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# Gene Therapy for Congenital Hearing Loss

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## Abstract

**Purpose of Review** This review provides the otolaryngology community with background on the basics of gene therapy for congenital hearing loss along with potential frontiers in this field.

**Recent Findings** In the last several years, there has been a tremendous increase in the amount of basic science research in gene therapy models for sensorineural hearing loss. Most work has been limited to murine models, but the transition to nonhuman primates has also occurred. There are still concerns with identifying the proper viral capsids, developing the most effective delivery approach, and timing the injection in humans to adequately restore hearing capability.

**Summary** Despite a growing interest and rapid advancement of the gene therapy research, there are still several challenges that remain before this can be utilized for children with congenital hearing loss.

**Keywords** Gene therapy · Congenital hearing loss · Adeno-associated virus

## Introduction

In the USA, at least 1 in 500 children are born with congenital hearing loss [1]. Delays in language acquisition and development from hearing impairment can impact quality of life, academic performance, and long-term earning potential [2, 3]. This recognized detriment prompted universal newborn hearing screening across America since the late 1990's to detect hearing loss and facilitate early specialist referrals [4, 5].

Sensorineural hearing loss (SNHL) results from defects in the cochlea or auditory nerve. Approximately 50% of congenital SNHL is genetically acquired of which 75% follow an autosomal recessive inheritance pattern, and 30% are associated with a syndrome [6]. A growing role for genetic testing has emerged to identify specific gene mutations responsible for pediatric hearing loss. Over 120 different genes that influence inner ear development or function have been identified with many loci resulting in SNHL [7–9].

Despite a recognition of genes responsible for SNHL, current treatment options remain limited to hearing amplification and cochlear implantation. Though these devices offer significant therapeutic benefit, they are not curative and cannot restore natural hearing. The potential for gene therapy has emerged as an exciting alternative to achieve improved sound resolution over cochlear implants, which are inherently limited [10], and to restore hearing function. The past decade has seen tremendous advances in the utilization and effectiveness of gene therapy for a variety of diseases [11]. Gene therapy is currently being used to treat Leber congenital amaurosis [12], hemophilia [13], Duchenne muscular dystrophy [14], cancer, and human immunodeficiency virus [15, 16]. This review offers a description of gene therapy for congenital SNHL with an appraisal of recent advancements and current limitations.

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## Discussion

### Inner Ear Considerations

The application of gene therapy in the inner ear requires several unique considerations. First, the inner ear is separated from blood vessels by the blood-labyrinthine barrier [17]. While this limits success of systemic therapy, the anatomic isolation restricts local vector delivery from disrupting other organ systems or disseminating elsewhere [18]. Additionally, this barrier protects the vector from immunologic breakdown and the fluid-filled labyrinth allows for diffusion of therapeutics [18, 19].

Second, the diverse molecular anatomy of the inner ear necessitates tailored delivery. The mature mammalian labyrinth is comprised of one auditory (cochlea) and five vestibular organs (posterior, lateral and superior semicircular canals, utricle, and saccule). These organs contain sensory epithelial cells (inner hair cells (IHC) and outer hair cells (OHC)), non-sensory supporting cells, and spiral ganglion neurons [20]. Monogenic mutations affecting the functions of hair cells, supporting cells, or the stria vascularis (SV) are three common causes of hearing loss. Other mutations are responsible for a range of functions in the cochlea such as organ development, stereocilia transduction, endocochlear potential (EP) maintenance, and neurotransmission between hair cells and spiral ganglion neurons [6, 20, 21]. Taken together, the delicate anatomy of the cochlea must be appropriately targeted based on the specific SNHL etiology. Researchers should be mindful of the molecular anatomy to ensure effective delivery without injury to surrounding structures.

### Definition of Gene Therapy

Gene therapy is defined as the delivery of genetic material into cells to exert a therapeutic benefit to stop or reverse an underlying disease process [18]. Gene therapy can be divided broadly into three categories: gene replacement, gene silencing, and gene editing. Gene replacement is the most common approach and occurs when a functional protein is delivered to a cell. This is an ideal method to rectify autosomal recessive, or loss of function, mutations. Conversely, gene silencing is best used to limit gain-of-function genes that produce an unfavorable protein and halt further production. Gene editing enables changing of pathologic genetic variants via gene editing systems such as with CRISPR/Cas9 [9]. These approaches are separate from cellular therapies, such as with stem cells, which have also been explored to restore hearing in a damaged cochlea [22].

