AAV1-hOTOF gene therapy for autosomal recessive deafness 9: a single-arm trial

Jun Lv, Hui Wang*, Xiaoting Cheng*, Yuxin Chen*, Daqi Wang*, Longlong Zhang, Qi Cao, Honghai Tang, Shaowei Hu, Kaiyu Gao, Mengzhao Xun, Jinghan Wang, Zijing Wang, Biyun Zhu, Chong Cui, Ziwen Gao, Luo Guo, Sha Yu, Luoying Jiang, Yanbo Yin, Jiajia Zhang, Bing Chen, Wuqing Wang†, Renjie Chai†, Zheng-Yi Chen†, Huawei Li†, and Yilai Shu†*

Summary

Background **Autosomal recessive deafness 9, caused by mutations of the *OTOF* gene, is characterised by congenital or prelingual, severe-to-complete, bilateral hearing loss. However, no pharmacological treatment is currently available for congenital deafness. In this Article, we report the safety and efficacy of gene therapy with an adeno-associated virus (AAV) serotype 1 carrying a human *OTOF* transgene (AAV1-hOTOF) as a treatment for children with autosomal recessive deafness 9.**

Methods **This single-arm, single-centre trial enrolled children (aged 1–18 years) with severe-to-complete hearing loss and confirmed mutations in both alleles of *OTOF*, and without bilateral cochlear implants. A single injection of AAV1-hOTOF was administered into the cochlea through the round window. The primary endpoint was dose-limiting toxicity at 6 weeks after injection. Auditory function and speech were assessed by appropriate auditory perception evaluation tools. All analyses were done according to the intention-to-treat principle. This trial is registered with Chinese Clinical Trial Registry, ChiCTR2200063181, and is ongoing.**

Findings **Between Oct 19, 2022, and June 9, 2023, we screened 425 participants for eligibility and enrolled six children for AAV1-hOTOF gene therapy (one received a dose of 9×10^{11} vector genomes [vg] and five received $1\cdot5\times10^{12}$ vg). All participants completed follow-up visits up to week 26. No dose-limiting toxicity or serious adverse events occurred. In total, 48 adverse events were observed; 46 (96%) were grade 1–2 and two (4%) were grade 3 (decreased neutrophil count in one participant). Five children had hearing recovery, shown by a 40–57 dB reduction in the average auditory brainstem response (ABR) thresholds at 0·5–4·0 kHz. In the participant who received the 9×10^{11} vg dose, the average ABR threshold was improved from greater than 95 dB at baseline to 68 dB at 4 weeks, 53 dB at 13 weeks, and 45 dB at 26 weeks. In those who received $1\cdot5\times10^{12}$ AAV1-hOTOF, the average ABR thresholds changed from greater than 95 dB at baseline to 48 dB, 38 dB, 40 dB, and 55 dB in four children with hearing recovery at 26 weeks. Speech perception was improved in participants who had hearing recovery.**

Interpretation **AAV1-hOTOF gene therapy is safe and efficacious as a novel treatment for children with autosomal recessive deafness 9.**

Funding **National Natural Science Foundation of China, National Key R&D Program of China, Science and Technology Commission of Shanghai Municipality, and Shanghai Refreshgene Therapeutics.**

Introduction

Up to 60% of cases of congenital deafness, which affects approximately 26 million people worldwide, are caused by genetic mutations.[1,2] Autosomal recessive deafness 9, characterised by severe-to-complete, congenital or prelingual, bilateral hearing loss, results from dysfunction of otoferlin (encoded by the *OTOF* gene)[3] and accounts for 2–8% of cases of congenital deafness.[4–7] Autosomal recessive deafness 9 has profound effects on speech development if not treated early in life.[8,9]

Gene therapy has previously shown success in treating various human diseases caused by mutation of a single gene,[10,11] and studies in animal models have established the efficacy of gene therapy for congenital hearing loss.[12] However, the safety and efficacy of gene therapy on congenital hearing loss in humans is poorly explored. We and other groups have reported restoration of auditory function in *Otof* (knockout) mouse models via gene replacement with the otoferlin coding sequence delivered by dual-adeno-associated virus (AAV) vectors, which permits circumvention of the size limitation of a single AAV (which cannot accommodate a full-length otoferlin coding sequence).[13–15] We subsequently designed the AAV1-hOTOF vector-based gene therapy carrying the human otoferlin coding sequence driven by *Myo15*, a hair cell-specific promoter, and verified its efficacy and safety in mice and the safety of AAV1 vector-carrying *MYO15* and a reporter transgene in non-human primates.[16] Here, we report the results of a single-arm trial in which we investigated the safety and efficacy of AAV1-hOTOF treatment in children with autosomal recessive deafness 9.

Published **Online** January 24, 2024 https://doi.org/10.1016/S0140-6736(23)02874-X

*Joint first authors

†Joint last authors

ENT Institute and Otorhinolaryngology Department, Eye & ENT Hospital (J Lv MMed, H Wang MD, X Cheng MD, Y Chen PhD, D Wang PhD, L Zhang MMed, Q Cao BMed, H Tang PhD, S Hu PhD, M Xun BMed, J Wang MD, Z Wang BMed, B Zhu PhD, C Cui BMed, Z Gao PhD, L Guo PhD, S Yu MD, L Jiang BMed, Y Yin MMed, J Zhang MMed, Prof B Chen MD, Prof W Wang MD, Prof H Li MD, Prof Y Shu MD)**, NHC Key Laboratory of Hearing Medicine** (J Lv, H Wang, X Cheng, Y Chen, D Wang, L Zhang, Q Cao, H Tang, S Hu, M Xun, J Wang, Z Wang, B Zhu, C Cui, Z Gao, L Guo, S Yu, L Jiang, Y Yin, J Zhang, Prof B Chen, Prof W Wang, Prof H Li, Prof Y Shu)**, State Key Laboratory of Medical Neurobiology and MOE Frontiers Center for Brain Science** (J Lv, C Cui, L Jiang, J Zhang, Prof H Li, Prof Y Shu)**, Institutes of Biomedical Science** (J Lv, C Cui, L Jiang, J Zhang, Prof H Li, Prof Y Shu)**, Fudan University, Shanghai, China; Research and Development Department, Shanghai Refreshgene Therapeutics, Shanghai, China** (K Gao PhD)**; State Key Laboratory of Digital Medical Engineering** (Prof R Chai DPhil)**, Department of Otolaryngology Head and Neck Surgery of Zhongda Hospital** (Prof R Chai)**, Advanced Institute for Life and Health** (Prof R Chai)**, Jiangsu Province High-Tech Key Laboratory for Bio-Medical Research** (Prof R Chai)**, Southeast University, Nanjing, China; Co-Innovation Center of Neuroregeneration, Nantong University, Nantong, China**

