Cochlear gene therapy for otoferlin-related hearing loss

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Purpose of review
There are currently five groups internationally involved in human clinical gene therapy trials for otoferlin-associated hearing loss. This includes (in alphabetical order) the Eye and ENT Hospital Fudan University (China), Lilly-Akouos (USA), Otovia (China), Regeneron (USA), and Sensorion (France). This review summarizes early work that led to these efforts and highlights early published data on clinical outcomes.

Recent findings
While published outcomes are currently limited, data emerging from each of these clinical trials is highly consistent. Using a dual vector approach to reconstitute full length Otoferlin, all groups report varying degrees of hearing improvement following cochlear gene therapy, with some cases of hearing restoration to normal levels. Recent data suggests that improvement is not limited only to young children but also adolescents and even young adults in some cases. The treatments all appear safe with limited adverse effects associated with the therapies reported.

Summary
Gene therapy for otoferlin-related deafness appears highly successful in most cases with limited reported adverse effects or outcomes. This success will undoubtably usher in a new era of gene therapy for other forms of genetic deafness.

Keywords
adeno-associated virus, cochlear gene therapy, hearing, otoferlin

INTRODUCTION

Gene therapy has long been advocated as the ultimate treatment for genetic hearing loss. Years of study in animal models of hearing loss have now paved the way for the first active gene therapy clinical trials for genetic deafness due to mutations in the gene coding for otoferlin. This review will highlight the research into animal models of otoferlin-related deafness that led to the clinical human trials and is followed by an overview of the active clinical trials currently occurring internationally.

OTOFERLIN BACKGROUND

Early epidemiologic studies documented that otoferlin-related deafness, termed DFNB9, accounts for ~2–4% of cases of nonsyndromic hearing loss in humans [1,2]. This hearing loss is characterized as a recessive auditory synaptopathy with moderate to profound hearing loss and the presence of normal otoacoustic emissions [3]. It is also believe to be the most common genetic defect seen in auditory neuropathy spectrum disorders [4]. In humans, DFNB9 manifests as a nonsyndromic bilateral loss of hearing before the acquisition of language. It was first discovered in an affected Lebanese family [5] and has since been found in many parts of the world [2,6–8].

Otoferlin is functionally expressed in the inner hair cells of the cochlea and sporadically throughout the central nervous system [9]. It is a member of the *ferlin* family of transmembrane proteins and thought to act as a calcium sensor for synaptic vesicle exocytosis in cochlear hair cells, acting with the SNARE proteins synaptobrevin, syntaxin 1A and SNAP-25 to play a critical role in afferent auditory synaptic transmission [10–13]. Anatomically, the gene expressing otoferlin (OTOF) is expressed in both cochlear and vestibular hair cells along the entire basolateral cell

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Curr Opin Otolaryngol Head Neck Surg 2025, 33:312–317
DOI:10.1097/MOO.0000000000001070

KEY POINTS

- Otoferlin is the first cochlear gene therapy available to treat genetic deafness.
- Otoferlin gene therapy requires a dual-vector approach to overcome the size limitations of the adeno-associated virus for gene delivery.
- Initial reported and published results for otoferlin gene therapy are variable, but all international efforts have shown extraordinary promise for restoring hearing to near normal levels in many cases.
- This is the first of what will hopefully be many more cochlear gene therapies available for other forms of genetic deafness.

membrane and within vesicular structures, and also is distributed throughout most of the hair cell cytoplasm [14]. Deletion of the OTOF gene in mice (*Otof*$^{-/-}$) results in profound deafness due to the loss of synchronous evoked glutamate release at the IHC-afferent synapse and progressive loss of spiral ganglion neurons, despite normal inner hair cell development and auditory ribbon synapse formation [12,15]. A unique feature of OTOF-related deafness is the relative excellent preservation of cellular structures, including hair cells, within the cochlea at birth despite the profound hearing loss seen. This feature has made it a very attractive target for gene therapy.

