GENE THERAPY IN TREATMENT OF HEARING LOSS: A MIRACLE COMING SOON

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Abstract:

Hearing loss is a widespread global issue, affecting millions of individuals, with genetic factors playing a significant role in its development. This condition, particularly in children, has far-reaching consequences on speech, language acquisition, and social development. Sensorineural hearing loss (SNHL) stands as the most prevalent sensory impairment. Its root causes encompass a wide range of genetic and environmental factors, which can result in hearing impairment of various types. Habilitation options usually center around amplification through wearable or implantable devices. Nevertheless, there are ongoing investigations into gene therapy-based approaches aimed at restoring and preventing hearing loss. Recent studies illustrate the therapeutic potential of molecular agents administered to the inner ear for the improvement of various types of hearing loss. While current interventions rely on hearing aids and cochlear implants, advances in genetic diagnosis and therapy offer hope for early intervention and improved outcomes in addressing sensorineural hearing loss.

Keywords: Hearing loss, Gene therapy, Gene editing

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INTRODUCTION:

The hearing loss is most prevalent sensorineural deficit. It constitutes a significant public health concern, impacting individuals across all age groups. [1] As per the World Health Organization, there are 466 million individuals globally who experience disabling hearing impairment, and among them, 34 million are children. Hearing loss has genetic origins in 70% of congenital cases and 25% of cases that develop in adulthood. [2,3] In children, congenital hearing loss can have adverse effects, including speech and language acquisition delays, as well as issues related to social and emotional development. [3,4] For adults, hearing impairment is linked to social and psychological challenges and, in the elderly, can be associated with more pronounced cognitive decline. [5,6]

Hearing loss can result from a combination of genetic and environmental factors, leading to various forms of hearing impairment, including congenital or late onset, stable or progressive, drug-induced, noise-induced, age-related, traumatic, or post-infectious. In otherwise healthy newborns with hearing loss, when factors like noise exposure, age-related causes, and trauma are ruled out, the most common etiologies are genetic factors or infections acquired prenatally, often linked to cytomegalovirus. [2]

While hearing loss can affect various cell types along the auditory pathway, one of the most prevalent types arises from the harm or loss of sensory hair cells and the connected auditory neurons that form synapses within the cochlea. This condition is collectively known as sensorineural hearing loss. Presently, the sole choices for improving sensorineural hearing loss (SNHL) are hearing aids or cochlear implants. [7-9]

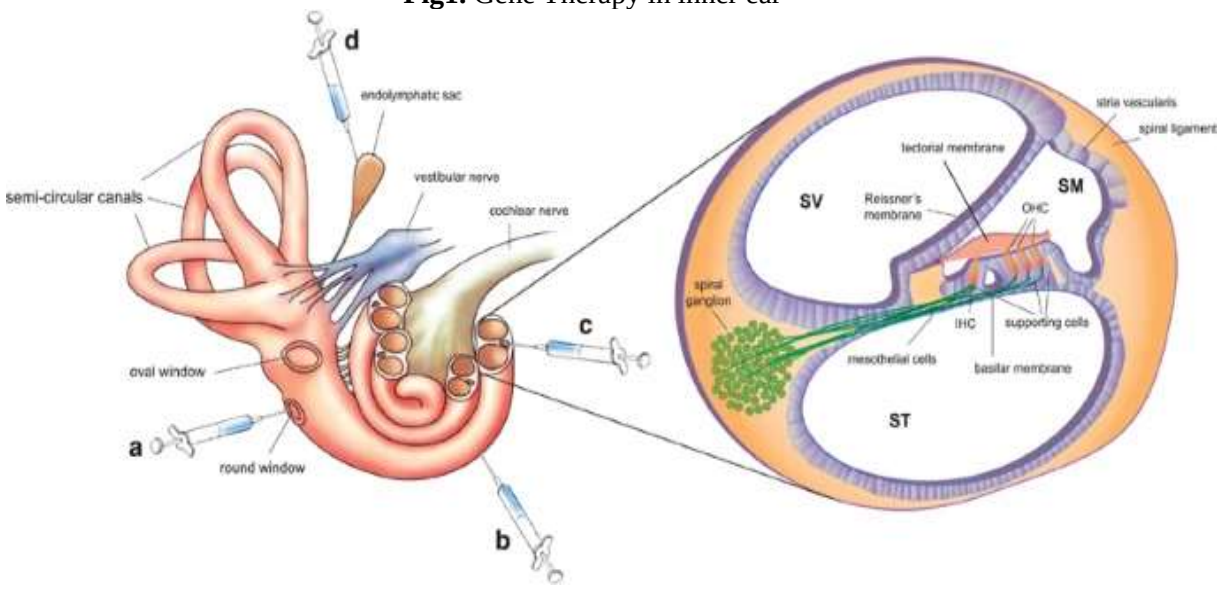
Utilizing genetic diagnosis for hearing loss enables the assessment of disease prognosis and opens the door to potential early therapeutic interventions. The discovery of genes linked to hearing loss has provided a more profound understanding of the fundamental mechanisms involved in normal hearing and the development of hearing-related conditions. [10]