(Prof R Chai); **Department of Neurology of Aerospace Center Hospital** (Prof R Chai), **School of Life Science** (Prof R Chai), **Beijing Institute of Technology, Beijing, China; Department of Otolaryngology-Head and Neck Surgery** (Prof Z Chen DPhil), **Graduate Program in Speech and Hearing Bioscience and Technology and Program in Neuroscience** (Prof Z Chen), **Harvard Medical School, Boston, MA, USA; Eaton-Peabody Laboratory, Massachusetts Eye and Ear, Boston, MA, USA** (Prof Z Chen)

Correspondence to:
Yilai Shu, ENT Institute and Otorhinolaryngology Department, Eye & ENT Hospital of Fudan University, Shanghai, 200031, China
yilai_shu@fudan.edu.cn

Research in context

Evidence before this study

We searched PubMed from inception to Oct 1, 2023, for all studies in English on *OTOF* mutations, their associations with congenital hearing loss, including autosomal recessive deafness 9 (DFNB9), and all related animal preclinical and human clinical trials. The search terms included "*OTOF*", "DFNB9", "hereditary hearing loss", "gene therapy", "DFNB9 trial", "DFNB9 mouse", or combinations thereof. We also searched ClinicalTrials.gov for related clinical trials. We found proof-of-principle of gene therapy for DFNB9 in animal models using recombinant adeno-associated viral vectors and four clinical trials. We found no reports on the safety or efficacy of human gene therapy to treat DFNB9.

Added value of this study

To our knowledge, this study is the first prospectively registered and the first-in-human clinical trial with the largest number of patients and the longest follow-up published to date of gene therapy targeting *OTOF* to treat autosomal recessive deafness 9. These data indicate that adeno-associated virus (AAV) administration in the human inner ear is safe and efficacious in treating genetic hearing loss. The study extends the utility of dual AAV to overcome the gene size limit to treat human diseases.

Implications of all the available evidence

Our study provides evidence of the safety and efficacy of gene therapy to treat autosomal recessive deafness 9 and lays a foundation for gene therapy as a novel treatment for other forms of genetic hearing loss. The process and techniques developed in this study are likely to advance the field of gene therapy for hearing loss.

Methods

Study design and participants

This single-arm trial was done at the Eye & ENT Hospital of Fudan University (Shanghai, China). Children (aged 1–18 years) of either sex were eligible if they had autosomal recessive deafness 9 due to biallelic pathogenic (or likely pathogenic) *OTOF* mutations and severe-to-complete hearing loss, as defined by average auditory brainstem response (ABR) threshold (0·5, 1·0, 2·0, and 4·0 kHz) at 65 dB or greater.[17] Before each hearing test, the participant's body temperature was measured to confirm that it was within a normal range of 36–37°C. Participants with bilateral cochlear implants were excluded. Blood samples were obtained from the participants and both their biological parents. The genotype of participants was assessed by whole-exome sequencing and *OTOF* variants in participants and their biological parents were also detected by Sanger sequencing. The pathogenicity of variants was confirmed by agreement from three independent geneticists (LG and SY plus another geneticist not otherwise affiliated with the trial) according to the latest version of the American College of Medical Genetics and Genomics' and Association for Molecular Pathology's Variant Interpretation Guidelines and ClinGen Hearing Loss Expert Panel Specifications. For safety reasons, the first three participants were required to be at least 3 years old; subsequent participants could be enrolled from the age of 1 year. Participants were excluded if they produced AAV1-neutralising antibodies at a titre of 1:2000 or greater. Detailed inclusion and exclusion criteria are listed in the protocol (appendix pp 76–78).

See Online for appendix

This study was done in accordance with the Declaration of Helsinki and Good Clinical Practice guidelines. The trial protocol ([2022]2022085-1) was approved by the Ethics Committee of the Eye & ENT Hospital of Fudan University in June, 2022, and subsequent amendments were approved by the same committee. Written informed consent was obtained from the legal guardians (both parents) of the children before any protocol-related procedure commenced.

Procedures

AAV1-hOTOF was developed by researchers at the Eye & ENT Hospital of Fudan University and Refreshgene Therapeutics (Shanghai, China), and manufactured by PackGene Biotechnology (Guangzhou, China). For each participant, a single, minimally invasive injection of AAV1-hOTOF was administered to one ear through the round window membrane with stapes fenestration, on the side with the most severe hearing loss or, for participants with a cochlear implant, on the side with no cochlear implant. The planned escalating doses were 30 µL (9×10^{11} vector genomes [vg]) per ear or 50 µL ($1·5\times10^{12}$ vg) per ear; all doses contained a 1:1 mixture of AAV1-hOTOF NT (the 5' terminal segment of the *OTOF* coding sequence) and AAV1-hOTOF CT (the 3□ terminal segment of the *OTOF* coding sequence). Participants were enrolled sequentially after a dose-limiting toxicity assessment was completed within 6 weeks for the first participant at each dose group (appendix p 10). Details of screening, enrolment, and surgical procedures are described in the appendix (pp 4, 10–11).

To minimise the risk of a potential inflammatory response, dexamethasone was given intravenously at 0·3 mg/kg per day for 8 consecutive days, starting 3 days before the AAV1-hOTOF injection. To minimise the risk of infection, ceftriaxone was given intravenously at 80 mg/kg per day for 5 consecutive days, starting on the day of AAV1-hOTOF injection. Either CT or MRI was done at baseline and at 6 weeks to investigate the structure of ear. At baseline, 3 days, 7 days, 2 weeks, 4 weeks, 6 weeks, 13 weeks, and 26 weeks, urine samples

were collected for routine urine tests and blood samples were collected for routine blood tests and for blood biochemistry, coagulation function tests, AAV1-neutralising antibodies tests, interferon-gamma enzyme-linked immunosorbent spot assays, or vector DNA in circulation. AAV1-neutralising antibodies and interferon-gamma were assessed at baseline, 6 weeks, and 13 weeks, and circulating vector DNA was assessed at baseline and at 1 week.

