nature medicine

Article

https://doi.org/10.1038/s41591-025-03773-w

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

Received: 3 October 2024

Accepted: 15 May 2025

Published online: 02 July 2025

Check for updates

A list of authors and their affiliations appears at the end of the paper

Gene therapy for congenital deafness has shown promising results in children but lacks data in older populations. We conducted a single-arm trial of adeno-associated virus (AAV)-*OTOF* gene therapy using the Anc80L65 capsid in ten participants with autosomal recessive deafness 9 aged 1.5 to 23.9 years at five sites in China. The primary endpoints were safety and tolerability within 5 years, and secondary endpoints assessed auditory function. Initial findings from the ten patients with 6–12 months of follow-up, including one patient who received two injections, revealed that the therapy was well tolerated, with 162 grade I/II adverse events. Decreased neutrophil percentage was the most common event (16 of 162). All ten participants had at least 6 months of follow-up and improved their pure-tone-average hearing level from baseline 106 ± 9 (mean ± s.d.) to 52 ± 30 decibels (dB). Other secondary endpoints showed similar improvements, including the average click auditory brainstem response (ABR) threshold, the tone-burst ABR threshold and the auditory steady-state response (101 ± 1 to 48 ± 26 dB, 91 ± 4 to 57 ± 19 dB and 80 ± 14 to 64 ± 21 dB, respectively). Post hoc analyses were conducted to evaluate the timecourse and factors contributing to the hearing improvement. Therapeutic effect was rapid, taking 1 month to achieve most of the overall hearing improvement. On an individual level, click and tone-burst ABR thresholds, but not the auditory steady-state response, reliably predicted the behavioral pure-tone-average thresholds after 4 months (R^2 = 0.68, 0.73 and 0.17, respectively). An age-dependent therapeutic effect was observed, with optimal outcomes in 5- to 8-year-olds. These preliminary results show that AAV-*OTOF* was safe and well tolerated in patients ranging from toddlerhood to adulthood. The trial remains ongoing and requires extended follow-up to confirm the long-term safety and efficacy. ClinicalTrials.gov registration: NCT05901480.

Gene therapy treats, or even prevents, diseases by altering genetic materials in living cells. One specific approach involves gene replacement—a process that delivers normal genes to produce functional proteins for the treatment of diseases caused by missing or faulty genes[1–7]. *OTOF* is a gene that is responsible for producing a protein called OTOFERLIN, important for transmitting sound signals from the ear to the brain[8]. Mutations in *OTOF* can cause severe-to-profound congenital deafness[9–11]. A synthetic adeno-associated virus (AAV), known as Anc80L65 (refs. 12,13), has been used to deliver the normal *OTOF* gene to the murine inner ear as a treatment for this type of deafness[14]. After safety and efficacy validation in mice and nonhuman primates[14], we initiated an open-label, single-arm, nonrandomized, interventional trial at a single center and previously reported clinical outcomes of two children with autosomal recessive deafness 9 (DFNB9)[15]. The two patients, a 5-year-old (unilateral injection) and an 8-year-old (bilateral injection), achieved almost normal hearing levels to enable daily conversation[15].

e-mail: maoli.duan@ki.se; zhadjun@fmmu.edu.cn; sunyu@hust.edu.cn; gaoxia@nju.edu.cn; sdphxl@email.sdu.edu.cn; fzeng@uci.edu; renjiec@seu.edu.cn

Collaborating with colleagues, we subsequently reported clinical trial results of unilateral ear gene therapy in six additional children, with effective hearing improvement in five[16]. Wang et al. later reported the results of bilateral ear gene therapy in five children, achieving hearing improvement without dosage-limiting toxicity or systemic toxicity[17]. The results from these trials indicated that *OTOF* gene therapy is effective in children from 1 to 11 years old. However, neither the efficacy of this therapy in adolescents and adults nor the optimal age was known. Here, we expanded from a single center to several centers, currently enrolling a total of 10 participants with 13 ears being treated across 5 hospitals. We presented not only the safety and efficacy of Anc80L65-*OTOF* across an age span from toddlers to adults, but also the important behavioral threshold outcomes that have been scarcely reported previously due to the age restriction.