Recent advancements in techniques for altering and rectifying genetic irregularities, coupled with our growing knowledge of the genes linked to deafness, have made gene-based therapy an attractive option for addressing hearing impairment. [11,12]

GENETIC APPROACHES TO HEARING LOSS:

The gene therapy has expanded beyond its initial definition, which involved treating genetic diseases by replacing a faulty gene with a functional one through gene transfer. Gene therapy is presently defined as the delivery of nucleic acids, either DNA or RNA, to address or prevent a disorder using a range of approaches, which includes gene replacement, gene silencing, as well as base and prime editing or in situ repair techniques. [13,14] The obstacles common to all these methods revolve around achieving precise, secure, and effective delivery of therapeutic materials (including DNA, RNA, oligonucleotides, siRNA, or the molecular components of the CRISPR) into target cells. This is often facilitated by employing a modified viral vector (as explained later) or, less frequently, non-viral vectors like liposomes. [1]

Fig1. Gene Therapy in inner ear [15]



Technological advances in editing genes to address hearing impairment

Gene Replacement:

Gene replacement therapy, the most commonly employed approach in gene therapy, has found extensive application in addressing recessive HHL (Hereditary Hearing Loss). [16] The inaugural successful instance of gene replacement therapy for HHL was documented in 2012, involving a VGlut3 knockout mouse model. [17] VGlut3, responsible for encoding vesicular glutamate transporter-3, plays a crucial role in glutamate release at the afferent synapse of inner hair cells (IHCs). Adeno-associated virus (AAV), a non-enveloped virus, can be genetically modified to transport DNA into specific target cells. In the case of neonatal VGlut3 knockout (KO) mice, delivering the VGlut3 cDNA using AAV1 through the round window membrane (RWM) into the inner ear significantly restored their auditory function, nearly returning it to normal levels. The remarkably high transduction efficiency, with approximately 100% of IHCs being transduced, likely contributed to the effectiveness of this treatment. [18]

HHL associated with VGlut3 mutations is uncommon and typically manifests as an autosomal dominant disorder (DFNA25). This genetic profile does not align well with the gene replacement strategy. However, following the confirmation of the strategy's efficacy in the inner ear, numerous studies focusing on alternative models of HHL have surfaced. [19]

The gene associated with deafness, TMC1, has emerged as one of the most prominent targets for gene therapy. TMC1 encodes a mechanosensitive ion channel located at the tips of hair bundles, and mutations in this gene are responsible for DFNB7/11 and DFNA36. Mouse models carrying recessive Tmc1 point mutations (c.A545G) or dominant Tmc1 mutations (c.T1253A), known as Baringo and Beethoven (Bth), respectively, serve as models for human DFNB7/11 and DFNA36 conditions. The Jeffrey R. Holt group has conducted three studies involving gene replacement therapy targeting Tmc1. [20-22]

In these investigations, they optimized AAV vectors to enhance the recovery of hearing function by improving the transduction efficiency of outer hair cells (OHCs). OHCs are typically challenging to transduce using traditional AAV serotypes. The initial study conducted in 2015 utilized AAV2/1 to introduce a functional Tmc1 gene into the Tmc1 knockout mouse model through the RWM. This intervention resulted in an improvement in hearing threshold by approximately 15–30 dB. However, the viral transduction primarily occurred in IHCs, with persistent dysfunction observed in OHCs. [20]

