, root and spindle cells from the spiral prominence, and marginal, intermediate, and basal cells from the stria vascularis. Several genes implicated in hereditary hearing loss are expressed in these cell populations, including *GJB2*, *GJB6*, *SLC26A4*, *KCNQ1*, *KCNE1*, *KCNJ10*, and *CLDN11*.

EXTRACELLULAR MATRIX

The cochlear extracellular matrix comprised key structural components, including spiral ligament, spiral limbus, and tectorial membrane. These structures provide tissue scaffolding and contribute to immune defense by furnishing space for innate immune cells, such as cochlear-resident macrophages. Furthermore, tectorial membrane is a key structure involved in mechano-electrical transduction of hair cells. Hearing loss genes primarily expressed in the extracellular matrix include *TECTA* and *COCH*.

NEURONAL STRUCTURES

Spiral ganglion neurons can be categorized into type I and type II: type I spiral ganglion neurons receive auditory input from inner hair cells and transmit signals to the central nervous system. They can be further subdivided into type IA, IB, and IC based on molecular and physiological characteristics. The function of type II neurons remains less well-understood, but they innervate outer hair cells and are thought to play a role in nociception (Liu et al., 2015). Spiral ganglion neurons are crucial in the progression of hereditary hearing loss, as hair cell

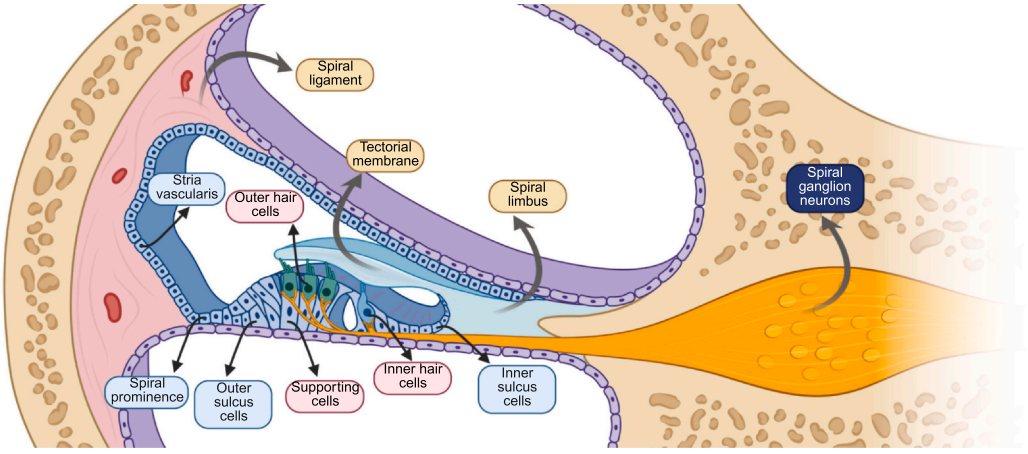

Fig. 1. Major inner ear cell types involved in hereditary hearing loss. Inner hair cells, outer hair cells, and supporting cells comprise the sensory epithelium. Inner sulcus cells, outer sulcus cells, spiral prominence, and stria vascularis make up the nonsensory epithelium, while spiral ligament, spiral limbus, and tectorial membrane constitute extracellular matrix–associated structures.

degeneration often leads to secondary degeneration of these neurons. Furthermore, certain deafness-associated genes, such as *TMPRSS3* (Du et al., 2023) and *PJVK* (Lu et al., 2022), have been implicated in the survival and maintenance of spiral ganglion neurons.

For effective inner ear gene therapy, therapeutic strategies should be tailored to the specific pathogenic mechanism underlying each form of hereditary hearing loss. In autosomal-recessive hearing loss, the majority cases result from biallelic loss-of-function variants, which are amenable to gene supplementation approach. Furthermore, variant-specific gene correction strategies, such as base editing and prime editing, can be employed to restore gene function in these cases. Deep intronic variants that interfere with normal mRNA splicing can be targeted using splicing-modulating antisense oligonucleotides (ASOs), and exon-skipping ASOs have been actively developed to bypass exons harboring pathogenic truncating or frameshift variants.

In contrast, autosomal-dominant hearing loss is typically caused by 1 of 3 mechanisms: haploinsufficiency, dominant-negative effects, and gain-of-function effects. For haploinsufficiency, both gene supplementation and precise gene correction strategies are considered appropriate. However, in cases involving dominant-negative or gain-of-function variants, gene supplementation is generally ineffective. Instead, targeted suppression or correction of the mutant allele—using CRISPR-Cas9-based allele disruption via nonhomologous end joining, base editing or prime editing, or RNase-H1-dependent ASOs—is considered a more appropriate therapeutic strategy.

VECTOR PLATFORMS AND ENGINEERING FOR INNER EAR GENE THERAPY

Efficient and safe delivery of therapeutic genes into the cochlear sensory epithelium is a fundamental prerequisite for successful gene therapy for hearing loss. Several vector platforms, including viral and non–viral delivery systems, have been explored for inner ear applications. Among these, AAV vectors have emerged as the gold standard owing to their favorable safety profile, stable transgene expression, and ability to transduce nondividing cells (Hudry and Vandenberghe, 2019).