Outcomes

The primary endpoint was dose-limiting toxicity, defined as haematologic toxicity of grade 4 or worse, non-haematologic toxicity of grade 3 or worse, or aural toxicity of grade 2 or worse within 6 weeks of injection, graded according to Common Terminology Criteria for Adverse Events (version 5.0). Secondary outcomes were preliminary efficacy (ie, auditory function and speech perception) and safety. Auditory function was assessed using ABR, auditory steady-state response, pure-tone audiometry, and distortion product otoacoustic emission test at baseline and at 4, 6, 13, and 26 weeks. The average thresholds of ABR, auditory steady-state response, or pure-tone audiometry were calculated as the arithmetic average thresholds at 0·5, 1, 2, and 4 kHz.[17] Addtionally, questionnaires were used to assess auditory function and speech perception: the Meaningful Auditory Integration Scale,[18] the Infant-Toddler Meaningful Auditory Integration Scale,[18] the Categories of Auditory Performance score,[19] the Speech Intelligibility Rating score,[20] and Meaningful Use of Speech Scale.[21] Speech perception was also assessed by Mandarin Speech Perception software (version 5.04.01)[22] and the Angel Test software (version 5.01.01).[23,24] Hearing recovery was defined as a 10 dB reduction in the average ABR threshold, as adopted from current guidelines for sudden sensorineural hearing loss.[25] Video head impulse test was used to assess vestibular function at baseline, 4, 6, 13, and 26 weeks. Safety was measured by the presence of adverse events, defined as any unfavourable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medical treatment or procedure that might or might not be considered related to the medical treatment or procedure. Adverse events will be recorded after treatment until the trial is complete at 52 weeks. Details of outcomes are described in the appendix (pp 5–9). Otoscopic examination was done at weeks 13 and 26 to confirm healing of the tympanic membrane after the injection.

Statistical analysis

All analyses were based on the intention-to-treat principle. All analyses, including demographic characteristics, safety, auditory function, and speech recognition, were descriptively summarised. This trial is registered at the Chinese Clinical Trial Registry, ChiCTR2200063181, and is ongoing.

Role of the funding source

The commercial funder of the study was involved in study design, protocol amendment, data analysis, interpretation of data, manuscript revisions, and decision for submission. All other funding sources had no role in study design, data collection, data analysis, data interpretation, the writing of the report, or the decision to submit the paper for publication.

Results

Between Oct 19, 2022, and June 9, 2023, we screened 425 participants for eligibility and enrolled six eligible participants (appendix p 10). One participant received an

	Participant 1	Participant 2	Participant 3	Participant 4	Participant 5	Participant 6
Sex	Female	Male	Female	Male	Female	Male
Age, years	4·8	5·0	6·2	2·1	3·3	1·0
Ethnicity	Han	Han	Han	Han	Han	Han
OTOF (HGNC:8515) mutations						
Mutation in allele 1	c.2985C>A (p.Cys995*)	c.2215-1G>C	c.4961-2A>C	c.2215-1G>C	c.3409-11A>G	c.5647C>T (p.Gln1883*)
Mutation in allele 2	c.5203C>T (p.Arg1735Trp)	c.5108delinsTCTT (p.Arg1703delinsLeuPhe)	c.5567G>A (p.Arg1856Gln)	c.4225A>T (p.Lys1409*)	c.5647C>T (p.Gln1883*)	c.5728G>A (p.Glu1910Lys)
Hearing threshold†						
Auditory brainstem response, dB	>95‡	>95	>95	>95	>95	>95
Auditory steady-state response, dB	80	111	98	100	>98	100
Pure-tone audiometry, dB	>115	100	106	NA§	NA§	NA§
Cochlear implant	Right ear	Left ear	Right ear	None	Right ear	None
Vector dose administered, vg	9×10^{11}	$1{\cdot}5\times10^{12}$	$1{\cdot}5\times10^{12}$	$1{\cdot}5\times10^{12}$	$1{\cdot}5\times10^{12}$	$1{\cdot}5\times10^{12}$

NA=not available. vg=vector genomes. *Nonsense mutation. †Average hearing threshold at 0·5–4·0 kHz; the symbol ">" in hearing threshold means no response at maximum sound intensity level. ‡Only click-evoked auditory brainstem response was tested at baseline in participant 1; at baseline, auditory brainstem response was measured at 0·25, 0·50, 1·00, 2·00, and 4·00 kHz in the other five participants. §Participants 4, 5, and 6 could not complete pure-tone audiometry due to their young age.

Table 1: **Baseline characteristics, genotype, and vector dose for each participant**

AAV1-hOTOF dose of 9×10^{11} vg and five participants received AAV1-hOTOF doses of $1{\cdot}5\times10^{12}$ vg. Median follow-up was 26 weeks (IQR 26–26) and all six participants completed the 26-week assessment. The median age of participants was 4·1 years (IQR 2·4–5·0), three were girls, three were boys, and all were of Han ethnicity (table 1). Participants 1, 2, 3, and 5 had a unilateral cochlear implant, and participants 4 and 6 had no cochlear implant. All identified variants in the *OTOF* gene were classified as pathogenic or likely pathogenic and all enrolled participants had complete hearing loss (no ABR response at a stimulus of 95 dB) at baseline (table 1).

In the participant who received the 9×10^{11} vg AAV1-hOTOF dose (participant 1), six adverse events (all grade 1–2) were observed within the 26-week follow-up (table 2). In the five participants who received $1{\cdot}5\times10^{12}$ vg AAV1-hOTOF, 42 adverse events were observed (table 2); all were grade 1–2, except for two grade 3 events of decreased neutrophil count in participant 5 (appendix p 15), which resolved spontaneously. No dose-limiting toxicity or serious adverse event was observed.

Two participants (4 and 5) in the $1{\cdot}5\times10^{12}$ vg group had three events of slightly prolonged activated partial thromboplastin time (<1·2 times the upper limit of normal [ULN] range; appendix p 15). Three participants (4, 5, and 6) had four events of transient reduction in fibrinogen, but no signs of haemorrhage. Increased lactate dehydrogenase (<1·5 times higher than the ULN range; appendix p 15) was observed in five participants (all but participant 2), without clinical manifestations. We found no increases in alanine aminotransferase or serum bilirubin concentrations in any participants.