In recent studies, researchers have presented evidence for the effectiveness of gene replacement therapy in treating HHL caused by mutations in Kcne1 and Syne4, marking a significant breakthrough. [23,24] Mutations in KCNE1, which is expressed in the stria vascularis (SV), are associated with Jervell and Lange-Nielsen syndrome type 2 (JLNS2). Wu et al. conducted a recent study demonstrating that the injection of AAV1-CB7-Kcne1 into the postnatal scala media (PSCC) of Kcne1-deficient mice at P0–P2 resulted in a dose-dependent improvement in auditory function. Specifically, in the low-dosage group, there was an improvement of 0–30 dB, while the high-dosage group exhibited an improvement of 40–70 dB in the 8–18 kHz frequency range. [23] In another study, the SYNE4 gene, responsible for encoding the nesprin-4 protein located in the outer nuclear membrane, was the focal point. Mutations in this gene give rise to DFNB76 in humans, a condition characterized by the mislocalization of OHC nuclei and subsequent OHC degeneration. [23]

Although gene replacement therapy has achieved considerable success in addressing HHL due to its efficiency and ease of manipulation, this strategy does come with certain drawbacks. For instance, it carries the potential risks of overexpression or ectopic expression of genes, as it cannot precisely regulate gene expression in accordance with cellular requirements. Furthermore, it presents challenges when attempting to introduce a sizable exogenous gene using multiple viral vectors due to capacity limitations. [19]

Gene Suppression:

RNA-level gene suppression therapies have the capacity to alter the expression of specific RNA molecules without disrupting DNA. It mainly includes two techniques RNAi and ASO.

RNAi:

RNA interference (RNAi) is a biological mechanism in which RNA molecules hinder gene expression or translation by targeting and deactivating specific messenger RNA (mRNA) molecules. In therapeutics, its primary focus often revolves around two distinct categories of small RNA molecules, namely small interfering RNA (siRNA) and microRNA (miRNA). [25] These small RNA agents constitute an innovative class of therapeutic interventions with the potential to address a wide spectrum of disorders, including cancer and infectious diseases. Numerous clinical trials involving siRNA and miRNA-based

medications have commenced, and there is now an RNAi-based therapeutic option available for the treatment of hereditary transthyretin-mediated amyloidosis in adults. The extensive range of miRNA functionality stems from its partial complementarity to multiple messenger RNAs (mRNAs). [26-28]

In the research study conducted by, Shibata et al. [29] devised an artificial miRNA designed to specifically target the mutation-carrying TMC1 allele in Beethoven mice, which serve as a murine model for human autosomal dominant non-syndromic hearing loss at the DFNA36 locus. They successfully attenuated the progressive hearing loss phenotype in mice treated at P1–2. Subsequently, Yoshimura et al. [30] conducted a follow-up study in which they treated older animals. They observed that a deceleration in the progression of hearing loss in animals treated at P15 and P30, the outcomes were less remarkable. Notably, in animals treated at P60, no discernible effect was observed. These findings imply that, for TMC1-related deafness, the window of opportunity for intervention through RNAi is temporally constrained, and beyond a specific point in time, targeted allele suppression ceases to have an effect.

In general, miRNAs exhibit lower specificity and typically regulate the expression of multiple genes. [26] They originate from a non-coding RNA primary transcript (pri-miRNA) that undergoes processing in the cell nucleus. This processing involves the conversion of pri-miRNA into a stem-loop pre-miRNA structure by the action of Drosha, an RNase III enzyme, and DGCR8, a double-stranded RNA-binding protein. Subsequently, the pre-miRNA is transported to the cytoplasm by Exportin 5, facilitated by GTP bound to the Ran protein. Once in the cytoplasm, the double-stranded ds RNA portion of the pre-miRNA is cleaved by Dicer, resulting in the production of a mature miRNA molecule. This mature miRNA can then be incorporated into the RNA-induced silencing complex (RISC). At this stage, both miRNA and siRNA utilize the same downstream intracellular machinery. The extensive regulatory capacity of miRNAs is attributed to their partial complementarity to multiple mRNA targets. [26-28]