The efficacy of AAV-mediated gene delivery is highly dependent on the serotype used, because different capsids exhibit variable tropisms for cochlear cell types (Table 1). Early studies have demonstrated that AAV2/1, AAV2/2, and AAV2/8 can transduce cochlear inner hair cells with modest efficiency (Stone et al., 2005). More recently, synthetic or evolved AAV variants such as Anc80L65, AAV9-PHP.B, and AAV9-PHP.eB have demonstrated enhanced transduction efficiency in hair cells—particularly in outer hair cells, which exhibit very low tropism for AAV2/1, AAV2/2, and AAV2/8—with minimal immunogenicity (György et al., 2019; Hu et al., 2019; Landegger et al., 2017). Another variant, AAV-ie, engineered by inserting a membrane-penetrating peptide into AAV-DJ, has demonstrated superior transduction capabilities in various cochlear structures. This includes both inner and outer hair cells, as well as supporting cells, specifically Deiter's and Hensen's cells, which AAV-DJ cannot efficiently target, and the lateral wall (Sun et al., 2025; Tan et al., 2019). Furthermore, a recent study demonstrated that AAV8BP2 and AAV8 are efficient options for targeting the cochlear lateral wall and endolymphatic sac, thereby expanding the AAV serotype toolkit for inner ear gene therapy.

Capsid engineering through rational design and directed evolution has been extensively applied to overcome limitations, such as narrow cell tropism and immune barriers. For example, Cre recombination–based AAV-targeted evolution has facilitated the discovery of AAV variants optimized for inner ear delivery in vivo (Deverman et al., 2016). Similarly, DNA shuffling and peptide insertion methods yielded capsids with enhanced permeability across the round window membrane (RWM) and improved specificity for target cell populations within the cochlea (Dalkara et al., 2013). In parallel, self-complementary AAV vectors, which bypass the rate-limiting step of second-

Table 1. Major AAV serotypes used for inner ear gene delivery

Serotype	Target cells in the scala media	Efficiency	Characteristics and applications
AAV1	Inner hair cells, stria vascularis, and spiral ligament	Medium to high	Used in a recent *OTOF* clinical trial
AAV2	Inner hair cells, supporting cells	Medium	-
AAV8	Inner hair cells, stria vascularis, spiral ligament, and endolymphatic sac	Medium to high	Effective for targeting lateral wall segment and endolymphatic sac
AAV9	Inner hair cells, spiral ganglion neurons	Medium	-
Anc80L65	Hair cells, supporting cells, and spiral ganglion neurons	Very high	Effective for targeting both inner and outer hair cells; used in a recent *OTOF* clinical trial
PHP.B	Hair cells, spiral ganglion neurons	Very high	A derivative of AAV9; superior BBB penetration capacity; effective for targeting both inner and outer hair cells
PHP.eB	Hair cells, spiral ganglion neurons	Very high	Enhanced CNS tropism compared with PHP.B; effective for targeting both inner and outer hair cells
AAV-DJ	Supporting cells, stria vascularis, and spiral ligament	Medium to high	Versatile and effective across various cell types
AAV-ie	Hair cells, supporting cells, stria vascularis, and spiral ligament	Very high	A derivative of AAV-DJ generated by inserting cell-penetrating peptides; enhanced tropism for hair cells, Deiter's cells, and Hensen's cells; currently the most efficient serotype for simultaneously targeting various inner ear cell types

BBB, blood-brain barrier; CNS, central nervous system.

strand synthesis, provide a faster onset and higher levels of transgene expression, which are important features for targeting transient developmental windows in neonatal gene therapy (McCarty, 2008), and for minimizing toxicity by reducing the AAV dosage required to achieve therapeutic effects (Hahn et al., 2025).

Although AAV remains dominant, other viral vectors such as lentiviruses, adenoviruses, and helper-dependent adenoviruses have been investigated for inner ear delivery. For instance, lentiviral vectors integrate into the host genome and may support long-term gene expression in dividing progenitor cells; however, they exhibit low transduction efficiency and potential for insertional mutagenesis (Pietola et al., 2008). Nanoparticle-based and exosome-mediated delivery systems are also under development, offering promising nonviral alternatives with the potential for repeated dosing and large cargo capacities (Gao et al., 2018a).

One of the notable limitations of AAV as a vector platform is its restricted packaging capacity (maximum 4.8-5 kb). Currently, 2 strategies are commonly employed to deliver genetic materials that exceed the single AAV packaging limit.

The first is dual-AAV with intein-mediated protein recombination. In this strategy, the genetic material is divided into 2 halves, with N- and C-terminal intein motifs appended to facilitate the recombination of the 2 N- and C-terminal fragments at the protein level (Fig. 2A). For efficient recombination, a cysteine residue is required at the beginning of the C-terminal