	9×10^{11} vg (n=1)			$1{\cdot}5\times10^{12}$ vg (n=5)		
	Grade 1	Grade 2	Grade 3	Grade 1	Grade 2	Grade 3
Increased lymphocyte count	0	1	0	0	5	0
Decreased neutrophil count	0	0	0	0	3	2
Decreased haemoglobin	0	0	0	3	0	0
Increased lactate dehydrogenase	1	0	0	5	0	0
Hyperglycaemia	2	0	0	0	0	0
Increased triglycerides	1	0	0	0	0	0
Decreased haptoglobin	0	0	0	3	0	0
Increased cholesterol	0	0	0	1	0	0
Prolonged activated partial thromboplastin time	0	0	0	3	0	0
Decreased fibrinogen	0	0	0	4	0	0
Influenza-like symptoms	1	0	0	0	0	0
COVID-19	0	0	0	2	0	0
Fever	0	0	0	7	0	0
Rhinobyon	0	0	0	1	0	0
Nausea	0	0	0	1	0	0
Decreased appetite	0	0	0	1	0	0
Constipation	0	0	0	1	0	0

No grade 4 or grade 5 adverse events occurred during the trial. vg=vector genomes.

Table 2: **Summary of adverse events**

Aspartate aminotransferase (reference range 15–37 U/L) increased in participant 1 (39 U/L at 1 week after injection) and participant 5 (39 U/L at 2 weeks after injection); neither participant reached Hy's law criteria for liver injury, and both cases of increased aspartate aminotransferase resolved spontaneously by 4 weeks after injection (appendix p 15). Vestibular function was normal in participants 1, 2, 3, and 5 at baseline and at 4, 6, 13, and 26 weeks after injection (appendix p 12); participants 4 and 6 could not finish the test because of their young age. Otoscopic examination showed healing of the tympanic membrane (appendix p 13); all participants had an otoscopic examination at 26 weeks, except participant 6, who had an examination at 13 weeks. No obvious ear abnormalities were observed by radiographic assessments in any participant (data not shown).

All participants had an increase in AAV1-neutralising antibodies from baseline to weeks 6 and 13 (table 3). T cell responses to the AAV1 capsid, as reflected by the concentration of interferon gamma, were negative for all participants at both 6 weeks and 13 weeks (table 3). Vector DNA in the blood was not detectable in any participant at 7 days (table 3).

In the participant injected with the 9×10^{11} vg dose (participant 1), the click-evoked ABR threshold was greater than 95 dB at baseline, and the average ABR threshold was 68 dB at 4 weeks, 70 dB at 6 weeks, 53 dB at 13 weeks, and 45 dB at 26 weeks (figure A; appendix p 16). The best recovery of the ABR threshold was 35 dB at 0·25 kHz and 2 kHz at 26 weeks (appendix p 16). The average auditory steady-state response threshold was 80 dB at 4 weeks, 73 dB at 6 weeks, 60 dB at 13 weeks, and 38 dB at 26 weeks (figure A). 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 signal-to-noise ratio of the distortion product otoacoustic emission test was slightly lower at 4 weeks compared with the baseline, but gradually recovered within 26 weeks (appendix p 14). The noise floor of the distortion product otoacoustic emission test results is provided in the appendix (p 17).

In the $1{\cdot}5\times10^{12}$ vg group, one participant (participant 2) did not show hearing improvement within the 26-week follow-up (figure B; appendix p 16). We found robust hearing improvement in participants 3, 4, 5, and 6 (figure C–F; appendix p 16). In participant 3, the average ABR threshold was greater than 95 dB at baseline, 60 dB at 4 weeks, 63 dB at 6 weeks, 63 dB at 13 weeks, and 48 dB at 26 weeks (figure C). The average auditory steady-state response was gradually reduced to 55 dB at 26 weeks from 98 dB at baseline, and the average pure-tone audiometry was gradually reduced to 45 dB at 26 weeks from 106 dB at baseline (figure C). In participant 4, the average ABR threshold was reduced from greater than 95 dB at baseline to 68 dB at 4 weeks, 55 dB at 6 weeks, 50 dB at 13 weeks, and 38 dB at

	Participant 1	Participant 2	Participant 3	Participant 4	Participant 5	Participant 6
AAV1-neutralising antibodies						
Baseline	<1:5	1:35	<1:5	<1:5	<1:5	<1:5
6 weeks	1:405	1:3645	1:405	1:135	1:1215	1:405
13 weeks	1:1215	1:3645	1:1215	1:135	1:1215	1:1215
Interferon gamma						
Baseline	Negative	Negative	Negative	Negative	Negative	Negative
6 weeks	Negative	Negative	Negative	Negative	Negative	Negative
13 weeks	Negative	Negative	Negative	Negative	Negative	Negative
Vector DNA						
Baseline	Negative	Negative	Negative	Negative	Negative	Negative
1 week	Negative	Negative	Negative	Negative	Negative	Negative

Negative indicates that the T cell responses to the AAV1 capsid or vector DNA were below the lower limit of detection. AAV1=adeno-associated virus serotype 1.