Adeno-associated virus (AAV) is currently the vector of choice for cochlear gene therapy for genetic hearing loss based upon its proven clinical safety profile [16,17], stable long-term transgene expression [18], lack of ototoxicity [19–21] and trophism towards auditory hair cells and other structures within the cochlea that results in relatively high levels of transfection [22]. Despite these advantages, the biggest limitation of AAV as a vector for gene therapy is its relatively small packaging capacity, allowing only ~5 kb of DNA as an insert, which greatly restricts the number of gene candidates that can potentially be corrected by AAV. This leads to one of the major hurdles for a gene therapy application utilizing the OTOF gene which is ~6.5 kb, exceeding the carrying capacity of AAVs.

A solution to this size dilemma was solved by Dyka *et al.* when they demonstrated a novel approach that allows AAV to shuttle larger genes into host cells by using dual AAV vectors [23]. This technique involves cleaving the target cDNA into two halves and packaging them in separate viral vectors with overlapping sequences (splice donor and acceptor sites); once both halves enter a cell, they recombine into the full-length functional cDNA, allowing translation into a normal protein. Using this technique, Dyka *et al.* were able to transfect cells to create full-length and functional Myo7A, a 6.7 dB gene that is mutated in Usher syndrome 1B.

There were two groups that simultaneously used the method developed by Dyka *et al.* to overcome the size limitations of the AAV vector to successfully delivery the OTOF gene into transgenic *Otof*$^{-/-}$ mice and restore hearing. Akil *et al.* [24] used this dual vector approach to restore the full length otoferlin mRNA sequence in hair cells in OTOF knockout mice and documented a high percentage of inner hair cell transfection and expression of otoferlin protein. Further, acoustic brainstem response (ABR) thresholds were restored to near normal levels. Encouragingly, in contrast to other mouse models of synaptic protein-related deafness [25], delivery at a later date (30 days postnatal) resulted in even better hair cell transfection and better hearing restoration. Al-Moyed *et al.* [26], also using a dual-vector approached in an OTOF knockout mouse model, were able to partially restore hearing and documented restoration of fast vesicle exocytosis at the inner hair cell ribbon synapse. Together these two studies demonstrated the feasibility of translating a treatment for OTOF-related deafness in mice into humans and paved the way for the current otoferlin gene therapy trials now underway.

CURRENT OTOFERLIN GENE THERAPY TRIALS – BACKGROUND

At present there are five active otoferlin gene therapy trials for OTOF-related deafness being run internationally. For a relatively rare form of hearing loss, it would at first seem unusual for so many different groups to be focused on the same gene. However as the discussion above alludes to, this particular form of deafness is very attractive as a gene therapy treatment for several reasons: in OTOF-related deafness, there is excellent cellular preservation in the cochlea after birth without the hair cell degeneration seen in many other forms of genetic deafness; within the cochlea the gene is localized to a single cell type – inner hair cells; current vectors used for cochlear gene therapy, AAV are highly trophic for inner hair cells; and, there is a potentially long window of intervention through childhood when treatment can be instituted.

The aim of these current clinical trials is to identify children who manifest OTOF-related hearing loss and intervene as soon as feasible. One of the initial challenges encountered is identifying babies with OTOF-related hearing loss, which has been facilitated by universal newborn hearing screening [27]. Because OTOF-associated hearing loss presents

as a loss of inner hair cell synaptic transmission with intact outer hair cell function, children will be born with intact otoacoustic emissions (OAEs) and absent acoustic brainstem response (ABR) thresholds [28]. Thus if a hospital or clinic uses OAEs to screen for hearing loss, those with OTOF-related hearing will likely pass their newborn hearing screening exam and be missed, whereas if ABRs are used, all cases will be identified. In a comprehensive review of otoferlin-related hearing loss cases in Japan by Iwasa *et al.* [29], all patients with documented otoferlin mutations passed newborn hearing screening by OAEs while in those who were screened with ABR 91% failed screening. When these same children were followed over several years, most of the children lost otoacoustic emissions by age 5. These data are very relevant to the current active clinical gene therapy trials since presence of otoacoustic emissions is an entry criterion for each of them and suggests that intervention after the age of 5 years may not be as successful for hearing restoration.