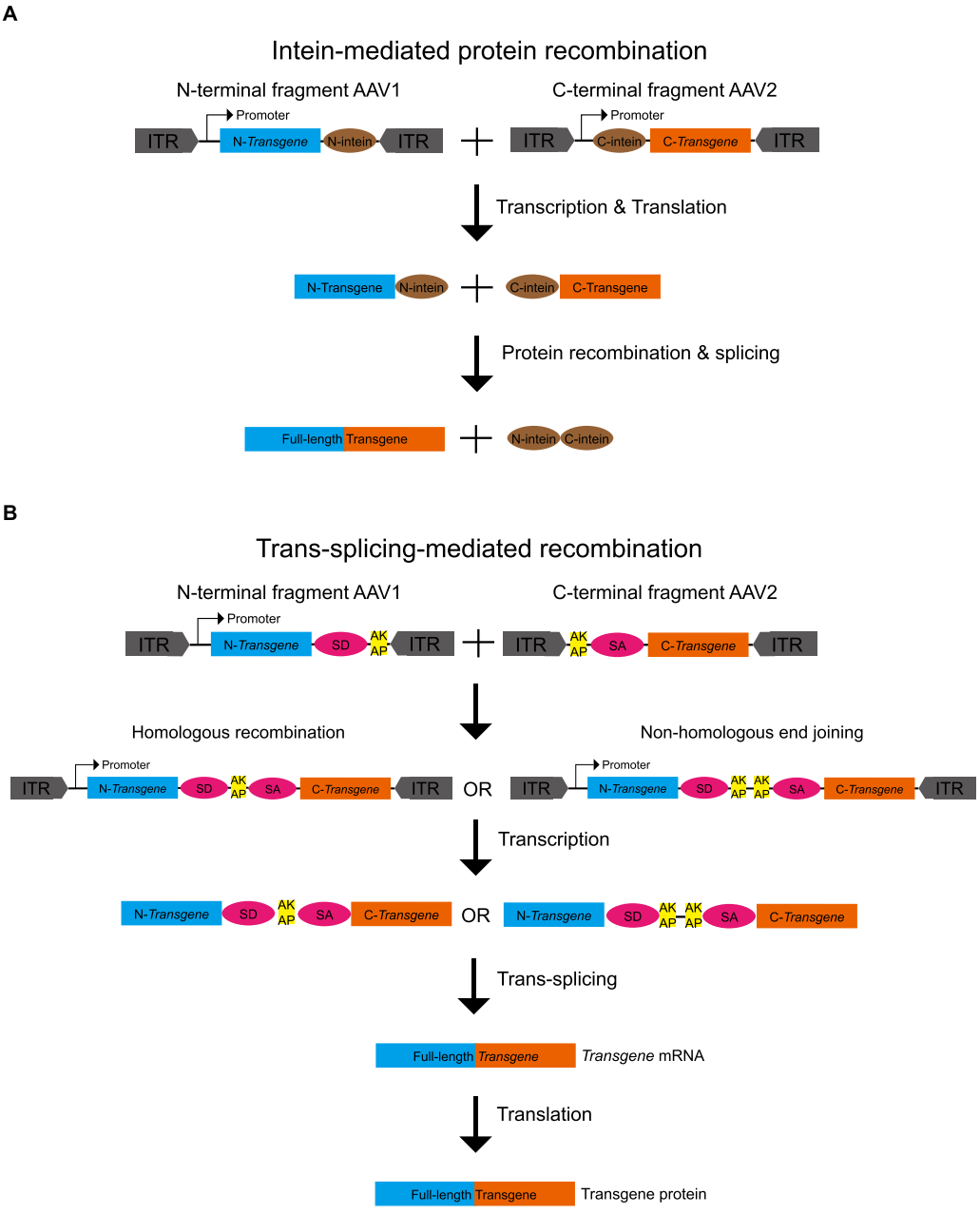

Fig. 2. Two common methods for delivering full-length transgenes using a dual-AAV system. (A) Intein-mediated protein recombination. (B) Trans-splicing-mediated recombination. ITR, inverted terminal repeats; SA, splice acceptor site; SD, splice donor site.

fragment. This strategy has been successfully applied to preclinical models of *OTOF*- and *STRC*-related hearing loss (Shubina-Oleinik et al., 2021; Tang et al., 2023). Furthermore, this approach is commonly used to deliver SpCas9 nucleases (Noh et al., 2022), base editors (Cui et al., 2025; Yeh et al., 2020), and prime editors that are size-compatible with dual-AAV delivery (Davis et al., 2024; Doman et al., 2023).

The second is dual-AAV with trans-splicing-mediated recombination. In this approach, the genetic material is divided into 2 halves with a splice donor (SD) and splice acceptor (SA) site appended, along with an additional homologous arm (eg, highly recombinogenic bridging sequences from human placental alkaline phosphatase [AP] or the filamentous F1 phage homology region [AK]) to facilitate homologous recombination of the AP or AK sequence and/or nonhomologous end joining of the inverted terminal repeats between the 2 fragments (Fig. 2B). Upon recombination, the transcribed mRNA undergoes trans-splicing to remove the SD and SA sites, thereby enabling the production of full-length mRNA and proteins. This strategy has been used in preclinical models of *OTOF*- and *PCDH15*-related hearing loss (Ivanchenko et al., 2024b; Qi et al., 2024b; Zhang et al., 2023) and in clinical applications in patients with autosomal-recessive deafness 9 (DFNB9) with *OTOF* variants (Lv et al., 2024; Qi et al., 2025; Wang et al., 2024). Recent reports on functional improvements following dual-AAV-*OTOF* gene therapy in patients with DFNB9 have highlighted the efficiency of this system in the human inner ear.

Although these approaches can be effective for genetic materials that fit within 2 AAVs, larger constructs exceeding 10 kb require at least 3 AAVs, and the efficient delivery of a triple AAV system to the inner ear has not been thoroughly investigated. Because several prevalent autosomal-recessive hearing loss genes, such as *MYO15A* and *CDH23*, have coding sequences larger than 10 kb, the development of novel strategies for efficient triple AAV delivery is imperative and will lay the foundation for treating hearing loss caused by loss-of-function variants in these genes.