Table 3: **Immunity response and vector shedding**

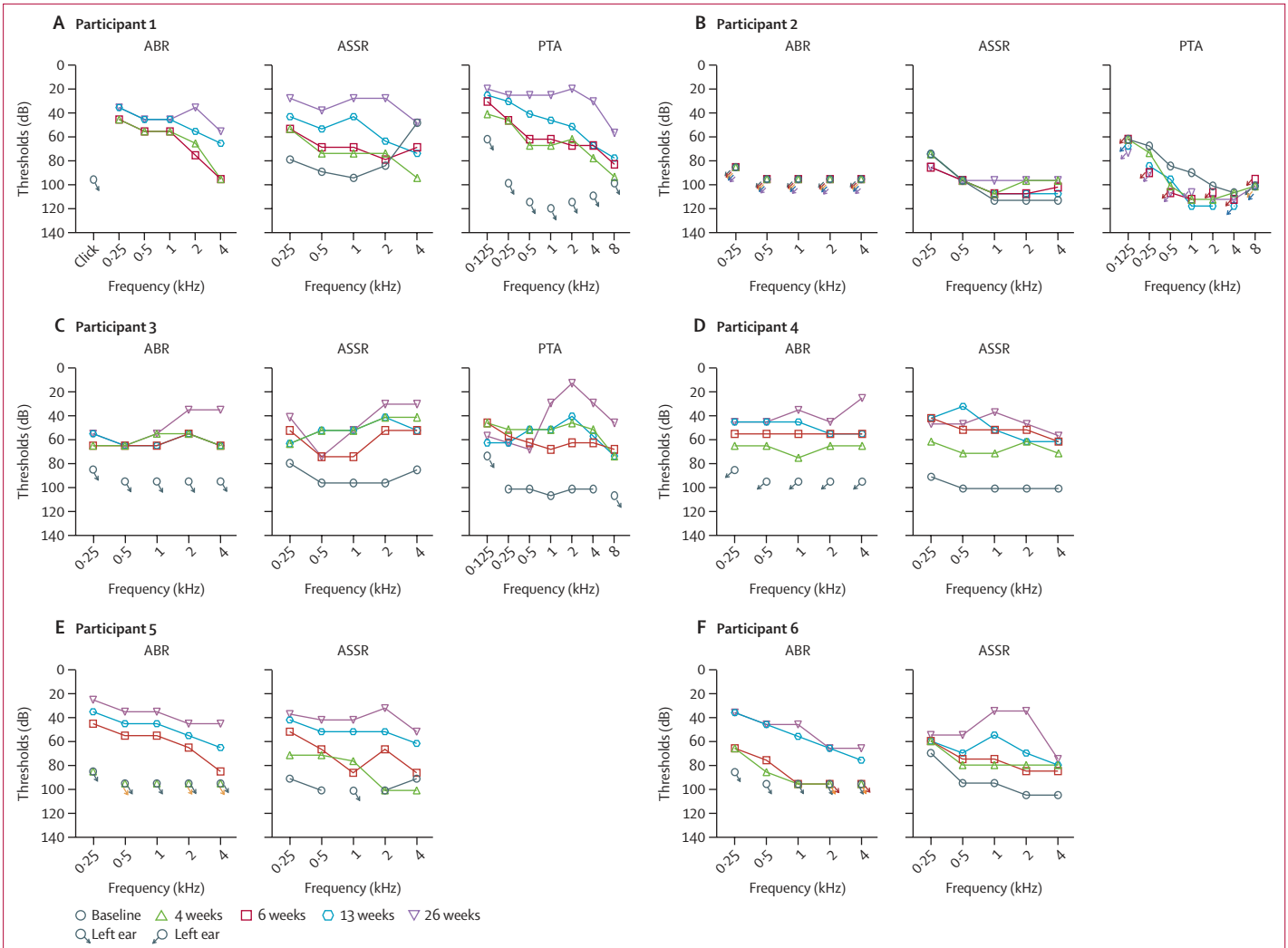

Figure: **Audiometric test before and after inner ear administration of AAV1-hOTOF**

Participant 1 received 9×10^{11} vg AAV1-hOTOF; all others received $1{\cdot}5 \times 10^{12}$ vg AAV1-hOTOF. Arrows indicate no response even at the maximum sound intensity level. Participants 4, 5, and 6 could not complete pure-tone audiometry due to their young age. ABR=auditory brainstem response. ASSR=auditory steady-state response. PTA=pure-tone audiometry. vg=vector genomes.

26 weeks (figure D). The best recovery of ABR threshold for participant 4 was 25 dB at 4 kHz at 26 weeks (appendix p 16). The average auditory steady-state response was gradually reduced to 50 dB at 26 weeks from 100 dB at baseline. In participant 5, the average ABR threshold was greater than 95 dB at baseline, greater than 95 dB at 4 weeks, 65 dB at 6 weeks, 53 dB at 13 weeks, and 40 dB at 26 weeks (figure E). The best recovery of ABR threshold for participant 5 was 25 dB at 0·25 kHz at 26 weeks (appendix p 16). The average auditory steady-state response was gradually reduced to 40 dB at 26 weeks from greater than 98 dB at baseline. In participant 6, the average ABR threshold was greater than 95 dB at baseline, greater than 93 dB at 4 weeks, greater than 90 dB at 6 weeks, 60 dB at 13 weeks, and 55 dB at 26 weeks (figure F). The average auditory steady-state response was gradually reduced to 60 dB at 26 weeks from 100 dB at baseline. Pure-tone audiometry was not done for participants 4, 5, and 6 because of their young age.

The signal-to-noise ratio of the distortion product otoacoustic emission test in participants 2, 4, 5, and 6 after treatment was lower at 4 weeks than at baseline (appendix p 14). The signal-to-noise ratio showed recovery in participants 2, 4, 5, and 6 in most frequencies. In participant 3, we found no apparent change in the signal-to-noise ratio after the treatment compared with the baseline (appendix p 14).

Changes in scores of auditory and speech perception from baseline to weeks 4, 13, and 26 are shown in table 4. In participants 1, 3, and 5, with the cochlear implant switched off, the Meaningful Auditory Integration Scale score, the Categories of Auditory Performance score, and the Meaningful Use of Speech Scale score were

	MAIS or IT-MAIS score	CAP score	SIR score	MUSS score	Ambient sound perception, %	Tone perception, %	Initial perception, %	Final perception, %
Participant 1								
Baseline	5	0	5	3	ND	ND	ND	ND
4 weeks	ND	ND	ND	ND	56·3%	62·5%	37·5%	20·8%
13 weeks	15	4	5	6	75·0%	37·5%	20·8%	29·2%
26 weeks	30	7	5	37	100%	100%	83·3%	91·7%
Participant 2								
Baseline	4	0	4	3	0	0	0	0
4 weeks	4	0	4	3	0	0	0	0
13 weeks	4	0	4	3	0	0	0	0
26 weeks	4	0	4	3	0	0	0	0
Participant 3								
Baseline	6	1	5	3	0	0	0	0
4 weeks	13	4	5	5	46·9%	18·8%	18·8%	39·6%
13 weeks	13	4	5	5	50·0%	43·8%	12·5%	41·7%
26 weeks	20	7	5	40	93·8%	93·8%	54·2%	100%
Participant 4								
Baseline	0	0	1	0	NA	NA	NA	NA
4 weeks	2	2	1	0	NA	NA	NA	NA
13 weeks	2	2	1	0	NA	NA	NA	NA
26 weeks	17	2	2	3	NA	NA	NA	NA
Participant 5								
Baseline	2	0	3	2	0	0	0	0
4 weeks	4	2	3	2	18·8%	0	0	0
13 weeks	6	4	3	2	68·8%	0	0	8·3
26 weeks	23	6	4	39	87·5%	68·8%	62·5%	70·8%
Participant 6								
Baseline	3	1	1	0	NA	NA	NA	NA
4 weeks	13	3	1	5	NA	NA	NA	NA
13 weeks	36	5	2	25	NA	NA	NA	NA
26 weeks	36	6	2	30	NA	NA	NA	NA

Participants 1, 2, 3, and 5 were tested with the cochlear implant switched off; participants 4 and 6 had no cochlear implants. MAIS was assessed in participants 1, 2, 3, and 5. IT-MAIS was assessed in participants 4 and 6. Participants 4 and 6 were too young to complete tests for speech perception. Perception of ambient sound, tone, initial, and final were assessed in a quiet environment. CAP=Categories of Auditory Performance. IT-MAIS=Infant-Toddler Meaningful Auditory Integration Scale. MAIS=Meaningful Auditory Integration Scale. MUSS=Meaningful Use of Speech Scale. NA=not applicable. ND=not done. SIR=Speech Intelligibility Rating.