Antisense oligonucleotides (ASO):

Antisense oligonucleotides (ASOs) are chemically modified nucleic acid sequences designed to bind to complementary RNA sequences through Watson–Crick base pairing. They exert control over gene expression through two primary mechanisms, which depend on their chemical properties and target. [31] ASOs, which are synthetically modified nucleic acid sequences, possess diverse functionalities, including up-regulating, down regulating, or modifying protein isoforms by binding to complementary RNA sequences. [32] The first mechanism, ASO knockdown, involves the cleavage of the RNA strand from the RNA–DNA duplex by ribonuclease-H (RNase-H). This cleavage leads to the degradation of the mRNA. The second mechanism, splice site switching, occurs when ASOs interfere with alternative splicing by targeting splice sites, exons, or introns. This interference results in the exclusion or inclusion of specific exons. [32] Currently, five ASOs have received approval from the US Food and Drug Administration, and numerous clinical trials are in progress. The first ASO to gain approval, fomivirsen, is employed for the treatment of cytomegalovirus-induced retinitis in patients with acquired immune deficiency syndrome. [33]

ASOs serve as potent gene suppression tools, capable of inhibiting the expression of mutant genes through either the degradation of target mRNA or the modulation of mRNA's alternative splicing. Additionally, various delivery routes are applicable for this treatment, encompassing intravenous injection, intraperitoneal injection, as well as local injection into sites like the inner ear, muscles, and nervous system. [34]

Wang and colleagues demonstrated that by directly microinjecting ASO-29 into the otic vesicle at embryonic day 12.5 (E12.5), they were able to restore harmonin expression and enhance auditory and vestibular function in Ush1c c.216A mutation mice. [35]

Since the therapeutic timeframe for restoring hearing in the Ush1c mice model with congenital deafness is approximately between P0 and P5, and considering that humans experience the onset of hearing much earlier than mice, it is crucial to conduct further investigations to determine the optimal treatment window in humans. This would involve assessing the effectiveness of early interventions within the inner ear. [36]

CRISPER Gene Therapy:

The remarkable efficiency and adaptability of CRISPR/Cas9 have positioned it at the forefront as a versatile tool for both preventing and treating human diseases. [2] Targeted genome editing has emerged as a potent tool in biological research. Among the three primary programmable nucleases—ZFNs (zinc finger nucleases), TALENs (transcriptional activator-like effector nucleases), and CRISPR (clustered regularly interspaced short palindromic repeats)/Cas9

(CRISPR-associated protein 9) ZFNs and TALENs necessitate the use of relatively large DNA fragments, typically in the range of 500 to 1500 base pairs. In contrast, Cas9 can precisely identify a target sequence with just a 20-base pair guide RNA (gRNA). Furthermore, CRISPR/Cas9 offers the advantage of multiplexing, as it allows for the delivery of multiple gRNAs to simultaneously target multiple genes within the same cell. [37]

Yeh and colleagues recently employed a targeted approach to address Tmc1 in the recessive Baringo mouse model, which carries the c.A545G mutation. Utilizing the mutation's specific nature, they harnessed a modified Cas9 nickase enzyme linked to cytidine deaminase. This innovative enzyme configuration enabled a single-base pair modification, facilitating the repair of the c.A545G mutation without the need for a double-stranded break. To deliver this cytosine base editor system, they packaged it into a dual AAV system, which was administered to the inner ear on post-natal day 1. [38]

Hsu and colleagues [39] have illustrated that limiting the concentration of single-guide RNA (single-gRNA) and Cas9 can enhance the on-target effects. In parallel, Kim et al. [40] have engineered RNA-guided nucleases to reduce the likelihood of producing off-target insertions and deletions (indels). In the context of auditory research, Gao et al. [41] conducted an experiment involving the injection of Cas9–gRNA–lipid complexes targeting the mutant TMC1 allele into P1 Beethoven mice. This intervention led to a substantial reduction in the progression of hearing loss. The study revealed significant preservation of hearing in the frequency range of 8 to 23 kHz, with average ABR thresholds being 15 dB lower in treated ears when compared to their untreated contralateral counterparts. Notably, this study marked the first demonstration of the potential of CRISPR/Cas9 for treating autosomal dominant hearing loss associated with hair cell dysfunction.