As noted earlier, there are five active gene therapy trials for Otoferlin-related hearing loss internationally. They all have in common several similarities, including use of the dual vector approach for gene delivery using an adeno-associated virus subtype, similar inclusion criteria, and requiring the presence of otoacoustic emissions as a measure of cochlear health. The primary differences between these efforts include such specifics as method of cochlear delivery, differing viral vectors used (though all of the AAV class) and various promoters used for gene expression within cells (Table 1). The following section describes the efforts by each of these groups with active gene therapy trials for otoferlin-related hearing loss, listed alphabetically.

EYE AND ENT HOSPITAL, FUDAN UNIVERSITY

The team from the Eye and ENT Hospital at Fudan University, led by Dr Yilai Shu, was the first group to both present and publish data on their ground-breaking gene therapy trial for OTOF-related hearing loss in humans, initially reported at the European Society of Gene and Cell Therapy Conference in Brussels Belgium, in October 2023 [30▪]. Preclinical work by this group in mice documented high rates of inner hair cell transfection and hearing restoration, while in primates they similarly showed excellent inner hair cell transfection rates without adverse effects [31]. From here the group moved to human clinical trials. Their initial publication in early 2024 [30▪], a first in human report, documented the results in six children who received the human otoferlin gene delivered via AAV serotype 1 (AAV1) employing a dual vector approach similar to that described by Akil [24] and Al-Moyed [26]. All children had bi-allelic mutations in the OTOF gene felt to be pathogenic and all had severe to profound sensorineural hearing loss at the onset of treatment. Patient ages ranged from 1 to 6 years and five out of the six children had a unilateral cochlear implantation and underwent the gene therapy delivery in the contralateral ear. Dosages of the study drug ranged from 9 x 10^{11}vg (initial patient) to 1.5 $\times$ 10^{12}vg (all subsequent subjects). The drug was delivered into the round window via a transcanal approach with a simultaneous fenestration through the stapes footplate to allow for egress of endolymph for full cochlear perilymph delivery. All patients developed significant hearing recovery except one. Further, in each of those subjects with hearing recovery, there was improved speech perception. The average pure-tone audiometry threshold was 71 dB at 4 weeks, 68 dB at 6 weeks, 55 dB at 13 weeks, and 30 dB at 26 weeks. The treatment was well tolerated with no dose-dependent toxicity recorded for any of the patients. The study is ongoing with additional patients being recruited and followed, but as of the writing of this review, no additional peer-reviewed publications have been identified.

LILLY-AKOUOS

Akouos, based out of Boston, MA also began working on a treatment for otoferlin-associated hearing loss with early preclinical studies being reported as early

Table 1. Active human otoferlin gene therapy clinical trials (listed alphabetically)

Sponsoring institution/company	ClinTrial.gov listing	Vector	Delivery route
Eye and ENT Hospital-Fudan University	NA	AAV1	Transcanal RWM with oval window fenestration
Lilly - Akouos	NCT05821959	AAV-Anc80	Transcanal RWM with oval window fenestration
Otovia Therapeutics	NCT05901480	AAV-Anc80	Transmastoid RWM
Regeneron	NCT05788536	AAV1	Transmastoid RWM + HSC Canalostomy
Sensorion	NCT063703510	AAV2	Transmastoid RWM + transcanal oval window fenestration

AAV, adeno-associated virus; HSC, horizontal semicircular canal; RWS, round window membrane.

as 2021 [32,33]. After receiving an Investigational New Drug approval in 2022 [34], and subsequent acquisition by Lilly in October of 2022, their clinical trial began in 2023 (ClinTrials.gov ID NCT05821959 – "A Trial of AAVAnc80-hOTOF Gene Therapy in Individuals With Sensorineural Hearing Loss Due to Otoferlin Gene Mutations"). This is a multicenter clinical trial with clinical sites in the United States, Canada, Taiwan and the United Kingdom. As with the other clinical trials, eligibility criteria include biallelic pathologic mutations in the OTOF gene, and bilateral profound sensorineural hearing loss as evidenced by ABR and preserved otoacoustic emissions. The initial cohort included older patients (between 7 and 17 years of age) to evaluate safety, and subsequent enrollees at younger ages. The vector used for gene delivery is a synthetic ancestral form of AAV termed Anc80 [34], and notable for its ability to transfect a variety of cell types within the cochlea. This study also employs a dual vector approach for gene delivery to allow the full-length mRNA transcript to recombine within the inner hair cells. Like the Fudan University Hospital group, delivery involves a trans-canal, endoscope assisted round window membrane delivery with a concomitant oval window fenestration to allow for egress of excess perilymph. To date there are no peer-reviewed publications documenting hearing outcomes, however presentations at clinical and research meetings, in addition to the popular press [35,36], have documented hearing recovery similar to what has been published by other groups internationally without significant adverse clinical events.