Table 4: **Scores of auditory and speech perception (without cochlear implant or with the cochlear implant switched off)**

improved by 26 weeks; in a quiet environment, the perception of ambient sound, tone, initial, and final was also improved by 26 weeks (table 4). In participants 4 and 6, without the cochlear implant, the Infant–Toddler Meaningful Auditory Integration Scale score, the Categories of Auditory Performance score, and the Meaningful Use of Speech Scale score, and the Speech Intelligibility Rating score, were improved by 26 weeks (table 4). Participant 1, with the cochlear implant switched off, was unable to recognise speech in steady-state noise or to complete the assessment for speech recognition thresholds of monosyllable, disyllable, and sentence conditions at baseline, 4 weeks, and 13 weeks (appendix p 19). However, at 26 weeks, her speech recognition thresholds in steady-state noise were improved in the monosyllable (–2·0 dB), disyllable (0·3 dB), and sentence (8·9 dB) conditions (appendix p 19). No improvements in auditory or speech perception were observed in participant 2 (table 4). Participant 3's perception of monosyllabic words was 0% at baseline and 74·0% at 26 weeks, perception of disyllabic words was 0% at baseline and 88·6% at 26 weeks, and perception of sentences was 0% at baseline and 73·6% at 26 weeks (appendix p 19). Participant 3, with the cochlear implant switched off, was unable to recognise speech in steady-state noise and unable to complete the assessment for speech recognition thresholds in the monosyllable, disyllable, and sentence conditions at baseline, 4 weeks, and 13 weeks (appendix p 19). However, at 26 weeks, her speech recognition thresholds in steady-state noise were improved in the monosyllable (6·4 dB), disyllable (9·7 dB), and sentence (29·0 dB) conditions (appendix p 19). Representative speech communication of participant 3 after treatment is presented in video 1.

Representative speech communication of participant 4 after treatment is presented in video 2.

Participant 5, with the cochlear implant switched off, she was unable to recognise speech in quiet environment at baseline, 4 weeks, and 13 weeks (appendix p 19). However, at 26 weeks, her speech perception in a quiet environment was improved in the monosyllable (54·0%), disyllable (62·9%), and sentence (23·6%) conditions (appendix p 19). Representative speech communication of participant 5 after treatment is presented in video 3.

Representative speech communication of participant 6 after treatment is presented in video 4.

Results for auditory and speech perception with the cochlear implant switched on (for participants 1, 3, and 5; participant 2 had no improvements in response) are shown in appendix (pp 18–20).

Discussion

In this trial, no dose-limiting toxicity was recorded during 26 weeks of follow-up after unilateral injection of AAV1-hOTOF at 9×10^{11} vg or $1{\cdot}5\times10^{12}$ vg. AAV1 has been previously used as a vector for gene therapy for lipoprotein lipase deficiency.[26] To further improve the safety profile of AAV1 for this study, a hair-cell specific promoter was used to minimise ectopic expression of otoferlin. Participants were given dexamethasone to minimise the risk of inflammation and ceftriaxone to minimise the risk of infection. Neither aural inflammation nor T cell responses to the AAV1 capsid were observed. 46 (96%) of all 48 adverse events were grade 1–2 and two (4%) were grade 3. None of the observed adverse events met Hy's law criteria for liver injury, an important concern with gene therapy.[27] We found no evidence that adverse events affected the treatment outcome. Although two participants had COVID-19 (participant 4 at 2 weeks after injection and participant 5 at 1 week after injection), their hearing was recovered by 4–6 weeks after injection. Altogether, these findings suggest that, with concomitant anti-inflammatory treatment, local and systemic inflammatory responses can be reduced to an acceptable level.

Our efficacy assessment revealed robust hearing recovery in all but one participant. Hearing recovery was first detected 4–6 weeks after injection in participants 1, 3, 4, 5, and 6. A key finding in this trial is a time-dependent hearing recovery, and participants are being followed up to further verify the temporal pattern. Treatment efficacy did not depend on vector dosage administered, but the number of participants included in this analysis is too small for any meaningful interpretation of a dose–response relationship, and further investigation in larger randomised trials would be required to generate adequate evidence regarding a dose–response relationship of treatment with AAV1-hOTOF gene therapy.

The signal-to-noise ratio of the distortion product otoacoustic emission test showed reductions at 4 weeks after treatment in five participants (participants 1, 2, 4, 5, and 6) (appendix p 14). The fairly stable signal-to-noise ratio of the distortion product otoacoustic emission test before and after injection in participant 3 might be due to less damage to the round window during surgery or to a reduced inflammatory reaction to AAV1-hOTOF compared with other participants. The signal-to-noise ratio of the distortion product otoacoustic emission test in participant 3 might also have changed before the first follow-up but recovered quickly after the injection, which could mean that change could not be detected at 4 weeks. Overall, the signal-to-noise ratio of the distortion product otoacoustic emission test of most participants decreased, followed by a recovery, although the degree of recovery varied among participants.

See Online for video 1–4

Children with congenital deafness face difficulties when learning spoken language because of not being able to hear spoken sounds; after hearing recovery, such children gradually acquire the ability of speech perception.[28,29] Therefore, besides objective audiometric tests, speech perception is also an important indicator of hearing recovery in children with hearing loss. Testing for speech perception showed improvement in all responding participants (participants 1, 3, 4, 5, and 6).

Participants 1, 3, and 5 had improvements in auditory and speech perception with the cochlear implant off by 26 weeks. At 4 and 13 weeks, with the cochlear implant off, participants 1 and 3 were unable to recognise speech in a noisy environment; however, by 26 weeks, both participants were able to recognise speech in a noisy environment and communicate using the telephone without difficulties. Participant 5, with the cochlear implant switched off, was also able to have a spoken conversation without difficulties (video 3). Children with cochlear implants generally need 1·0–1·5 years of speech rehabilitation to achieve good improvement in sound perception and speech recognition.[30–32] The improvement in participants 1, 3, and 5 might be partly due to the continuous hearing recovery after gene therapy and the benefit of speech rehabilitation. Participants 4 and 6 did not receive a cochlear implant and scored 0 on all measures of speech perception before the injection. After injection, speech perception was improved to different degrees in participants 4 and 6, which might be caused by differences in the participants' individual abilities or different speech rehabilitation education. Notably, children with autosomal recessive deafness 9 might need time to further develop speech perception after an improvement in hearing, and the development of speech or language skills is variable from child to child. Some participants were too young to complete some tests. In the future, more objective and comprehensive speech assessments need to be developed and explored.