Results

Participants

Thirty-one participants with *OTOF* mutations and hearing loss were screened in the clinical trial from 26 July 2023 to 27 June 2024 at the five centers, based on genetic screening, neutralizing antibodies (NAb) testing and audiologic examinations from a total of 393 candidates (Fig. 1). Four participants were excluded due to the titer of blood NAb > 1:128, whereas the fifth was excluded due to failed distortion product otoacoustic emission (DPOAE). Ten participants enrolled and received the AAV-*OTOF* (Anc80L65 capsid) injection, with five participants at center 1, one participant each at centers 2, 3 and 5 and two participants at center 4. Information regarding the ten participants is detailed in Table 1. The age range of participants was from 1.5 to 23.9 years, with a median age of 6 years. Participants had compound heterozygous or homozygous mutations in *OTOF* (Table 1, Extended Data Fig. 1 and Extended Data Table 1). Through minigene assays, the c.2214+27 G>A mutation in participant 4 was demonstrated to induce aberrant *OTOF* mRNA splicing with 28-bp intronic retention, offering a mechanistic basis for the patient's functional impairment (Extended Data Fig. 2). All ten participants had profound hearing loss (no click-ABR or pure-tone average (PTA) at maximum signal intensity levels; Table 1), but elicited click cochlear microphonic (CM) responses and DPOAE at one or more frequencies (Table 1 and Extended Data Table 2), indicating preserved outer hair cells. Seven participants received gene therapy in one ear due to the unilateral cochlear implantation, while three received therapies in both ears. Nine participants tested negative for NAb, while the adult participant had a NAb titer of 1:40 (≤1:128) (Table 1). Intervention of the treated ear, if any, and the mode of communication before gene therapy were described (Extended Data Table 3). Participants received viral delivery into the cochlea by a needle via the round window membrane, which was exposed through the trans-mastoid facial recess—a standard procedure used commonly in cochlear implantation. The surgeons involved the present five-site trial preferred this commonly used approach rather than going through the external auditory canal[16], which may limit the accuracy of need punctuation into the round window. Most importantly, our surgeons have demonstrated in cynomolgus monkeys that the trans-mastoid facial recess approach provides consistently accurate round window injection and high virus transduction efficiency[14].

Primary endpoints: safety and tolerability

Adverse events (AEs) occurring throughout the study were assessed and graded in accordance with the NCI-CTCAE v.5.0. The safety evaluation encompassed otological examinations, laboratory assessments and other reported otological or systemic AEs. AEs were categorized and summarized based on type and frequency. During the 12-month follow-up, 162 AEs in ten patients were observed, which were all graded as I or II (Table 2). No serious AEs (SAEs) occurred. Among the 49 distinct types of AE identified, the most frequently observed were decreased neutrophil percentage (16 of 162), increased platelet counts (10 of 162) and anemia (10 of 162).

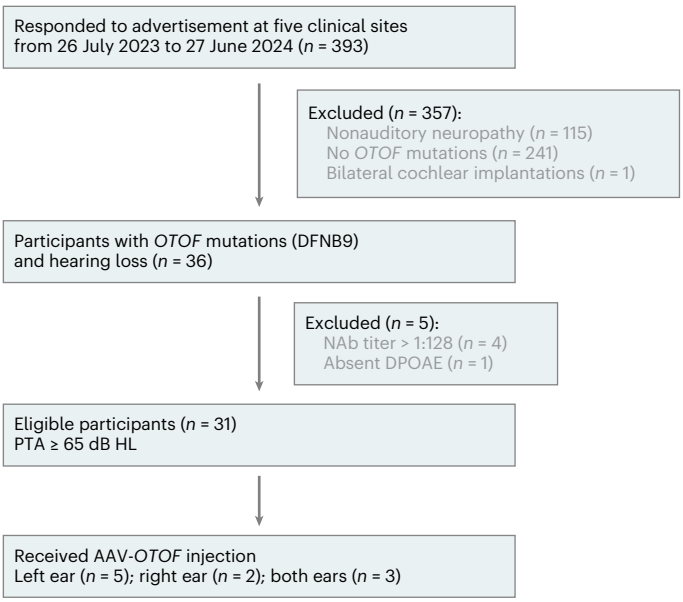


Fig. 1 | Screening and enrollment. Flowchart of participant screening and enrollment; 31 participants were found to be eligible out of 393 patients with DFNB9. Ten enrolled participants received AAV-*OTOF* injection in a single-arm, interventional study.

Despite receiving similar doses of AAV-*OTOF* (maximum/minimum = 1.3), large variations occurred in both blood AAV vector levels and NAb concentrations (Fig. 2). For blood AAV vector levels (green lines), all, except for the oldest participant 9 who showed an undetectable level, had a peak AAV level on the first day after virus injection. Participants 5, 7, 8 and 10 had remarkable higher blood AAV levels than the other participants. The peak AAV level ranged from undetectable (participant 9) to 2.1 million copies μg^{-1} (participant 7). For NAb concentrations (yellow lines), participants 1 to 9 showed a delayed peak against AAV-*OTOF*, ranging from W1 in participant 4 to M6 in participant 2. The peak NAb titer from the first injection ranged from 1:80 in participants 1 and 6 with the unilateral AAV-*OTOF* injection to 1:10,240 in participant 7 with the bilateral injection. The greatest peak NAb, or 1:10,240, was also recorded 3 weeks after the second injection in participant 1 (dashed yellow line in Fig. 2a).