OTOVIA THERAPEUTICS

Otovia Therapeutics is biotechnology company that was founded in January 2022 in China. Preclinical data in mice and nonhuman primates utilizing an Anc80 AAV and Myo15 hair cell-specific promotor to transfect inner hair cells demonstrated both the safety and efficacy of their approach [37,38]. A follow up report included two patients – a 5 year old with profound hearing loss and a cochlear implant in one ear (who received contralateral gene delivery) and an 8 year old with profound hearing loss who received bilateral gene delivery [38]. The drug was delivered via a transmastoid, facial recess approach to the round window, similar to a cochlear implant surgical approach. The study documented significant hearing improvement in both patients in the treated ear(s). The 5-year-old was reported to have an ABR threshold near 30 dB hearing loss (HL) and after several months was able to respond to voice without amplification. The 8-year-old had similar improvements in one ear and moderate hearing thresholds (50 dB HL) in the other.

Otovia is currently in active clinical trials (ClinTrials.gov ID NCT05901480) and has recently published a study that extends their findings in 10 patients, including importantly, an older age cohort [39▪]. This study included patients aged 1.5–23 years of age with 6–12 months of follow-up. One patient received two injections into the same ear. Patients demonstrated an improvement in pure-tone-average hearing levels from a baseline of 106 dB to 52 dB (range ±30 dB). The authors reported the therapeutic effect was rapid, taking one month to achieve most of the overall hearing improvement. Individually, click and tone-burst ABR thresholds, but not the auditory steady-state response, reliably predicted the behavioral pure-tone-average thresholds after 4 months. The authors also reported an age-dependent therapeutic effect, with optimal outcomes in 5–8-year-old patients. One patient showed a rapid improvement from profound hearing loss to 25 dB HL pure tone average at 2 months postdelivery. No serious adverse events were reported in this series. This study was particularly unique regarding two factors: Firstly, the age of the patients was much greater than what has been previously reported and documenting that the treatment appears to be effective in this older cohort. Surprisingly, the oldest patient progressed from a profound hearing loss to a pure tone average threshold of 40 dB HL at 4 months. Secondly one of the patients in the study achieved only modest hearing improvement yet had undergone two injections in the *same* ear. There is an unresolved question in the field of cochlear gene therapy whether a second injection in the same ear could elicit an immune response against the viral vector that could in turn cause scarring or inflammation in the cochlea leading to deafness. This appears not to be the case, and at least in this patient, was well tolerated. This brings hope that future additional injections for incomplete responses or in those patients where the therapy fails over time could possibly result in recovering the hearing once again. Clinical trials with this team are ongoing.

REGENERON (FORMERLY DECIBEL THERAPEUTICS)

Decibel Therapeutics originally started working on a gene therapy approach for otoferlin-related hearing loss in 2019. They advanced their technique through preclinical studies in mice and nonhuman primates [40,41]. In August of 2023 Decibel Therapeutics was acquired by Regeneron who then took over control of the developing clinical trial. The resulting clinical trial, termed the CHORD study (AAV-based gene therapy, in children/infants with hearing loss due to otoferlin mutations) has been active since 2023