Inner-ear injection through the round window membrane is a common surgical approach to deliver AAV vectors in mice and non-human primates.[33,34] However, to our knowledge, no study has investigated the same approach to deliver AAV vectors to the human cochlea. In this trial, AAV1-hOTOF was injected through the round window membrane via the external auditory canal under direct vision with an endoscope rather than cortical mastoidectomy to minimise the risk of damage to the mastoid cavity and tympanic sinus. Injection via the round window membrane without fenestration might lead to variable hair-cell transduction efficiency along the tonotopic positions inside the cochlea and can carry a risk of injection-induced hearing loss.[35,36] To further minimise the risk and improve the transduction efficiency, we used a round window membrane injection with a small fenestration introduced to the stapes footplate to promote lymph flow.

The auditory function of participant 2 was not improved by 26 weeks after injection, for reasons that could not be established. One possibility might be the higher concentrations of neutralising antibodies at baseline (1:135 in participant 2, compared with <1:5 in other participants) and after treatment (1:3645 at 6 weeks), as previously reported for AAV-mediated gene therapy.[37–39] An alternative explanation is a possible leakage of the AAV1-hOTOF solution from the round window membrane during or after surgery.

In conclusion, we found that a single injection of AAV1-hOTOF resulted in robust hearing recovery in five of six children with autosomal recessive deafness 9, and in improved speech perception in those who had hearing recovery, without dose-limiting toxicities at either administered dose. This study supports the continuous investigation of gene therapy to treat hearing loss in children with autosomal recessive deafness 9. Trials with larger sample sizes and longer follow-ups are needed to further examine the efficacy of gene therapy compared with that of cochlear implants.

Contributors

YS and HL were the principal investigators of the study and conceived the trial. YS, ZC, JL, HW, XC, YC, DW, HT, and KG contributed to the study design. JL, HW, and QC enrolled participants. JL, HW, XC, QC, and YY collected the data. XC collected the questionnaires. QC prepared the videos. JL, HW, XC, YC, DW, YS, ZC, and KG analyed and interpreted data and wrote the manuscript. JL, HW, XC, YC, DW, YS, and RC accessed and verified the data. SH, BZ, RC, HT, CC, LJ, and ZG contributed to the revision of the manuscript. YS, HL, ZC, and YC obtained funding. YS, WW, HL, BC, JL, HW, and LZ participated in the surgery. MX, ZW, JW, and JZ processed the blood sample. LG, SY, DW, and HT confirmed the genotype of participants. All authors vouch for the fidelity of the protocol and the accuracy and completeness of the reported data. All authors reviewed and approved of the manuscript before submission. JL, HW, XC, YC, DW, YS, and ZC had full access to all data in the study and had final responsibility for the decision to submit for publication.

Declaration of interests

KG is a staff of the Shanghai Refreshgene Therapeutics. ZC is a cofounder of Salubritas Therapeutics. All other authors declare no competing interests.

Data sharing

To respect the privacy of participants, individual participant data is anonymised. De-identified data (text, figures, tables, and appendices) in the manuscript are available. The redacted trial protocol is available in the appendix. These data will be available from the corresponding author.

Acknowledgments

The Eye & ENT Hospital of Fudan University sponsored the study. The study was supported by the National Natural Science Foundation of China (82225014, 82171148, and 82192864), the National Key R&D Program of China (2020YFA0908201, 2021YFA1101302, and 2023YFC2508400), Science and Technology Commission of Shanghai Municipality (21S11905100), Shanghai Municipal Health Commission (20224Z0003), Shanghai Municipal Education Commission (2023ZKZD12), and Fudan University (yg2022-23). The study was also funded by Shanghai Refreshgene Therapeutics. ZC was supported by the Ines and Fredrick Yeatts Fund. We thank the participants and their families for support of the study. We thank the physicians and staff at the Eye & ENT Hospital of Fudan University for laboratory testing, audiometric examination, aural endoscopy, and vestibular function examination, and the nurses for professional care of participants during hospitalisation. We thank Yongfu Yu from Fudan University for assisting with project design. Writing and editorial assistance was provided by Kehong Zhang from Ivy Medical Editing (Shanghai, China).

References

1 Morton CC, Nance WE. Newborn hearing screening—a silent revolution. *N Engl J Med* 2006; **354:** 2151–64.

2 Spencer L. Global, regional, and national incidence, prevalence, and years lived with disability for 354 diseases and injuries for 195 countries and territories, 1990-2017: a systematic analysis for the Global Burden of Disease Study 2017. *Lancet* 2018; **392:** 1789–858.

3 Roux I, Safieddine S, Nouvian R, et al. Otoferlin, defective in a human deafness form, is essential for exocytosis at the auditory ribbon synapse. *Cell* 2006; **127:** 277–89.

4 Sloan-Heggen CM, Bierer AO, Shearer AE, et al. Comprehensive genetic testing in the clinical evaluation of 1119 patients with hearing loss. *Hum Genet* 2016; **135:** 441–50.

5 Rodríguez-Ballesteros M, Reynoso R, Olarte M, et al. A multicenter study on the prevalence and spectrum of mutations in the otoferlin gene (*OTOF*) in subjects with nonsyndromic hearing impairment and auditory neuropathy. *Hum Mutat* 2008; **29:** 823–31.

6 Iwasa YI, Nishio SY, Sugaya A, et al. OTOF mutation analysis with massively parallel DNA sequencing in 2265 Japanese sensorineural hearing loss patients. *PLoS One* 2019; **14:** e0215932.

7 Choi BY, Ahmed ZM, Riazuddin S, et al. Identities and frequencies of mutations of the otoferlin gene (OTOF) causing DFNB9 deafness in Pakistan. *Clin Genet* 2009; **75:** 237–43.

8 Gallo-Terán J, Megía López R, Morales-Angulo C, et al. Estudio de una familia con hipoacusia neurosensorial secundaria a la mutación q829x en el gen otof. *Acta Otorrinolaringol Esp* 2004; **55:** 120–25.