To drive robust transgene expression using AAV, universal overexpression promoters, such as CMV and CAG, have traditionally been employed in AAV-based gene supplementation. However, several recent studies have reported ototoxicity attributable to the ectopic expression or overexpression-induced cytotoxicity of transgenes driven by these universal promoters (Aaron et al., 2023; Du et al., 2023; Sun et al., 2025). These findings have prompted the search for cell-type- and transgene-specific regulatory elements to precisely control gene expression in the desired cell types. Consistent with these efforts, current *OTOF* clinical trials have used the mouse *Myo15* promoter to restrict *OTOF* transgene expression in hair cells (Lv et al., 2024; Qi et al., 2025; Wang et al., 2024). Furthermore, to circumvent the toxicity caused by the ectopic expression of *Gjb2*, several studies have adopted *Gjb2*-specific regulatory elements or supporting cell-specific promoters to accurately confine expression to target cells (Ivanchenko et al., 2024a; Sun et al., 2025). Moreover, a recent study demonstrated a rationale-based strategy for identifying and engineering cell-type-specific enhancers based on chromatin accessibility, sequence motifs, and evolutionary conservation across multiple species (Zhao et al., 2025). To further advance this approach, integrated multiomics data (single-nucleus RNA sequencing combined with single-nucleus ATAC sequencing) of the mature cochlea are required to systematically identify and characterize cell-type-specific regulatory elements for diverse cell populations within the inner ear (Ben-Simon et al., 2025; Li et al., 2025).

Taken together, the development of inner ear–specific vector platforms and innovative engineering strategies has considerably advanced the translational potential of gene therapy for hereditary deafness. Continued refinement of capsid tropism, payload capacity, and transgene expression regulation is essential for safe and effective clinical application.

DELIVERY STRATEGIES FOR INNER EAR GENE THERAPY

The inner ear is a compact, fluid-filled, and anatomically secluded organ, which makes gene delivery challenging and uniquely accessible. Effective delivery strategies must ensure the safe and targeted administration of vectors to the cochlear sensory and neural tissues while avoiding iatrogenic injury. Over the past 2 decades, significant progress has been made in developing surgical approaches and minimally invasive techniques tailored to the inner ear anatomy (Fig. 3).

The most widely used method in preclinical and clinical settings is the injection of viral vectors through the RWM, which provides direct access to the scala tympani of the cochlea without disrupting the otic bony capsule. This approach allows for a high local concentration of the therapeutic agent and a relatively uniform distribution across cochlear turns, particularly in neonatal mouse models (Landegger et al., 2017). However, variability in membrane thickness, fibrosis, and vector leakage into the central nervous system through the cochlear aqueduct may affect transduction efficiency and safety (Han et al., 2024; Salt and Plontke, 2009). In adult mice and primates, including cynomolgus macaques and humans, canal or stapes footplate fenestration is often combined with RWM injection to minimize hydrostatic pressure–related injury and leakage into the cochlear aqueduct, both of which have been shown to be safe and effective for preserving preoperative hearing thresholds (Du et al., 2023; Lv et al., 2024; Omichi et al., 2020; Yoshimura et al., 2018; Zhang et al., 2023).

Cochleostomy, which involves a small fenestration in the otic capsule to access the scala media or scala tympani, enables deeper and more controlled delivery into the cochlea. Although this method can increase the area of transduction, it carries a higher risk of mechanical trauma, inflammation, and hearing threshold shifts (Géléoc and Holt, 2014). It is often used in larger animal models (eg, guinea pigs and nonhuman primates) where cochlear dimensions permit greater surgical manipulation (Géléoc and Holt, 2014).

Direct delivery to specific inner ear compartments, such as the scala media, has been achieved via canalostomy or direct endolymphatic injection, primarily in rodent models. These techniques allow the transduction of sensory hair cells and supporting cells with high specificity; however, they are technically demanding and pose a risk of iatrogenic hearing loss (Wu et al., 2021; Zhu et al., 2021).

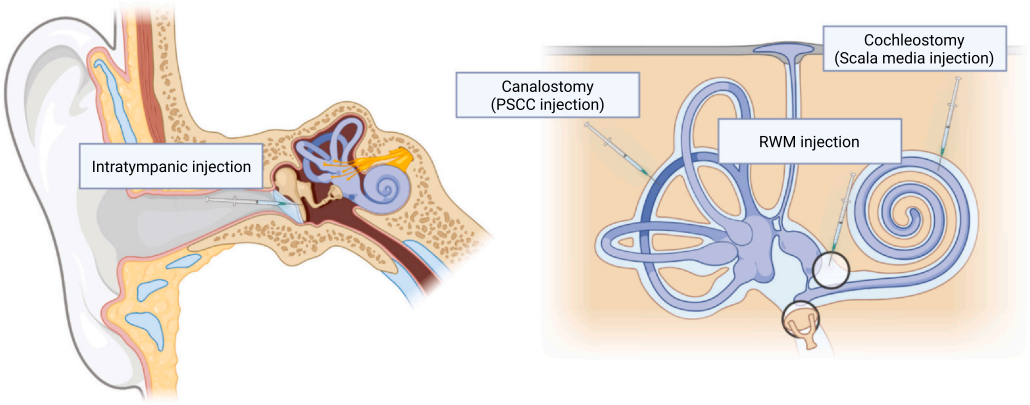

Fig. 3. Commonly used local delivery strategies for the inner ear. A schematic of the human ear depicting various methods for delivering therapeutics to the inner ear. These include indirect middle ear approaches such as intratympanic injection (left). Direct injection methods (right) target specific inner ear compartments, including cochleostomy into the scala media, posterior semicircular canal (PSCC) injection through canalostomy, and scala tympani delivery through the round window membrane (RWM) injection.