Secondary endpoint: efficacy

The ten participants underwent pre- and postoperative hearing assessments, with all being followed for at least 6 months, eight for at least 9 months and three for at least 12 months. The assessments included Click-ABR, tone-burst-ABR (TB-ABR), auditory steady-state response (ASSR), DPOAE tests and pure-tone audiometry.

The presence of otoacoustic emission with abnormal or absent ABR is a hallmark of auditory neuropathy, including those with *OTOF* mutations. Before surgery, 13 pretreated ears in all ten participants had DPOAE present (signal-to-noise ratio (SNR) > 6 dB) at one to nine of the nine tested frequencies. Two weeks after injection, nine ears showed a decreased number of present DPOAE at all tested frequencies, two showed an increasing number (participants 2 and 7, left ear), and two were not tested due to missed appointments (participants 5 and 1, with second injection). Over the course of 1–12 months, the number of present DPOAE recovered either partially or fully in most participants (Extended Data Fig. 3 and Extended Data Table 2). Because DPOAE was a binary passing or failing screening measure, its SNR was not correlated positively with the pure-tone threshold at the corresponding tested frequency (Extended Data Fig. 4).

All patients showed hearing improvement after treatment (Extended Data Table 4). The degree of hearing recovery can be seen in

https://doi.org/10.1038/s41591-025-03773-w

Table 1 | Baseline information for all enrolled participants

Participant	1	2	3	4	5	6	7	8	9	10
Sex	Male	Female	Female	Female	Male	Male	Male	Male	Female	Male
Age (years)	3.9	5.3	8.0	7.1	1.6	14.5	1.8	6.7	23.9	1.5
Mutations in *OTOF* allele 1[a]	c.4819C>T	c.4275G>A	c.1066T>G	c.1129G>A c.3498_3499delinsG	c.2377G>T	c.5108delinsTCTT	c.2887C>T	c.4669_4677del	c.709C>T	c.5098G>C
Mutations in *OTOF* allele 2[a]	c.4819C>T	c.2377_2401del	c.3987G>A	c.2214+27G>A	c.4225A>T	c.5108delinsTCTT	c.1697G>C	c.5212_5214del c.1927G>A	c.5108delinsTCTT	c.5098G>C
NAb in serum	_[b]	–	–	–	–	–	–	–	1:40	–
Click-ABR[c]	>100	>100	>100 (L)[e] >100 (R)	>99	>99	>100	>100 (L) >100 (R)	>100	>100	>100 (L) >100 (R)
PTA[d]	116	112	95 (L) 116 (R)	105	112	105	101 (L) 99 (R)	100	101	121 (L) 121 (R)
Click-CM	Elicited	Elicited	Elicited	Elicited	Elicited	Elicited	Elicited	Elicited	Elicited	Elicited
Injection ear	Left	Left	Both	Right	Left	Left	Both	Right	Left	Both
CI ear[f]	Right	Right	No CI	Left	Right	Right	No CI	Left	Right	No CI

[a]Matched annotation from NCBI and EMBL-EBI (MANE) Selected transcript: NM_194248.3. [b]Negative. [c]'>100' or '>99' indicates no response at this maximum sound intensity level. [d]Average hearing thresholds at 0.5, 1, 2 and 4 kHz. [e]L, left ear; R, right ear. [f]Ear with cochlear implantation (CI).

individual thresholds for Click-ABR, TB-ABR, pure-tone audiometry thresholds (Fig. 3) and ASSR (Extended Data Fig. 5).

Participant 1 received two separate injections in the same ear (Fig. 3f). After the initial injection, participant 1 was followed for 4 months. The Click-ABR and TB-ABR indicated minimal hearing recovery, but the PTA improved from 116 dB at baseline to 89, 83 and 95 dB at 1, 2 and 4 months, respectively. After 4 months, a second AAV-*OTOF* injection was administered, resulting in Click-ABR improvement from 99 dB at the new baseline to 80 dB at 6 months. The mean TB-ABR and ASSR thresholds fluctuated less than 10 dB within the baseline at different timepoints after surgery. The PTA improved to 68 dB at 2 months, but returned to 86 dB, or 7 dB lower than the 95 dB baseline at 9 months. Together, the two injections improved the click-ABR threshold by at least 19 dB, the TB-ABR by 6 dB and the PTA by 28 dB within 9 months after the second injection or 13 months after the first injection.