### Viral Vector Delivery

Viral vectors utilize an inherent infectivity to insert genetic material into a cell. Developing adequate vectors requires replacing elements of the viral genome that contribute to replication, virulence, and disease with genes of interest, while retaining infectivity (cis-acting regulatory sequence) [19, 21]. The type of cells the virus infects, the DNA carrying capacity, as well as ability to evade immune response, are all factors that determine which virus to use to deliver different types of gene therapy.

Adeno-associated virus (AAV) has emerged as the most favorable virus for transfection of cochlear cells. AAV is a linear, single-stranded DNA parvovirus that is endogenous to many mammalian species. It preferentially targets chromosome 19 in humans and latently infects cells without harming the host. Integration of the AAV gene into the host genome has shown high frequency and stability through 150 passages. Despite these advantages, AAV is small and has a carrying capacity of 4.5 kb of foreign DNA, limiting the DNA amount utilized [18, 23–25]. Splitting the trans gene into two or three parts can address this issue [26], and a dual AAV approach has been utilized in mouse models [23].

AAV was the first viral vector to successfully deliver inner ear gene therapy in an animal model [27]. Mice with a mutation in VGLUT3, which encodes a glutamate transporter at the IHC–afferent nerve synapse, have been shown to be congenitally deaf [28]. Functional copies of Vglut3 cDNA were delivered to the cochlea of Vglut3 knockout mice, which resulted in correction of hearing loss in animals who were less than 12 days old. The normal ABR thresholds in these animals were sustained for up to 1.5 years after the gene therapy. Via immunohistochemical staining, the group demonstrated that the Vglut3 expression was restored to the IHCs of the mice [27].

Additional cells have also been successfully transfected by AAV in murine models. Harmonin-b found near the tip-link insertion point of stereocilia were recovered using AAV2 in a model of Usher Syndrome Type Ic [30]. In Whirler mice, a model for Usher Syndrome Type II, successful restoration of the whirlin protein at the tip of stereocilia with AAV8 increased IHC survival [31].

Perhaps a large interest for gene therapy is for connexin 26 mutations, which account for the greatest percentage of genetic hearing loss in humans [6, 32–34]. GjB2 encodes the gap junction protein beta 2, which is important for maintaining cochlear ion homeostasis. More than 100 mutations in this gene have been described with the majority through autosomal recessive inheritance [7]. Yu et al. were able to reestablish normal murine cochlear gap junctions by AAV-delivered gene therapy resulting in decreased cell death in the organ of Corti [35]. Importantly, this study only observed

the cellular impact of therapy and did not report on hearing outcomes. To date, AAV vectors have not shown robust transduction of OHC or supporting cells [23], which express GJB2, and in the outer sulcus and spiral prominence cells, where SLC26A4 is expressed [9]. This gene encodes for the transmembrane protein pendrin responsible for Pendred syndrome that is characterized by severe to profound SNHL, bilateral enlarged vestibular aqueduct with or without cochlear hypoplasia, and an abnormal perchlorate discharge test or thyroid goiter [6].

Promising audiometric results have been recorded with gene therapy delivery in animal models. Deafened guinea pigs obtained improved ABR thresholds and hair cell restoration after AAV vector delivery of the Atoh1 gene, which encodes a basic helix-loop-helix transcription factor important for hair cell differentiation, via a cochleostomy approach [36]. In another study, guinea pigs with noise-induced hearing loss were inoculated with Atoh1 adenoviral vectors via a round window approach 7 days after exposure and demonstrated improved ABR thresholds at 1 month. Additionally, electron microscopy demonstrated that the damaged stereocilia bundles were repaired [37]. These studies provided the groundwork for the first gene therapy clinical trial in humans for hearing loss which was initiated in 2014. In this trial, Atoh1 analog in humans (Hath1) was delivered via adenoviral vector 5 into adult subjects via a round window approach. Unfortunately, the study stopped recruitment in 2019 as the changes and effect on ABR thresholds were minimal [38].