9 Migliosi V, Modamio-Høybjør S, Moreno-Pelayo MA, et al. Q829X, a novel mutation in the gene encoding otoferlin (OTOF), is frequently found in Spanish patients with prelingual non-syndromic hearing loss. *J Med Genet* 2002; **39:** 502–06.

10 Bainbridge JW, Mehat MS, Sundaram V, et al. Long-term effect of gene therapy on Leber's congenital amaurosis. *N Engl J Med* 2015; **372:** 1887–97.

11 Chowdary P, Shapiro S, Makris M, et al. Phase 1-2 trial of AAVS3 gene therapy in patients with hemophilia B. *N Engl J Med* 2022; **387:** 237–47.

12 Jiang L, Wang D, He Y, Shu Y. Advances in gene therapy hold promise for treating hereditary hearing loss. *Mol Ther* 2023; **31:** 934–50.

13 Akil O, Dyka F, Calvet C, et al. Dual AAV-mediated gene therapy restores hearing in a DFNB9 mouse model. *Proc Natl Acad Sci USA* 2019; **116:** 4496–501.

14 Al-Moyed H, Cepeda AP, Jung S, Moser T, Kügler S, Reisinger E. A dual-AAV approach restores fast exocytosis and partially rescues auditory function in deaf otoferlin knock-out mice. *EMBO Mol Med* 2019; **11:** e9396.

15 Tang H, Wang H, Wang S, et al. Hearing of Otof-deficient mice restored by trans-splicing of N- and C-terminal otoferlin. *Hum Genet* 2023; **142:** 289–304.

16 Zhang L, Wang H, Xun M, et al. Preclinical evaluation of the efficacy and safety of AAV1-hOTOF in mice and nonhuman primates. *Mol Ther Methods Clin Dev* 2023; **31:** 101154.

17 WHO. World report on hearing. Geneva: World Health Organization, 2021. https://www.who.int/publications/i/item/9789240020481 (accessed Dec 1, 2022).

18 Robbins AM, Renshaw JJ, Berry SW. Evaluating meaningful auditory integration in profoundly hearing-impaired children. *Am J Otol* 1991; **12** (suppl)**:** 144–50.

19 Archbold S, Lutman ME, Nikolopoulos T. Categories of auditory performance: inter-user reliability. *Br J Audiol* 1998; **32:** 7–12.

20 Cox RM, McDaniel DM. Development of the Speech Intelligibility Rating (SIR) test for hearing aid comparisons. *J Speech Hear Res* 1989; **32:** 347–52.

21 Robbins AM, Osberger MJ. Meaningful Use of Speech Scale (MUSS). Indianopolis: Indiana University School of Medicine, 1990.

22 Fu QJ, Zhu M, Wang X. Development and validation of the Mandarin speech perception test. *J Acoust Soc Am* 2011; **129:** EL267–73.

23 Cheng X, Liu Y, Shu Y, et al. Music training can improve music and speech perception in pediatric mandarin-speaking cochlear implant users. *Trends Hear* 2018; **22:** 2331216518759214.

24 Tao D, Deng R, Jiang Y, Galvin JJ 3rd, Fu QJ, Chen B. Melodic pitch perception and lexical tone perception in Mandarin-speaking cochlear implant users. *Ear Hear* 2015; **36:** 102–10.

25 Chandrasekhar SS, Tsai Do BS, Schwartz SR, et al. Clinical practice guideline: sudden hearing loss (update). *Otolaryngol Head Neck Surg* 2019; **161:** S1–45.

26 Mingozzi F, Meulenberg JJ, Hui DJ, et al. AAV-1-mediated gene transfer to skeletal muscle in humans results in dose-dependent activation of capsid-specific T cells. *Blood* 2009; **114:** 2077–86.

27 US Food and Drug Administration. Guidance for industry. Drug-induced liver injury: premarking clinical evaluation. Rockville, MD: Food and Drug Administration, 2009. https://www.fda.gov/media/116737/download (accessed June 1, 2023).

28 Dornhoffer JR, Reddy P, Meyer TA, Schvartz-Leyzac KC, Dubno JR, McRackan TR. Individual differences in speech recognition changes after cochlear implantation. *JAMA Otolaryngol Head Neck Surg* 2021; **147:** 280–86.

29 Benchetrit L, Ronner EA, Anne S, Cohen MS. Cochlear implantation in children with single-sided deafness: a systematic review and meta-analysis. *JAMA Otolaryngol Head Neck Surg* 2021; **147:** 58–69.

30 Santarelli R, Scimemi P, Costantini M, Domínguez-Ruiz M, Rodríguez-Ballesteros M, Del Castillo I. Cochlear synaptopathy due to mutations in *OTOF* gene may result in stable mild hearing loss and severe impairment of speech perception. *Ear Hear* 2021; **42:** 1627–39.

31 Santarelli R, del Castillo I, Cama E, Scimemi P, Starr A. Audibility, speech perception and processing of temporal cues in ribbon synaptic disorders due to OTOF mutations. *Hear Res* 2015; **330:** 200–12.

32 Zheng D, Liu X. Cochlear implantation outcomes in patients with *OTOF* mutations. *Front Neurosci* 2020; **14:** 447.

33 Zhao Y, Zhang L, Wang D, Chen B, Shu Y. Approaches and vectors for efficient cochlear gene transfer in adult mouse models. *Biomolecules* 2022; **13:** 38.

34 Akil O, Seal RP, Burke K, et al. Restoration of hearing in the VGLUT3 knockout mouse using virally mediated gene therapy. *Neuron* 2012; **75:** 283–93.

35 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:** 2557–64.

36 Yoshimura H, Shibata SB, Ranum PT, Smith RJH. Enhanced viral-mediated cochlear gene delivery in adult mice by combining canal fenestration with round window membrane inoculation. *Sci Rep* 2018; **8:** 2980.

37 Jiang H, Couto LB, Patarroyo-White S, et al. Effects of transient immunosuppression on adenoassociated, virus-mediated, liver-directed gene transfer in rhesus macaques and implications for human gene therapy. *Blood* 2006; **108:** 3321–28.

38 Manno CS, Pierce GF, Arruda VR, et al. Successful transduction of liver in hemophilia by AAV-Factor IX and limitations imposed by the host immune response. *Nat Med* 2006; **12:** 342–47.

39 Wang L, Herzog RW. AAV-mediated gene transfer for treatment of hemophilia. *Curr Gene Ther* 2005; **5:** 349–60.