Participant 4 represented the best-case scenario, showing rapid improvement to nearly normal hearing with gene therapy (Fig. 3i). The click-ABR threshold decreased from 101 dB at baseline to 40 dB at 2 weeks, stabilizing near 25 dB at 2 months and thereafter. The average TB-ABR threshold was 96 dB at baseline, improving to 53 dB at 2 weeks, and further to ~35 dB by 4 months and thereafter. The average ASSR threshold showed less improvement than the other two ABR measures (−3 to 23 dB at various timepoints; Extended Data Fig. 5i). Crucially, PTA improved from 105 dB at baseline to 16 dB, 18 dB, 13 dB and 10 dB at 4, 6, 9 and 12 months, respectively, with all frequencies below 30 dB by then, including the lowest threshold of −5 dB at 8 kHz, which is better than the population average (Fig. 3i). Participant 4 was able to carry on daily conversation with her mom 4 months after treatment without activating the cochlear implant and lipreading (Supplementary Video 1). Similar hearing improvement was also observed in participants 2, 8 and 3 (Fig. 3g,h,j,k). One month after gene therapy, participant 8 could identify words like 'scissors,' 'excavator,' 'tomato,' and 'banana' in a closed-set word choice task, again without the implant and lipreading (Supplementary Video 2).

Participant 6, aged 14.5 years, was older than any participant in any AAV-*OTOF* clinical trial known to date[15–17] (Fig. 3l). The Click-ABR threshold improved from 101 dB at baseline to 55 and 80 dB at 4 and 6 months after injection. Over 6 months, the average TB-ABR threshold improved from 101 to 61 dB, the PTA from 105 to 59 dB and the average ASSR threshold from 94 to 58 dB (Extended Data Fig. 5l). The relatively less improvement in the Click-ABR threshold was a result of poor hearing at high frequencies.

Participant 9 was the only adult participant to receive this gene therapy so far (Fig. 3m). The Click-ABR threshold improved from 101 dB at baseline to 45 dB at 2 months, and 40 dB at 4 and 6 months. The corresponding TB-ABR threshold improved from 101 to 78 dB (M2), 78 dB (M4) and 79 dB (M6), and PTA from 101 to 73 dB (M2), 65 dB (M4) and 70 dB (M6). The average ASSR threshold fluctuated less than 13 dB within the baseline at different timepoints after surgery (Extended Data Fig. 5m and Extended Data Table 4). One month after treatment, this adult participant did not have any open-set speech recognition, but could correctly identify the number of spoken words or handclapping (Supplementary Video 3).

Post hoc analyses

When the data were pooled for group-level analysis, three interesting and important patterns emerged in gene therapy for hearing loss. First, hearing improvement is rapid and stabilizes 1 month after AAV-*OTOF* treatment (Fig. 4a). On average, PTA (the most important behavioral threshold) improved by 28 dB at 2 weeks, 34 dB at 1 month, 43 dB at 2 months, 50 dB at 4 months and 55 dB at 6 months (Fig. 4a; red dots and lines). The most rapid phase of hearing improvement occurred in the first month, accounting for 62% (78%) of the total PTA (TB-ABR) improvements at 6 months. We performed similar analysis on the eight participants previously reported who completed 26-week follow-up[16,17]. Auditory

https://doi.org/10.1038/s41591-025-03773-w

Table 2 | Adverse events

Incidence of AEs during treatment period (safety analysis set)[a]				
AE	**Number of participants (percent)**	**Number of times**		
Deaths	0 (0)	0		
AE	10 (100)	162		
SAE	0 (0)	0		
AE occurring in ten participants				
AE	**Number of participants (percent)**	**Number of times**	**Grade I (*n*)**	**Grade II (*n*)**
Neutrophil percentage decreased	7 (70)	16	7	0
Platelet count increased	5 (50)	10	5	0
Anemia	4 (40)	10	4	0
Lymphocyte ratio increased	4 (40)	8	4	0
Leukocyte counts increased	6 (60)	7	6	0
Upper respiratory tract infection	3 (30)	7	0	3
Blood triglycerides increased	4 (40)	6	4	1
Urine ketone body present	4 (40)	6	4	0
Lymphocyte count increased	4 (40)	6	4	0
Albumin-globulin ratio increased	2 (20)	5	2	0
Neutrophil count decreased	3 (30)	4	1	2
Eosinophil percentage increased	2 (20)	4	2	0
Total protein decreased	2 (20)	4	2	0
Globulins decreased	2 (20)	4	2	0
Cough	3 (30)	3	2	1
Total cholesterol increased	3 (30)	3	3	0
Eosinophil percentage decreased	3 (30)	3	3	0
Eosinophil counts decreased	3 (30)	3	3	0
Basophil percentage decreased	1 (10)	3	1	0
Blood urea increased	2 (20)	3	2	0
Proteinuria present	3 (30)	3	3	0
Neutrophil count increased	3 (30)	3	3	0
Glucose urine present	3 (30)	3	3	0
Blood albumin decreased	1 (10)	2	1	0
Fever	2 (20)	2	2	0
Hypercholesterolemia	1 (10)	2	1	0
Hematocrit decreased	1 (10)	2	1	0
Erythrocyte counts decreased	1 (10)	2	1	0
Respiratory tract infection	1 (10)	2	0	1
Urinary leukocytes positive	2 (20)	2	2	0