## Autosomal Dominant Mutations

For autosomal dominant or gain of function mutations, alternative gene therapy strategies have been developed. RNA-based therapy prevents the formation of an autosomal dominant deleterious protein by preventing transcription of mRNA [39]. In RNA interference (RNAi), microRNAs (miRNAs) or small-interfering RNA (siRNA) which are small non coding sequences of RNA bind to RNA sequences of the protein of choice. While generally similar in appearance, siRNAs tend to be more specific [40], and miRNAs can affect multiple different genes at once [41]. This double strand of RNA is unable to be translated and forms into a nuclease complex of degradation called RNA induced silencing complex [42]. In a study by Shibata et al. miRNA were engineered to rescue progressive hearing loss in *Beethoven (Bth)* knockout mice, a model analog for human autosomal-dominant non-syndromic hearing loss (DFNA36) [43]. These mice carry a semi-dominant *Tmc1* allele, which is responsible for encoding a transmembrane protein important for the mechanoelectrical transduction complex within the cochlea [44]. In this study, miRNA carried on AAV was injected into the cochlea of mice with $Tmc1^{Bth/+}$, and the hearing thresholds of these mice were tested against controls. The mice receiving the injection had average ABR thresholds 40 dB higher than the controls [39]. Maeda et al. used siRNA was to treat an autosomal dominant form of GjB2 mutation. Wild-type mice were injected with GjB2 with a mutation of p.R75W, which causes abnormal gap junction formation, via liposomes. At the same time, some of the mice were also injected with RNAi that specifically silenced the expression of this gene. The mice who received the RNAi had significantly improved ABR thresholds compared to the ones who did not receive the RNAi [45].

Another modality by which autosomal dominant mutations can be silenced is by gene editing. Gene editing has emerged in the last decade as an efficient and specific way of mediating targeted gene disruption or repair and has been used in other genetic diseases such as muscular dystrophy [46]. CRISPR (clustered regularly interspaced short palindromic repeats)/Cas9 (crispr associated protein 9) has been shown to be able to target DNA with only 20 bp of guide RNA (gRNA) and is able to target multiple genes in the same cells by receiving multiple types of guide RNA [46]. This technology has been applied to hearing research in an animal model. Gao and colleagues demonstrated that by delivering Cas9-gNRA-lipid complexes targeting the mutant allele of the previously mentioned TMC1 into Beethoven mice, this could disrupt the mutant allele and the ears which received the therapy had ABR thresholds on average 15 dB higher than the untreated ear [47]. The therapeutic time window in this study was in the early postnatal period. Though gene editing is efficient, off target mutations from their application can have serious side effects, the dangers of which must be mitigated before this can be utilized in human trials [9].

## Gene Therapy Delivery

Delivery of gene therapy to the inner ear has been described through several different approaches. Commonly deployed techniques by which gene therapy has been delivered to the cochlea are (1) round window membrane (RWM) [48, 49], (2) canalostomy [50, 51], (3) cochleostomy into the endolymph or perilymph [48, 52], and (4) RWM combined with canal fenestration (CF) [53] (Fig. 1). Stapedotomy is another approach, though technically demanding, but one used by the recent gene therapy trial among nongenetic hearing loss for Hath1 [54•]. While these delivery methods have been shown to be effective in animal studies and a human clinical trial, they are often invasive, and may not be the preferred method approach in children [9].

In general, gene therapy to the human inner ear might be delivered across the tympanic membrane or directly into the cochlea itself. The trans-tympanic or intratympanic approach requires the vector to enter the middle ear cleft with subsequent diffusion into the inner ear. This might