The research revealed that the CRISPR-CasRx system achieved a substantial 70% reduction in Tmc1 Bth mRNA expression in an in vivo setting. Importantly, this reduction was accomplished with minimal off-target effects. Consequently, this intervention led to improvements in auditory function by enhancing the survival rate of hair cells and reducing the extent of hair bundle degeneration. [42]

Cochleostomy:

Cochleostomy provides a direct route for delivering vectors into the endolymph by creating a hole between the basal turn of the cochlea and the round window. In adult mice, this surgical procedure presents more challenges because their cochlear bone is less pliable compared to neonatal mice. In neonatal mice, the trauma caused by the procedure can seal automatically with minimal endolymph leakage. Kilpatrick et al. demonstrated that, in adult mice, the virus successfully transduced hair cells (HCs) and supporting cells (SCs) through cochleostomy with minimal high-frequency hearing loss. [43] In contrast, Kawamoto et al. conducted a comparison between cochleostomy and canalostomy, revealing that while cochleostomy resulted in higher transduction efficiency, it also caused more significant hearing loss in adult mice. [44] Furthermore, Chien et al. reported that cochleostomy inflicted more substantial trauma compared to the RWM injection, although their transduction rates were similar. [45] Therefore, cochleostomy is considered invasive in adult mice, potentially disrupting the cochlear structure or inner ear homeostasis by mixing endolymph and perilymph. Additionally, this method has limitations regarding the volume of injections. The human cochlea is larger, which may facilitate future interventions in human patients. However, further validation is required. This approach allows direct access to the scala media, enabling substantial transduction in the marginal cells of the stria vascularis (SV). This is challenging to replicate with perilymph injection methods. [46]

Canalstomy:

Kawamoto et al. introduced the canalostomy technique as an alternative to the injection of RWM in the field of mouse cochlear gene therapy. In this study, they employed a method involving injecting an adenovirus carrying the bacterial lac Z gene through a fenestration made in the posterior semicircular canal, with the cannula directed toward the crus commune. [44] This method allows for the introduction of viral vectors into both the cochlea and vestibule without causing hearing impairment or obvious vestibular dysfunction in adult mice.[44,47] However, it remains challenging to determine whether the transgenes are effectively delivered into the endolymph or perilymph, a factor contingent on the orientation of the tube during the surgical procedure.[44] Later, Suzuki et al. [47] made modifications to this technique by using AAV2/Anc80L65 and targeting the injection toward the ampulla. Importantly, this modification did not compromise hearing, and it achieved a 100% transduction rate in inner hair cells (IHCs) and 80–90%, 35–70%, and 20–35%

transduction rates in apical, mid, and basal outer hair cells (OHCs), respectively.

Summary of Gene therapy

Author	Gene	Expression site	Vector	Route
Akil et al. (2012) [17]	VGlut3	IHCs	AAV2	RWM, cochleostomy
Askew et al. (2015) [20]	TMC1	HCs	AAV2	RMW
Taiber et al. (2021) [24]	SYNE4	OHCs	AAV9-PHP.B	PSCC
Wang et al. (2020) [35]	Ush1c c.216G>A	HCs	-	Otocysts
Lentz et al. (2020 [34]	Ush1c c.216G>A	HCs	-	RWM, trans-tympanic membrane topical tympanic membrane
Wu et al. (2021) [23]	KCNE1	SV	AAV1	PSCC
Yoshimura et al. (2019) [29]	TMC1	HCs	AAV2/9(miRNA)	RWM+CF
Zheng et al. (2022) [17]	TMC1	HCs	AAV9-PHP.eB (CasRx RNA editing)	RWM
Gao et al.101 (2018) [41]	TMC1	HCs	Lipofectamine 2000	PSCC
Yeh et al. (2020) [38]	TMC1	HCs		inner ear
Shibata et al. (2016) [30]	GJB2	SCs	rAAV2/9 (miRNA)	RWM