(ClinTrials.gov ID NCT05788536). This is an international multicenter study based in the United Kingdom, Spain, and the United States. Eligibility criteria include the presence of a bi-allelic pathogenic mutation in the OTOF gene, under the age of 18 years, bilateral severe to profound sensorineural hearing loss (>90 dB HL), and the presence of otoacoustic emissions in the ear(s) to receive the drug, termed DB-OTO. If an OAE cannot be obtained, alternatively the presence of a cochlear microphonic must be verified in the ear(s) to be treated. In contrast to the delivery technique from Fudan University Hospital (transcanal round window delivery with oval window fenestration) and Lilly-Akouos, the DB-OTO is delivered via a transmastoid facial recess approach to the middle ear, similar to what is done for cochlear implantation. In addition, to allow for egress of perilymph and ensure complete cochlear perilymph perfusion, a small canalostomy is made in the horizontal semicircular canal, similar to that described by Isgrig and Chien, and sealed after delivery [42]. While there are no peer-reviewed published reports from the trial group, there have been presentations at a number of clinical and research conferences in addition to the popular press [43,44] that have documented similar hearing results as seen in other clinical trials and no reported significant adverse events associated with the drug delivery technique.

SENSORION

Sensorion is a biotechnology company based in France that is focused on developing novel therapeutics for treating and preventing hearing loss. The Sensorion team includes Dr Christine Petit from the Pasteur Institute who was responsible not only for identifying the OTOF gene but also was involved in elucidating the pathophysiology of DFNB9, the deafness associated with OTOF mutations. Further, Dr Petit and her team at the Pasteur were part of one of the 2 research groups that developed the dual vector approach for treating OTOF deafness in mice [24]. The Sensorion contribution to the OTOF gene therapy space includes the development of SENS-501 (OTOF-GT). This is a dual vector approach utilizing AAV subtype 2 (AAV2). Mouse and nonhuman primate studies have documented efficacy and safety of their construct [24,45].

Based on these preclinical studies there is an active human clinical trial that is ongoing (ClinicalTrials.gov ID: NCT063703510). The study, termed 'Audiogene' consists of a dose-escalation part, comprising two cohorts of three patients each, assessing a low and high dose of SENS-501 (1.5×10^{11} vg/vector/ear) and a higher dose of SENS-501 in Cohort 2 (4.5×10^{11} vg/vector/ear). For this initial cohort, the primary endpoint for the dose expansion part includes ABR thresholds. Delivery of the therapy is via a transmastoid approach with round window access through the facial recess. In addition, a trans-canal approach is utilized for an oval window stapedotomy for venting during drug delivery. Preliminary data for the initial patients were announced in a recent press release by Sensorion on July 1, 2025 [46]. Preliminary positive data from the first cohort (low dose) of SENS-501 documented that SENS-501 and the surgical procedure to instill the drug were well tolerated by all participating children (6–31 months of age) without serious adverse events or serious side effects reported. Of the three patients enrolled, in one patient aged 11 months at the time of injection, a clinical response was observed including positive ABR responses at two frequencies, with the best frequency reaching 70 dB, improvement of hearing levels across two speech frequencies with best frequency reaching 90 dB PTA. Further, this patient had meaningful changes in responses to sounds and voices as reported by the parents with an Infant-Toddler Meaningful Auditory Integration Scale (IT-MAIS) score increase of 16 points (145% relative improvement from baseline), and met expected auditory milestones based on an age-based parent questionnaire and according to the patient's age (LittlEARS). Ongoing recruitment in the higher dose cohort 2 is ongoing per the company press release. At present only unilateral delivery of SENS-501 has been used, but bilateral delivery is planned at a later stage in the clinical study.

SUMMARY

Years of research in basic and translational sciences by investigators from around the world have led inexorably to the first human clinical gene therapy trials for OTOF related hearing loss. The fact that efforts from five different investigative groups, each with a unique drug for this condition, have yielded similar remarkable hearing outcomes is a testament to the efficacy of this treatment. While all these trials are in their earliest stages, the initial successes by each are a promising early indicator that these treatment effects and hearing recovery will be enduring. Otoferlin may be the first out of the gate, but the successes seen in these early trials is sparking the development of treatments for a number of other forms of genetic deafness with similar levels of enthusiasm. We are truly on the cusp of a new era of treatment for genetic hearing loss.

Acknowledgements

Dr Lawrence Lustig is a site Principal Investigator for the Regeneron CHORD Study for DB-OTO at Columbia

University Vagelos College of Physicians and Surgeons and NewYork Presbyterian Hospital.

Financial support and sponsorship

None.

Conflicts of interest

There are no conflicts of interest.

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- ■ of special interest
- ■■ of outstanding interest

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