Table 2 (continued) | Adverse events

Incidence of AEs during treatment period (safety analysis set)[a]				
AE	**Number of participants (percent)**	**Number of times**		
Urinary occult blood positive	2 (20)	2	2	0
Eosinophil counts increased	2 (20)	2	2	0
Aspartate aminotransferase increase	2 (20)	2	2	0
Bronchopneumonia	2 (20)	2	0	2
Electrocardiogram ST segment elevation	1 (10)	1	1	0
Alanine aminotransferase increased	1 (10)	1	1	0
Low-density cholesterol increased	1 (10)	1	1	0
Sinus bradycardia	1 (10)	1	1	0
Sinus arrhythmia	1 (10)	1	1	0
High-density cholesterol increased	1 (10)	1	1	0
Respiratory syncytial virus infection	1 (10)	1	1	0
Resting tremor	1 (10)	1	1	0
Uric acid increased	1 (10)	1	1	0
Hemoglobin decreased	1 (10)	1	1	0
Lymphocyte ratio decreased	1 (10)	1	1	0
Neutrophil percentage increased	1 (10)	1	1	0
Blood bilirubin increased	1 (10)	1	1	0
Direct bilirubin	1 (10)	2	1	0
Blood glucose	1 (10)	1	1	0

[a]The safety analysis set included all patients who received the study drug and have at least one post-treatment safety evaluation data; the safety analysis set is used primarily for the analysis of safety data.

assessments were restricted to TB-ABR and ASSR thresholds due to unavailable Click-ABR data and partial PTA availability (n = 3, 4.8-, 5.0-, 6.2-year-olds, respectively). A similar pattern was observed in ten ears from these seven patients, that the hearing improvement in 4–6 weeks accounted for 65% of the total TB-ABR improvement at 26 weeks (Fig. 4a; thick gray dots and lines). Similar trends were also observed in ASSR thresholds improvements (Extended Data Fig. 6a). All improvement curves were fitted accurately by a single-time-constant exponential function (Fig. 4a and Extended Data Fig. 6a; thick red and gray lines).

Second, correlation between objective and subjective hearing outcomes is time dependent. Between 0.5 and 1 month, the Click- and TB-ABR thresholds were modestly correlated with PTA (coefficient of determination (R^2) = 0.31 and 0.40, respectively; Fig. 4b). Between 4 and 12 months, the correlation increased dramatically, with the Click- and TB-ABR thresholds being able to explain 68% and 73% variability in the PTA, respectively (Fig. 4c). In contrast, the ASSR thresholds accounted for much less variability in the PTA (0.5–1 month, R^2 = 0.003; 4–12 months, R^2 = 0.17; Extended Data Fig. 6b).

Third, the efficacy of AAV-*OTOF* gene therapy seems to depend on age. The PTA result showed that the most improvement (>80 dB) occurred in participants aged between 5 and 8 years old, whereas participants younger and older than this age range showed much less improvement (Fig. 4d). The Click- and TB-ABR thresholds showed a