Recent studies have explored the systemic administration of vectors that cross the blood-labyrinth barrier. AAV2/9 and certain engineered AAV capsids, such as AAV9-PHP.B, can transduce inner ear tissues following intravenous injection in neonatal mice (Chan et al., 2017; Shibata et al., 2017). However, translation to humans remains uncertain owing to differences in blood-labyrinth barrier permeability, immune response profiles, and the accumulation of viral genomes in other major organs, including the liver and spleen.

Intratympanic injection, a clinically established route of drug delivery to the middle ear, has also been investigated for use in gene therapy. In this approach, a vector is applied to the middle ear cavity, from which it diffuses across the RWM and into the cochlea. Although minimally invasive, transduction efficiency is often limited by poor penetration, variable RWM permeability, and rapid clearance through the Eustachian tube (Staecker et al., 2001).

The timing of vector delivery is critical for effective gene therapy in the inner ear. A key developmental difference exists between preclinical mouse models and humans. Although the mouse inner ear continues to develop until postnatal day 10 to 14 (P10-14), inner ear development in humans is largely completed before birth (approximately gestational week 27). Many hereditary forms of hearing loss involve genes that are expressed during early cochlear development. Thus, P1 to 5 in mice is considered the optimal therapeutic window, particularly for targeting differentiating hair cells (Wang et al., 2018). However, this developmental period in mice corresponds to the in utero stage in humans, which makes clinical translation technically and ethically challenging. Moreover, the therapeutic benefits of inner ear gene supplementation gradually decline with age in various preclinical mouse models. Several factors may contribute to these findings. First, in mature cochleae, the cellular accessibility and transduction efficiency of AAV decrease, particularly in outer hair cells, emphasizing the need for improved capsids or delivery enhancers in adult therapeutic applications (Mendia et al., 2024). Second, in some preclinical models of hereditary hearing loss, sensory epithelium degeneration progresses so rapidly that therapeutic interventions beyond P14 are too late to prevent further deterioration. Therefore, to ensure successful clinical translation, therapeutic efficacy in mice should be demonstrated by interventions beyond P14, which is equivalent to early human infancy.

RECENT ADVANCES AND TARGET GENES FOR GENE SUPPLEMENTATION

Hereditary hearing loss is genetically heterogeneous, with over 150 genes implicated in its pathogenesis. Several genes have been prioritized as targets for gene supplementation therapy because of their well-characterized molecular functions, expression patterns within the inner ear, and availability of robust animal models. Successful preclinical studies targeting these genes have laid the foundation for translational efforts in gene supplementation, gene silencing, and genome editing strategies.

OTOF (Otoferlin)

OTOF encodes otoferlin, a calcium sensor that is critical for synaptic vesicle fusion at inner hair cell ribbon synapses. Biallelic variants in *OTOF* lead to DFNB9, a form of profound prelingual sensorineural hearing loss (Roux et al., 2006). The large cDNA sequence of the *OTOF* gene exceeds the packaging limit of a single AAV vector. To address this, dual-AAV strategies have been developed to deliver and reconstitute full-length otoferlin in cochlear hair cells, as discussed above (Akil et al., 2019; Al-Moyed et al., 2019; Qi et al., 2024b; Shubina-Oleinik et al., 2021; Tang et al., 2023; Zhang et al., 2023).

In *Otof*-knockout mice, both strategies (ie, intein-mediated recombination and trans-splicing-mediated recombination) have demonstrated the recovery of auditory brainstem response (ABR) thresholds, restoration of ribbon synapse formation, and sustained auditory improvements lasting weeks to months, even after interventions in adult mice. These findings validate dual-AAV gene therapy as a viable intervention for DFNB9.

Qi et al. (2024b) tested dual-AAV delivery in *Otof*-deficient mice and nonhuman primates. The treated mice exhibited restored ABRs and normal auditory behavior. No significant toxicity was observed in the nonhuman primates. Based on this success, a clinical trial of AAV-based *OTOF* gene therapy is

currently underway. Qi et al. (2024a) treated 2 children with AAV-*OTOF*. One patient regained normal hearing within 1 month, whereas the other exhibited notable improvements in speech perception. Lv et al. (2024) administered AAV1-*OTOF* unilaterally to 6 pediatric patients with DFNB9. Four patients demonstrated hearing gains of 40 to 57 dB and improved speech recognition. No dose-limiting toxicities were observed. Wang et al. (2024) subsequently administered bilateral AAV1-*OTOF* to 5 children. All patients exhibited bilateral hearing threshold recovery, improved sound localization, and better speech understanding in noisy environments, without serious adverse events. More recently, Qi et al. (2025) reported the results of Anc80L65-*OTOF* gene supplementation in patients with DFNB9 across a broad age range, from toddlerhood to adulthood. All patients showed improvements in hearing thresholds; however, the functional gains declined with age, with the most pronounced effect observed in 5- to 8-year-olds. The reason for the increased efficacy in this age group remains unclear. Notably, most hearing improvements occurred within 1 month after delivery, with additional but modest gains observed for up to 6 months after treatment. Furthermore, a second injection was administered to 1 patient 4 months after the first injection. This second injection did not trigger a systemic immune response or serious adverse events and led to modest hearing improvement, despite the presence of neutralizing antibodies against AAV in the systemic circulation from the initial dose.