This chart provides an overview of different genes, their expression sites, vector types, and routes of administration in studies related to inner ear research. It is essential to acknowledge that this is a limited selection of studies. Presently, over 20 pre-clinical investigations employing knockout mice or mouse models simulating human deafness conditions have demonstrated varying levels of restored hearing function after the replacement of the faulty gene within the cochlea. [1]

Conclusions and Future Directions:

Gene therapy holds significant promise as a curative strategy for HL, as it has the potential to offer targeted treatment to fully restore auditory function, an accomplishment that conventional medical approaches cannot achieve. In this context, we have explored gene therapy as a potential treatment for hearing loss, focusing on three primary strategies: gene replacement, gene suppression, and gene editing which are currently employed in gene therapy. To advance the use of gene therapy for hearing loss in clinical settings, there is an ongoing need for significant advancements and breakthroughs.

Numerous reports have detailed gene therapy experiments in neonatal and adult mouse models of human deafness, showcasing varying degrees of success. Hearing loss is a widespread issue affecting millions globally. Nevertheless, it is crucial to recognize that there are numerous challenges to address before gene therapy for hearing loss can be administered to patients. This process could extend for several years beyond the successful conclusion of clinical trials. Furthermore, we require a thorough understanding of potential treatment side effects, encompassing factors such as the immune response and the treatment's durability, both of which remain poorly understood, particularly when applied to humans.

References:

1. Lahlou G, Calvet C, Giorgi M, Lecomte M-J, Safieddine S. Towards the Clinical Application of Gene Therapy for Genetic Inner Ear Diseases. J Clin Med. 2023;12:1046. [https://doi.org/10.3390/jcm12031046]
2. Omichi R, Shibata SB, Morton CC, Smith RJ. Gene therapy for hearing loss. Hum Mol Genet. 2019;28(R1):R65-79.
3. Smith RJH, Bale JF, White KR. Sensorineural Hearing Loss in Children. Lancet. 2005; 365:879–890.
4. François M, Boukhris M, Noel-Petroff N. Schooling of Hearing-Impaired Children and Benefit of Early Diagnosis. Eur Ann Otorhinolaryngol Head Neck Dis. 2015;132:251–255.
5. Lin FR, Yaffe K, Xia J, Xue QL, Harris TB, Purchase-Helzner E, Satterfield S, Ayonayon HN, Ferrucci L, Simonsick EM. Hearing Loss and Cognitive Decline in Older Adults. JAMA Intern Med. 2013;173:293–299.
6. Cunningham LL, Tucci DL. Hearing Loss in Adults. N Engl J Med. 2017;377:2465–2473.