https://doi.org/10.1038/s41591-025-03773-w

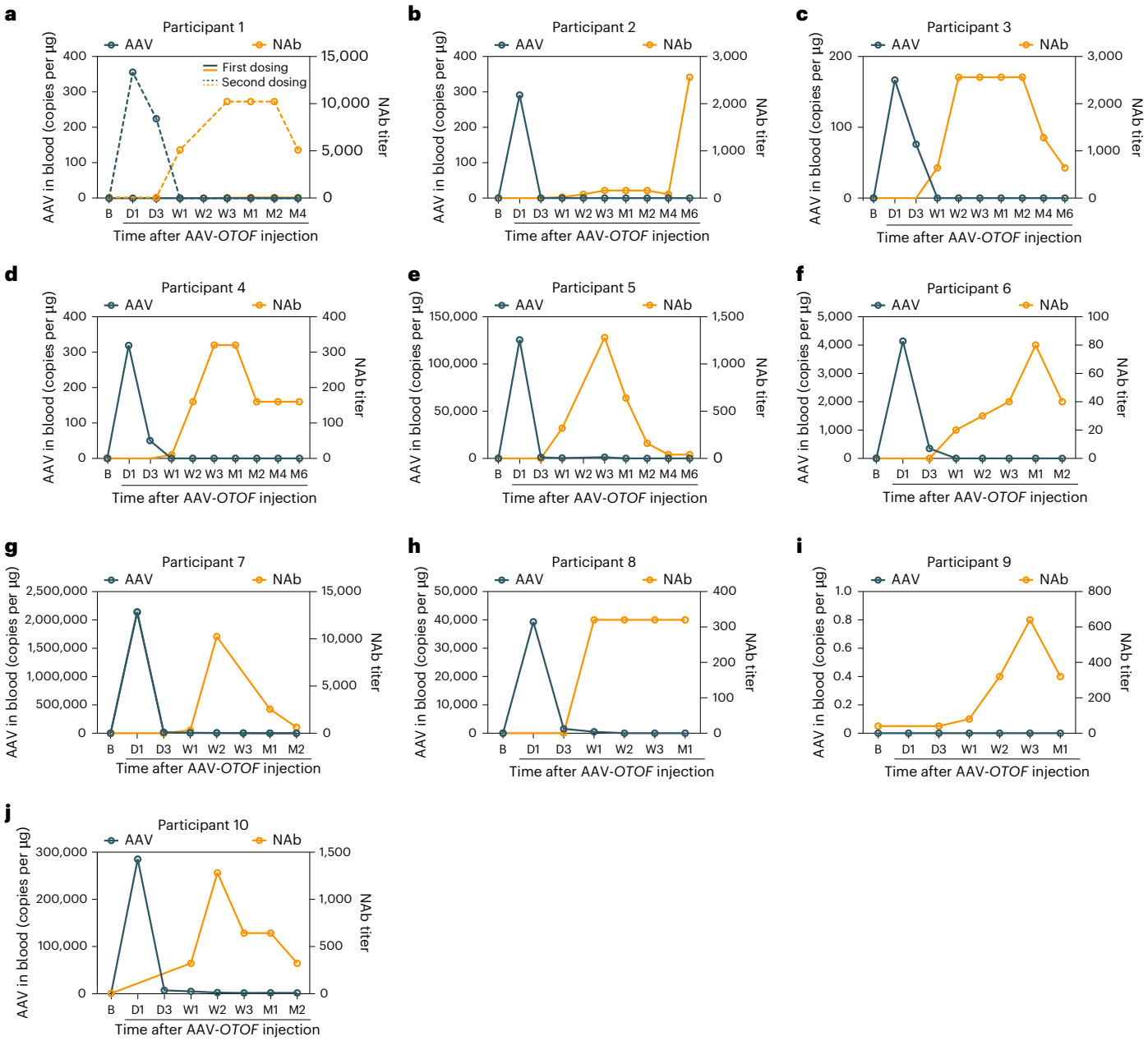


Fig. 2 | AAV distribution in whole blood and NAb distribution in serum. **a**–**j**, Genomic AAV-OTOF levels in blood (copies/µg; dark green lines, left *y* axis) and NAb titers (yellow lines, right *y* axis) for participants 1 (**a**), 2 (**b**), 3 (**c**), 4 (**d**), 5 (**e**), 6 (**f**), 7 (**g**), 8 (**h**), 9 (**i**), and 10 (**j**) at the following time points: baseline (B), 1 day (D1), 3 days (D3), 1 week (W1), 2 weeks (W2), 3 weeks (W3), 1 month (M1), 2 months (M2), 4 months (M4), and 6 months (M6). Participant 1 (**a**) received two AAV-*OTOF* injections, with the second injection (dashed lines) being performed 4 months after the first (solid lines overlap with *x* axis because all values were zero, except that the NAb level increased from 10 at W2 to 80 at M1, and stayed at 80 throughout M4).

trend similar to that of the PTA result (Fig. 4d; solid lines). Again, the ASSR showed a different pattern of results with the participants of 1–2 years old showing an amount of improvement similar to those of 5–8 years old (Fig. 4d; solid lines). We also performed similar analysis on the previously reported eight participants. The TB-ABR thresholds showed similar correlations between the efficacy and age to our result, except that the participants of 2–3 years old had greater improvement. The ASSR thresholds from the published eight participants also showed a similar trend (Fig. 4d; dashed lines).

Discussion

To our knowledge, a multicenter clinical trial of gene therapy for deafness involving adult participants has not been reported previously. Our results show that the present ten participants, aged between 1.5 and 23.9 years old, all tolerated AAV-*OTOF* injection well without any SAEs. Importantly, the present gene therapy is able to improve hearing rapidly with the first month of drug delivery, albeit to various degrees, in all ten participants, including one older adolescent and one adult. Compared with previous studies[16,17], which reported behavioral pure-tone thresholds in only three out of 11 participants, the present study reports this important functional outcome in ten of ten participants.