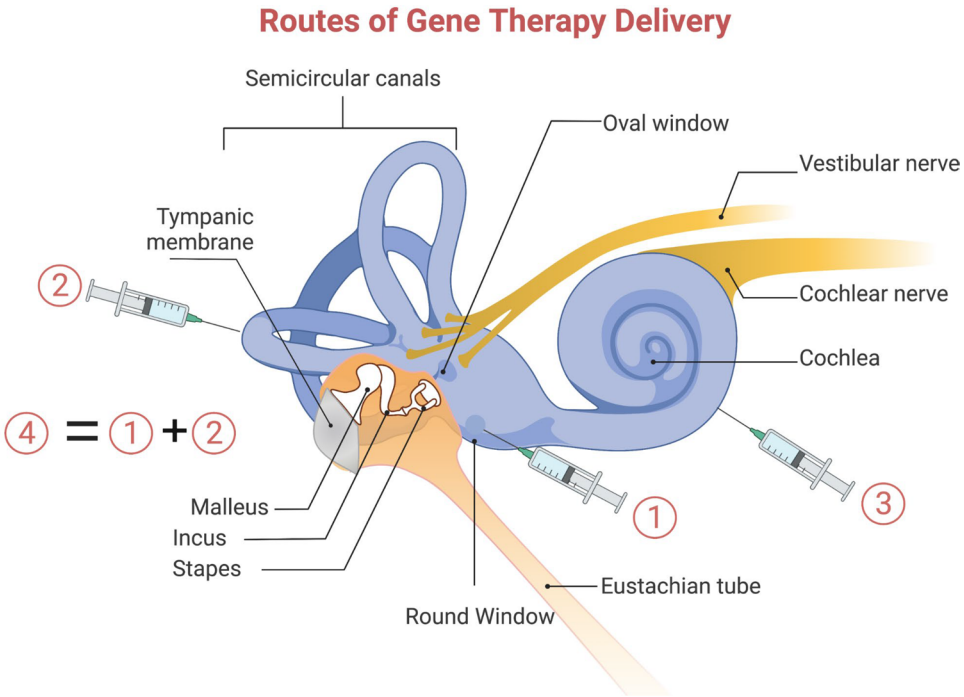


**Fig. 1** Schematic of common delivery routes for inner ear gene therapy*. *This figure was created with BioRender.com

1. Round window membrane injection
2. Semicircular canal injection (canalostomy)
3. Cochleostomy
4. Combined round window membrane and semicircular canal injection

have advantages over intracochlear delivery such as avoiding potential damage to the delicate inner ear anatomy and decreased risk of perilymph leakage. It could also help preserve any residual hearing the child may have. An intratympanic technique has been used for several other disease processes in otology and can be approached through the external auditory canal across the tympanic membrane. However, for children, the limitation is how to ensure adequate time for diffusion once delivered to the middle ear. Drug diffusion through the RWM has been described as dependent on duration of contact time [55] that would be influenced by eustachian tube loss through swallowing. Other considerations for this approach are RWM permeability particle size, charge and concentration [56], and success of delivery to the inner ear that can be improved by things like sustained delivery techniques [57].

Another option could be cerebral spinal fluid (CSF) delivery to the inner ear via the cochlear aqueduct. Utilization of this approach offers a method to avoid disruption of the delicate inner ear anatomy. The cochlear aqueduct connects the CSF to perilymph of the inner ear but is often obliterated in humans [54•]. In a guinea pig model, successful AAV vector delivery of gene therapy via the contralateral inner ear suggests feasibility of this pathway [58]. Future studies will be needed to determine if CSF delivery is a viable option for inner ear delivery with concerns such as diffusion to other structures and central nervous system effects. Until the blood labyrinthine barrier can be altered, there needs to be a technique that offers direct access with as little ramification of local and distant destruction as possible. Most of the inner ear approaches need to be mindful of CSF leak and perilymph flow disruption if not adequately sealed.

Lastly, there have been some studies looking at whether a cochlear implant (CI) electrode may help with accessing the scala media. For example, neurotrophin 3 (NT3) a factor that contributes to health of nerve cells, were delivered to deafened guinea pigs through an AAV vector via a cochleostomy. When a CI electrode was introduced, 78% of the deafened ears that were inoculated with AAV.Ntf3 showed better SGN survival the deafened-control ears [59]. In a slightly different approach, CI with steroid eluting electrodes improved residual hearing and reduce trauma from insertion in Mongolian gerbils [60]. Therefore, combining gene therapy with cochlear implantation may be the ideal transfection, and therapeutic modality while gene therapy delivery to the inner ear is still in the developing stages.

## Advanced Animal Models

To date, the overwhelming majority of gene therapy research for hearing loss has been described and developed in murine models. Mice, like other rodents, have a well-characterized genome, are robust to manipulation, are cost-effective, and have resemblance to human inner ear [54•]. The timing of most of these studies occur during the murine neonatal period [30]. An important challenge is the murine timeline of deafness. In one report, gene therapy was successful in improving ABR thresholds if delivered to mice less than 12 days old while less successful between days 12 and 15 [27]. The mouse cochlea is not mature at birth and matures around 2 weeks of life. This contrasts with human cochlea, which are mature at birth [29]. Mice are born deaf and the development of organ of Corti occurs until P14 postnatally. This timing is difficult to recreate in humans since this occurs in utero. Many of the cellular and molecular process occurs in utero making effective postnatal changes difficult. Inner ear for human matures 26 weeks of gestation but not until 15 days after birth for mice [9, 53]. This calls into question whether degradation has already occurred by the time diagnosis would have been made and whether looking at human fetal delivery is the more effective technique.