7. Liberman MC. 2017. Noise-induced and age-related hearing loss: new perspectives and potential therapies. F1000Res. 6:927. [doi:10.12688/f1000research.11310.1]
8. Richardson GP, Petit C. 2019. Hair-bundle links: genetics as the gateway to function. Cold Spring Harb Perspect Med. 9:a033142. [doi:10.1101/cshperspect.a033142]
9. Taiber S, Gwilliam K, Hertzano R, Avraham KB. 2022. The genomics of auditory function and disease. Annu Rev Genomics Hum Genet. 23:275–299. [doi:10.1146/annurev-genom-121321-094136]
10. Morgan M, Schott JW, Rossi A, Landgraf C, Warnecke A, Staecker H, Lesinski-Schiedat A, Schlegelberger B, Büning H, Auber B, Schambach A. Gene therapy as a possible option to treat hereditary hearing loss. Medizinische Genetik. 2020;32(2):149-159.
11. Géléoc GS, Holt JR. Sound strategies for hearing restoration. Science. 2014;344: 1241062. [doi:10.1126/science.1241062]
12. Liu SS, Yang R. Inner ear drug delivery for sensorineural hearing loss: current challenges and opportunities. Front Neurosci. 2022; 16:867453. [doi:10.3389/fnins.2022.867453]
13. Tao J, Bauer DE, Chiarle R. Assessing and Advancing the Safety of CRISPR-Cas Tools: From DNA to RNA Editing. Nat Commun. 2023;14:212.
14. Chen C, Yang Z, Tang X. Chemical Modifications of Nucleic Acid Drugs and Their Delivery Systems for Gene-Based Therapy. Med Res Rev. 2018;38:829–869.
15. Sacheli R, Delacroix L, Vandenackerveken P, et al. Gene transfer in inner ear cells: a challenging race. Gene Ther. 2013;20:237–247. [https://doi.org/10.1038/gt.2012.51]
16. Ahmed H, Shubina-Oleinik O, Holt JR. Emerging gene therapies for genetic hearing loss. J Assoc Res Otolaryngol. 2017;18:649–670.
17. Akil O, Seal RP, Burke K, Wang C, Alemi A, During M, Edwards RH, Lustig LR. Restoration of hearing in the VGLUT3 knockout mouse using virally mediated gene therapy. Neuron. 2012;75:283–293.
18. Zhao X, Liu H, Liu H, Cai R, Wu H. Gene therapy restores auditory functions in an adult Vglut3 knockout mouse model. Hum Gene Ther. 2022;33:729–739.
19. Jiang L, Wang D, He Y, Shu Y. Advances in gene therapy hold promise for treating hereditary hearing loss. Mol Ther. 2023 Feb 8.
20. Askew C, Rochat C, Pan B, Asai Y, Ahmed H, Child E, Schneider BL, Aebischer P, Holt JR. Tmc gene therapy restores auditory function in deaf mice. Sci Transl Med. 2015;7:295ra108.
21. Nist-Lund CA, Pan B, Patterson A, Asai Y, Chen T, Zhou W, Zhu H, Romero S, Resnik J, Polley DB, et al. Improved TMC1 gene therapy restores hearing and balance in mice with genetic inner ear disorders. Nat Commun. 2019;10:236.
22. Wu J, Solanes P, Nist-Lund C, Spataro S, Shubina-Oleinik O, Marcovich I, Goldberg H, Schneider BL, Holt JR. Single and dual vector gene therapy with AAV9-PHP.B rescues hearing in Tmc1 mutant mice. Mol Ther. 2021;29:973–988.
23. Wu X, Zhang L, Li Y, Zhang W, Wang J, Cai C, Lin X. Gene therapy via canalostomy approach preserves auditory and vestibular functions in a mouse model of Jervell and Lange-Nielsen syndrome type 2. Nat Commun. 2021;12:697.
24. Taiber S, Cohen R, Yizhar-Barnea O, Sprinzak D, Holt JR, Avraham KB. Neonatal AAV gene therapy rescues hearing in a mouse model of SYNE4 deafness. EMBO Mol Med. 2021; 13: e13259.
25. Adams D, Gonzalez-Duarte A, O'Riordan WD, Yang CC, Ueda M, Kristen AV, Tournev I, Schmidt HH, Coelho T, Berk JL, et al. Patisiran, an RNAi therapeutic, for hereditary transthyretin amyloidosis. N Engl J Med. 2018;379:11–21.
26. Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. Cell. 2004;116:281–297.
27. Ha M, Kim VN. Regulation of microRNA biogenesis. Nat Rev Mol Cell Biol. 2014; 15: 509–524.
28. Lam JK, Chow MY, Zhang Y, Leung SW. siRNA versus miRNA as therapeutics for gene silencing. Mol Ther Methods Clin Dev. 2015; 4:e252.
29. Yoshimura H, Shibata SB, Ranum PT, Moteki H, Smith RJH. Targeted allele suppression prevents progressive hearing loss in the mature murine model of human TMC1 deafness. Mol Ther. 2019;27:681–690.
30. Shibata SB, Ranum PT, Moteki H, Pan B, Goodwin AT, Goodman SS, Abbas PJ, Holt JR, Smith RJH. RNA interference prevents autosomal-dominant hearing loss. Am J Hum Genet. 2016;98:1101–1113.
31. Studzinska S. Review on investigations of antisense oligonucleotides with the use of mass spectrometry. Talanta. 2018;176:329–343.