Three major *OTOF* gene therapy trials, AK-OTOF (Akouos/Eli Lilly, https://clinicaltrials.gov/study/NCT05821959), DB-OTO (Regeneron, https://clinicaltrials.gov/study/NCT05788536), and SENS-501 (Sensorion), have advanced to Phase 1/2 clinical trials. Early results indicate ABR recovery and improved speech perception in pediatric patients. However, further evaluation is needed to determine the long-term durability of gene supplementation and to compare the auditory outcomes between AAV-*OTOF* gene supplementation and cochlear implantation.

GJB2 (Gap Junction Beta-2, Connexin 26)

Variants in the *GJB2* gene, which encodes connexin 26, lead to impaired gap junction function, which is critical for cochlear homeostasis, resulting in DFNB1A, the most prevalent genetic cause of profound congenital sensorineural hearing loss (Kelley et al., 1998). Gene therapy targeting *Gjb2* is more challenging because of its expression in the supporting cells and nonsensory epithelium rather than hair cells, and the lack of relevant animal models owing to the embryonic lethality of whole-body *Gjb2* knockout. Nonetheless, an initial study demonstrated the feasibility of gene therapy for DFNB1A using *Gjb2*-conditional knockout mice and AAV1-mediated *Gjb2* supplementation, which achieved modest hearing improvement following a single neonatal delivery (Iizuka et al., 2015). To advance these findings toward clinical application, recent studies have reported successful transduction and partial hearing recovery using a supporting cell-specific promoter (SCpro) in mouse models. Sun et al. (2025) employed a combined dual-AAV (AAV1 and AAV-ie) system to restore functional *Gjb2* expression driven by SCpro, effectively overcoming the ototoxicity caused by ectopic *Gjb2* expression in hair cells and restoring hearing in *Gjb2*-conditional knockout mice. In addition, the combined AAV system successfully transduced the supporting cells and nonsensory epithelium within the inner ear of Bama miniature pigs, and its administration into the inner ear of cynomolgus monkeys resulted in no hearing impairment and minimal systemic toxicity. These findings support the efficacy and safety of this gene supplementation approach in large-animal models. Similarly, Ivanchenko et al. (2024a) used AAV9-PHP.B with *Gjb2*-specific gene-regulatory elements identified through chromatin accessibility (ATAC-seq) analysis to specifically deliver and express *GJB2* in the supporting cells and nonsensory epithelium. The gene-regulatory elements-driven *GJB2* expression vector successfully achieved the desired cell-specific expression pattern and showed minimal systemic toxicity in nonhuman primates.

Sensorion, in collaboration with the Institut Pasteur, developed an AAV-based gene therapy named GJB2-GT, designed to target nonsensory cells of the cochlea that naturally express connexin 26. The preclinical evaluation involved a conditional *Gjb2* knockout mouse model ($Gjb2^{cKO1/cKO1}$). Upon administration of GJB2-GT via round window membrane injection, the treated animals exhibited significant auditory recovery at both 3- and 7-week post-treatment time points. GJB2-GT specifically transduced nonsensory cochlear cells without affecting the hair cells or inducing ototoxicity. Similar selective transduction and safety profiles have been confirmed in nonhuman primates, reinforcing the therapeutic potential and specificity of GJB2-GT in clinical applications. Currently, *GJB2* gene therapy remains in the preclinical stage. Sensorion is advancing toward clinical trials, targeting initiation by 2025, pending regulatory approval (IND/CTA).

RECENT ADVANCES IN GENE EDITING AND VARIANT-SPECIFIC APPROACHES

While gene supplementation has formed the foundation of most current gene therapy strategies for hereditary hearing loss, genome editing technologies offer a new frontier, particularly for dominant-negative/gain-of-function variants and the precise correction of pathogenic loss-of-function alleles. Unlike conventional gene supplementation approaches, genome editing enables variant-specific interventions, potentially restoring the normal functions of endogenous genes and preserving physiological gene regulation.

The most widely used genome editing system, CRISPR-Cas9, employs a guide RNA (gRNA) to direct the Cas9 endonuclease to a specific genomic locus, enabling targeted double-stranded DNA cleavage, which leads to permanent allele disruption. This system has been successfully applied to various preclinical models of autosomal-dominant hearing loss caused by dominant-negative variants. An initial study attempted to preferentially disrupt the *Tmc1* dominant-negative (*Tmc1* Beethoven [*Bth*]) allele, which resulted in significant hearing improvement in $Tmc1^{Bth/+}$ mice following neonatal injection (Gao et al., 2018b). Furthermore, allele-specific disruption of the dominant-negative *Kcnq4* p.W277S variant using CRISPR-Cas9 led to selective silencing of the mutant allele while preserving the wild-type allele and restoring auditory function in mice (Noh et al., 2022). Similarly, Tao et al. (2023) employed liposome-based delivery of SpCas9-sgRNA ribonucleoprotein complexes to treat hearing loss in a mouse model harboring a