We used an adeno-associated virus serotype, Anc80L65 (AAV-Anc80L65)-mediated *OTOF* gene delivery vector. AAV-*OTOF* was detected rapidly in the blood of nine out of ten participants, indicating systemic circulation after local injection, but the AAV level varied greatly among participants (0 to 2,141,000 copies μg^{-1}). It is not clear

https://doi.org/10.1038/s41591-025-03773-w

Fig. 3 | ABR and behavioral threshold test results. a–**m**, Click-ABR (left), TB-ABR (middle) and pure-tone (right) thresholds for participants 10 (**a**,**b**), 5 (**c**), 7 (**d**,**e**), 1 (**f**), 2 (**g**), 8 (**h**), 4 (**i**), 3 (**j**,**k**), 6 (**l**) and 9 (**m**) in order of age. Arrows indicate no response at the tested maximum sound intensity, with the direction of the arrow representing either the left (-L) or right (-R) ear, respectively. **f**, Data from the first (dashed lines) and second (asterisks) injections for participant 1.

https://doi.org/10.1038/s41591-025-03773-w

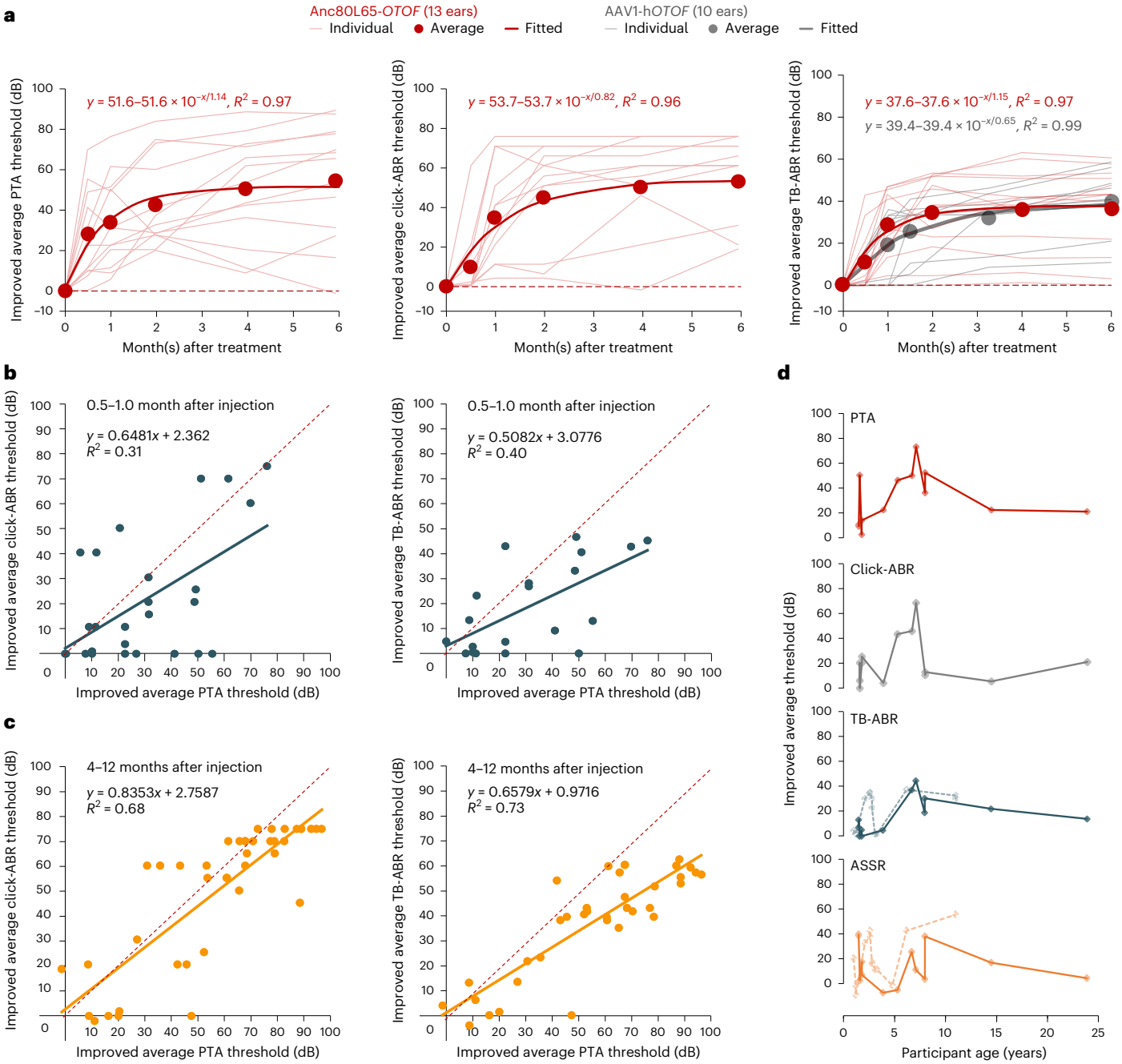


Fig. 4 | Group data analysis on the efficacy of gene therapy. a, Timecourse of gene therapy shown as the improved thresholds (*y* axis) by PTA (left), Click-ABR (middle) and TB-ABR (right). Red and gray dots represent average data; other light lines represent individual data. The thick red and gray lines showed the fitted exponential growth functions of the improved thresholds R^2 as shown. Red dots and lines, thresholds for participants in this trial; gray dots and lines, thresholds for ten ears from the seven patients with hearing improvement and 26-week follow-up in published articles. **b**, Correlation between improved PTA thresholds and improved Click-ABR (left) thresholds and TB-ABR thresholds (right) at 0.5–1 month after injection. Solid green lines represent a linear fit to the data, with the regression equation and R^2 as shown. The red dashed diagonal line represents a theoretical perfect fit with $R^2 = 1$. **c**, As in **b** except for data at 4–12 months. **d**, Average improved thresholds at 1 month (*y* axis) as a function of participant age (*x* axis) for PTA, Click-ABR, TB-ABR and ASSR. Solid lines, thresholds for participants in this trial; dashed lines, thresholds for ten patients in published articles.

how large molecules such as present AAVs permeate the blood–labyrinth barrier in the cochlea. A possible pathway is through the connection between the perilymphatic fluid in the cochlear aqueduct and the cerebrospinal fluid[18]. Clearance time depended on concentration: rapid clearance within 3 days at low levels (≤352.75 copies μg^{-1}) and up to 7 days at high levels (≥4,330 copies μg^{-1}). High blood AAV levels did not result in any SAEs, indicating the safety of the gene therapy protocol. Participant 1 received a second AAV injection. Despite the high NAb level (1:10,240), the second injection did not lead to SAEs. Not only did the second injection further improve hearing but, more importantly, the present participant offered direct evidence for the safety of two injections in the same ear, opening the door to several injections to maintain or even improve recovered hearing in the future.

Although the AAV could not be detected in the blood of the adult participant, her NAb was detectable at a level similar to that of other participants. At present, we do not know whether this paradoxical result