32. Goyal N, Narayanaswami P. Making sense of antisense oligonucleotides: a narrative review. Muscle Nerve. 2018;57:356–370.
33. Urban E, Noe CR. Structural modifications of antisense oligonucleotides. Farmaco. 2003;58: 243–258.
34. Lentz JJ, Pan B, Ponnath A, Tran CM, Nist-Lund C, Galvin A, Goldberg H, Robillard KN, Jodelka FM, Farris HE, et al. Direct delivery of antisense oligonucleotides to the middle and inner ear improves hearing and balance in Usher mice. Mol Ther. 2020;28:2662–2676.
35. Wang L, Kempton JB, Jiang H, Jodelka FM, Brigande AM, Dumont RA, Rigo F, Lentz JJ, Hastings ML, Brigande JV. Fetal antisense oligonucleotide therapy for congenital deafness and vestibular dysfunction. Nucleic Acids Res. 2020;48:5065–5080.
36. Hastings ML, Brigande JV. Fetal gene therapy and pharmacotherapy to treat congenital hearing loss and vestibular dysfunction. Hear Res. 2020;394:107931.
37. Zou B, Mittal R, Grati M, Lu Z, Shu Y, Tao Y, Feng Y, Xie D, Kong W, Yang S, et al. The application of genome editing in studying hearing loss. Hear Res. 2015;327:102–108.
38. Yeh W-H, Shubina-Oleinik O, Levy JM, Pan B, Newby GA, Wornow M, Burt R, Chen JC, Holt JR, Liu DR. In Vivo Base Editing Restores Sensory Transduction And Transiently Improves Auditory Function In A Mouse Model Of Recessive Deafness. Science Translational Medicine. 2020;12(546).
39. Hsu PD, Scott DA, Weinstein JA, Ran FA, Konermann S, Agarwala V, Li Y, Fine EJ, Wu X, Shalem O, et al. DNA targeting specificity of RNA-guided Cas9 nucleases. Nat Biotechnol. 2013;31:827.
40. Kim S, Kim D, Cho SW, Kim J, Kim JS. Highly efficient RNA-guided genome editing in human cells via delivery of purified Cas9 ribonucleoproteins. Genome Res. 2014;24: 1012–1019.
41. Gao X, Tao Y, Lamas V, Huang M, Yeh W-H, Pan B, Hu YJ, Hu JH, Thompson DB, Shu Y, et al. Treatment of autosomal dominant hearing loss by in vivo delivery of genome editing agents. Nature. 2018;553:217–221.
42. Zheng Z, Li G, Cui C, Wang F, Wang X, Xu Z, Guo H, Chen Y, Tang H, Wang D, et al. Preventing autosomal-dominant hearing loss in Bth mice with CRISPR/CasRx-based RNA editing. Signal Transduct Target Ther. 2022; 7:79.
43. Kilpatrick LA, Li Q, Yang J, Goddard JC, Fekete DM, Lang H. Adeno-associated virus-mediated gene delivery into the scala media of the normal and deafened adult mouse ear. Gene Ther. 2011;18:569–578.
44. Kawamoto K, Oh SH, Kanzaki S, Brown N, Raphael Y. The functional and structural outcome of inner ear gene transfer via the vestibular and cochlear fluids in mice. Mol Ther. 2001;4:575–585.
45. Chien WW, McDougald DS, Roy S, Fitzgerald TS, Cunningham LL. Cochlear gene transfer mediated by adeno-associated virus: comparison of two surgical approaches. Laryngoscope. 2015;125:2557–2564.
46. Chang Q, Wang J, Li Q, Kim Y, Zhou B, Wang Y, Li H, Lin X. Virally mediated Kcnq1 gene replacement therapy in the immature scala media restores hearing in a mouse model of human Jervell and Lange-Nielsen deafness syndrome. EMBO Mol Med. 2015;7:1077–1086.
47. Suzuki J, Hashimoto K, Xiao R, Vandenberghe LH, Liberman MC. Cochlear gene therapy with ancestral AAV in adult mice: complete transduction of inner hair cells without cochlear dysfunction. Sci Rep. 2017; 7:45524.