heterozygous dominant-negative *Atp2b2* variant. They achieved mutant allele–specific disruption, improved hearing loss, and prevented hair cell degeneration after neonatal administration. However, because of double-stranded breaks induced by SpCas9, a large deletion (~1.7 kb in size) was observed in the mutant allele, raising potential safety concerns regarding clinical translation. Recently, Zhu et al. (2024) used AAV2-based delivery of a SaCas9-KKH-sgRNA construct to target a dominant-negative *miR-96* variant. Treatment of adult mutant mice led to significant improvements in hearing thresholds and hair cell survival. However, low-level integration of the AAV vector genome into the *miR-96* target site (~0.26%) was detected in the treated mice, and this integration frequency increased with higher viral titers in vitro. These findings raise concerns regarding the potential for insertional mutagenesis at the double-stranded break site and underscore the importance of careful dose optimization to balance editing efficiency with genomic integrity.

Base editors, including cytosine base editors and adenine base editors, enable single-nucleotide conversions, specifically from cytosine to thymine or adenine to guanine, without causing double-stranded breaks or relying on homology-directed repair, which is inefficient in postmitotic cells such as hair cells. These systems show promise in correcting transition variants, which represent a significant proportion of deafness-related point mutations (Cui et al., 2025; Yeh et al., 2020). The strength of base editing lies in its potential to address a wide range of pathogenic mechanisms, including loss-of-function, dominant-negative, and gain-of-function variants caused by single transitional changes. However, a current limitation of base editors is the occurrence of bystander edits, owing to their relatively wide editing windows. Although various engineered versions of base editors have been developed to minimize bystander editing and confine the editing window, careful evaluation of the sequence context surrounding the target variant remains essential to avoid unintended missense mutations resulting from bystander editing, which could compromise the desired therapeutic effects (Lee et al., 2025). Recently, prime editing has emerged as a next-generation tool that enables all types of base substitutions, insertions, and deletions without inducing double-stranded breaks (Anzalone et al., 2019). Although it has not yet been tested in cochlear systems, ongoing advances in prime editing efficiency and reduction in construct size suggest that this technology could enable the precise correction of small indels and all types of single-nucleotide changes commonly found in human deafness alleles in the near future.

ASOs are a variant-specific approach. These short synthetic nucleotides can modulate pre-mRNA splicing (steric-blocking ASO) or degrade target transcripts by recruiting RNase-H1 (RNase-H1-dependent ASO). Most studies in the inner ear have investigated the therapeutic potential of steric-blocking ASOs. In a pioneering study, intraperitoneal injection of a steric-blocking ASO (ASO-29) targeting the *USH1C* c.216G > A variant, which creates a cryptic SD site, restored correct splicing and improved auditory and vestibular functions in a mouse model of type I Usher syndrome (Lentz et al., 2013). Building on this work, subsequent studies have explored various therapeutic time windows (from in utero to adulthood) (Depreux et al., 2016; Ponnath et al., 2018; Wang et al., 2020) and alternative delivery methods (ranging from topical tympanic application to RWM injection) (Lentz et al., 2020) using ASO-29 in a mouse model of type I Usher syndrome. In contrast, despite widespread use of RNase-H1-dependent ASOs in various central nervous system disorders to target dominant-negative or gain-of-function variants, their therapeutic potential in the inner ear has been less explored than that of steric-blocking ASOs. Notably, we recently demonstrated that an RNase-H1-dependent ASO (ASO-123) preferentially knocked down the *Kcnq4* p.W277S dominant-negative allele over the wild-type allele in the mouse inner ear, thereby mitigating progressive hearing loss in a DFNA2 mouse model while enhancing the survival and electrophysiologic function of outer hair cells (Jang et al., 2025). This study highlights the therapeutic value of ASOs in targeting dominant-negative or gain-of-function variants primarily affecting hair cells. ASO therapies also provide safety advantages owing to their temporary effects at the mRNA level, unlike the permanent DNA changes induced by gene editing approaches. However, because of its transient nature, repeated administration is required to maintain therapeutic concentrations in the target tissues. Therefore, for the successful clinical translation of ASO-based strategies to the inner ear, the development of reliable and minimally invasive delivery methods to the cochlea remains a critical area of research.

SAFETY AND REGULATORY CONSIDERATIONS

The transition of gene therapy for hereditary hearing loss from preclinical studies to human trials has accelerated in recent years, owing to the success of AAV-based therapies in the fields of ophthalmology and neurology. Several clinical trials are currently underway or planned to evaluate the safety, feasibility, and efficacy of gene therapy for monogenic forms of deafness.