As described, there has been a lot of in vivo work for animal models with varying degrees of measured success. The next logical step would be to replicate gene therapy studies in nonhuman primate (NHP) models. NHPs offer a closer and more accurate model to human inner ear anatomy and function than would be seen in rodents. There have been a few early series describing the successful delivery of AAV9 uptake to inner and outer hair cells juvenile NHP (*Macaca fascicularis*) [61, 62••]. Using a RWM membrane delivery with oval window venting, AAV1 or Anc80L65, which is a vector that was derived from AAV 1, 2, 8, and 9 lineages, was injected into Rhesus macaque cochleae. Expression 7–14 days following injection showed that for two animals, Anc80L65 transduced up to 90% of apical inner hair cells but transduction for both vectors declined from apex to base. This unexpected finding necessitates further investigation but was hypothesized to be a result of morphological and metabolic differences in supporting cells, tectorial membrane, and IHCs [63]. There are logical correlates to human inner ear anatomy from NHP namely a closer approximation of the inner volume [54•]. Notably absent, however, are the audiometric measurements of improved hearing in these models that have been seen in the murine model. This will undoubtedly be an import step moving forward especially with regard to the timing of hearing restoration.

## Limitations and Outlook

There remain important unanswered questions about the potential for gene therapy for congenital SNHL. While the last 10–15 years have offered tremendous growth in the basic science for this area of research, there are uncertainties about how to translate its use in children. Most agree that AAV represents the ideal vector to load gene therapy for delivery to the inner ear. Continuing to identify which AAV strain is best suited for specific cells of the inner ear involved with hearing loss is of great interest. The proper viral capsids will likely need to be engineered to ensure delivery that is specific and effective.

The surgical approach is still another important area with which future clarity is necessary. Particularly in the pediatric patient, morbidity associated with violation of the inner ear needs to be carefully weighed. While animal models have delivered the viral capsids to the inner ear successfully and shown adequate cellular uptake, potential for harm to surrounding structures exists in these surgeries. Damage to the delicate structure of the ear must be considered as a complication of inner ear gene therapy which may include the loss of residual hearing [64]. The common surgical risks associated with advanced otologic surgery, such as bleeding, infection, and pain, also need to be weighed. Even the potential pressure-induced wave injury to hair cells, offset by lateral canal fenestration techniques in mice [53], would be considered a suboptimal procedure in humans. Researchers must continue to refine approaches to deliver gene therapy in an atraumatic fashion.

Perhaps the biggest concern is the timing of delivery. While an improved ability to identify and detect specific mutations of SNHL exists, the timing with which this identification occurs is a limitation. If the assumption that there is a very narrow window of applicability for congenital SNL, then delivery of gene therapy will be a major logistical challenge. This begins to call into question whether fetal delivery may offer the highest likelihood of success despite the obvious challenges with this approach. Recognition that the virus can reach and infect specific cochlear cell types has improved in recent years with a more limited success in audiometric data. Especially as a growth in NHP studies is anticipated, recognizing when might be too late for gene therapy is crucial to understand the limitations of applicability in children.

Some groups have tried to look at different approaches to cellular regrowth potential. While cochlear hair cells within the mammalian cochlea do not have the ability to regenerate, hair cells within the avian cochlea can do so by mitosis and trans-differentiation of supporting cells [65]. Therefore, embryonic stem cell transplantation has been an area that scientists have been exploring. While this represents an

exciting frontier outside of gene therapy, current limitations include the lack of specificity and an inability to regenerate enough hair cells in the correct orientation to restore hearing [23].

Finally, the future of this research will benefit from the alignment of key stakeholders. This would include not only basic scientists but also clinician scientists in the field of otolaryngology and neurotology. Addressing many of the barriers to inner ear gene therapy will necessitate multiple unique perspectives and the skill sets of different individuals. Finding ways to connect these groups will be of the utmost importance. Additional perspectives from audiology, pediatrics, neonatology, and geneticists will also have valuable roles. Finally, the question of cost is oftentimes left out of this discussion. Not only is there a tremendous cost in the development and progression of this research or trial, but the cost for the surgical procedures, should that be attained, will need to be considered. Certainly, as the translation from bench to clinical research progresses, the multidisciplinary approach to this type of endeavor will be crucial for the success and longevity of a viable gene therapy for congenital SNHL.

## Conclusion

The potential for gene therapy as a therapeutic modality for congenital SNHL appears to be on the horizon. Growth in this area of basic research has been also fueled by success for gene therapy in other sectors of medicine. There remain many barriers to overcome, but there is clearly an interest and scientific rationale to harness gene therapy for hearing loss. Otolaryngologists will undoubtedly be part of translational steps needed to apply gene therapy to management of congenital hearing loss.

## Declarations

**Conflict of Interest** The authors declare no competing interests.

**Human and Animal Rights and Informed Consent** This article does not contain any studies with human or animal subjects performed by any of the authors.

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