Safety is of paramount importance in gene therapy, particularly in the inner ear, where irreversible damage to sensory cells can result in permanent hearing loss due to the lack of hair cell regeneration in mammals. Major safety concerns include the following:

- The immunogenicity of vector capsids and transgene products, which can induce inflammation or off-target immune responses. Recent clinical data indicate that AAV can enter the systemic circulation and lead to the formation of neutralizing antibodies even after local delivery to the inner ear (Qi et al., 2024a, 2025). Therefore, systemic immune responses should be meticulously assessed when considering repeated AAV administration.
- Dose-dependent toxicity, particularly with high-titer AAVs known to cause hepatotoxicity and dorsal root ganglion damage during systemic delivery (Hinderer et al., 2018). Furthermore, cytotoxicity resulting from ectopic or uncontrolled overexpression of transgene products should also be evaluated.
- Insertional mutagenesis associated with the integration of the vector genome when delivering the Cas9-nuclease that creates double-stranded breaks.
- Large structural variants at on-target loci, particularly with Cas9-nuclease-based approaches, in which double-stranded breaks are continuously formed over a prolonged

period owing to AAV-based delivery. These structural variants can activate the p53-mediated DNA damage response, leading to cellular apoptosis (Haapaniemi et al., 2018; Kosicki et al., 2018; Shin et al., 2017). To mitigate the potential side effects associated with the long-term expression of genome editing tools, transient delivery strategies such as lipid nanoparticle–based mRNA delivery or engineered virus-like particle-based ribonucleoprotein delivery may offer a safer alternative for genome editing.

- Off-target effects of genome editing, particularly in CRISPR-based systems, may induce unintended DNA modifications at nontarget loci.

Preclinical safety studies using optimized AAV vectors in rodents and nonhuman primates have not demonstrated significant ototoxicity when administered appropriately (Qi et al., 2024b). However, the long-term surveillance of cochlear function, immune responses, and potentially delayed effects is critical in human trials.

Gene therapy products are regulated under the Advanced Therapy Medicinal Product guidelines of the European Union and the Food and Drug Administration Center for Biologics Evaluation and Research in the United States. In South Korea, the Ministry of Food and Drug Safety serves as the primary regulatory authority overseeing the development of gene therapy products under the Act on Safety and Support for Advanced Regenerative Medicine and Advanced Biopharmaceuticals (commonly known as the Advanced Regenerative Bio Act). Developers must provide comprehensive chemistry, manufacturing, and controls documentation, preclinical biodistribution and toxicology data, and long-term monitoring plans for trial participants.

Pediatric indications, such as congenital deafness, raise additional ethical issues. Once these therapies are approved, they will include informed consent, timing of intervention relative to the critical periods of auditory development, and equity of access. Regulatory agencies typically require risk-benefit justifications for early-age interventions, along with robust efficacy endpoints such as ABR thresholds and language development metrics (Bell et al., 2011). Despite these challenges, the designation of several hearing loss gene therapies, such as orphan drugs or rare pediatric diseases, by the Food and Drug Administration and European Medicines Agency, has facilitated expedited review pathways and potential priority vouchers (https://www.fda.gov/industry/medical-products-rare-diseases-and-conditions/rare-pediatric-disease-designation-and-priority-review-voucher-programs).

FUTURE DIRECTIONS

As gene therapy for hereditary hearing loss transitions from bench to bedside, the field stands at a transformative juncture. The success of early clinical trials, together with technological innovations in vector design and genome engineering, has laid a strong foundation for a new era of precision medicine. Nonetheless, significant opportunities and challenges remain in ensuring the long-term efficacy, safety, and accessibility of these therapies.

One of the key limitations of current gene therapy strategies is their narrow therapeutic window. Most studies have shown optimal outcomes when treatment is administered during the neonatal stage, prior to irreversible hair cell degeneration. Expanding this window to include older children or adults requires improved vector tropism and potency in mature cochlear cells, the use of in situ hair cell regeneration strategies in combination with gene therapy, and neuroprotective or anti-inflammatory adjuncts to preserve the auditory circuits.

Because mammals lack endogenous cochlear hair cell regeneration, a promising strategy is to combine gene therapy with cellular reprogramming techniques. Recent studies have shown that the combinational AAV-mediated delivery of transcription factors, such as *SIX1*, *ATOH1*, *GFI1*, and *POU4F3,* can induce the transdifferentiation of supporting cells into hair cell–like phenotypes (McGovern et al., 2024; Zhang et al., 2024). The integration of regenerative cues with genetic correction may offer a curative approach for late-stage hearing loss.

The expanding catalog of deafness-associated variants (> 10,000 variants) underscores the need for personalized therapeutic strategies. Future therapies may be tailored using patient-specific genome editing tools, RNA-based interventions, and multimodal diagnostics using next-generation sequencing and single-cell transcriptomics to stratify patients according to their molecular subtypes. Ongoing efforts to build variant databases and genotype-phenotype correlations will facilitate the design of individualized interventions (Joo et al., 2025; Kim et al., 2025; Oh et al., 2021; Rim et al., 2021).

However, achieving effective and durable gene transfer remains a challenge. Future advances are likely to include next-generation capsids with enhanced tissue penetration, lower immunogenicity, and larger packaging capacity (Dalkara et al., 2013), non–viral delivery systems such as lipid nanoparticles or exosome mimetics that allow repeat dosing, and the development of minimally invasive delivery devices suitable for clinical application in humans. These innovations will be critical for safe application in pediatric populations and therapies requiring readministration.

AUTHOR CONTRIBUTIONS

Hyeong Gi Song: Writing – review & editing. **Jinsei Jung:** Writing – review & editing. **Seung Hyun Jang:** Writing – review & editing, Writing – original draft, Formal analysis, Data curation, Conceptualization. **Heon Yung Gee:** Writing – review & editing, Writing – original draft, Funding acquisition, Conceptualization.

DECLARATION OF COMPETING INTERESTS

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper. The author Heon Yung Gee is an Associate Editor for Molecules and Cells and was not involved in the editorial review or the decision to publish this article.

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