was unique to the age or other factors. Nevertheless, this adult's hearing was partially recovered, suggesting that the immune response NAb, rather than the AAV level, is an appropriate biomarker for potentially successful gene delivery.

An important finding is that the effect of gene therapy takes place with a rapid timecourse of about 1 month, which accounted for 62% of the total improved hearing in terms of PTA improvement at 6 months. On the one hand, this finding allows us to predict the long-term hearing improvement of gene therapy on an individual basis 1 month after surgery. On the other hand, this finding sheds light on the physiological mechanisms underlying the present gene therapy for hearing loss. OTOFERLIN helps synaptic vesicles fuse with the cell membrane and neurotransmitter release in the inner hair cells. Mutations in *OTOF* disrupt the transmission of auditory signals from hair cells to the auditory nerve, as evidenced by the abnormal or absent Click- and TB-ABR in the presence of otoacoustic emission[19]. The lack of reliable correlation between objective and behavioral thresholds within the first month of gene therapy indicates ongoing repair following *OTOF*-mediated release of neurotransmitters at the synapses between inner hair cells and auditory nerve fibers. We speculate that behavioral thresholds are improved faster than objective thresholds. Before gene therapy, no spikes are generated at the auditory nerve level; initially, after gene therapy, limited and asynchronous spikes are probably generated, which can be sufficient to produce behavioral perception but not ABRs. Indeed, this phenotype is typically used to differentiate auditory neuropathy from conventional sensorineural hearing loss. Highly reliable correlation between the two measures after 4 months suggests completion of this synaptic repair. We further speculate that the obvious discrepancy between the ABR and ASSR results reflects peripheral and central influences. Although both the ABR and ASSR can be used to estimate behavioral thresholds, they reflect different neural responses. The ABR reflects the auditory nerve and brainstem's synchronous response to the onset of a stimulus, whereas the ASSR reflects the central auditory system's synchronous response to the temporal envelope of an ongoing stimulus[19,20]. Even if gene therapy has repaired the synaptic deficiency, the central system may take much longer than the peripheral system to recover[21].

Despite the overall promising results from the present and previous studies, gene therapy produces great individual variability in treatment efficiency and phenotypes. Many factors may contribute to this variability, including *OTOF* splicing sites, AAV serotypes, volume of AAV solution, drug administration and currently unknown or uncontrolled processes in the diffusion and transduction of AAV in the inner ear. Participant 1 and participant 9 represent two contrasting conditions in the present sample. Participant 1 (3.9 years old) produced only modest hearing improvement after two injections. He was homozygous with a rare missense variant in *OTOF* but he might have other genetic mutations that cannot be detected by existing genetic testing technology. Participant 9 (23.9 years old) also produced modest hearing improvement, but the causes of hearing loss could be due to environmental factors such as medication, infection, noise exposure and other unknown risks during the 23.9-year period. At present, we cannot differentiate between the nature versus nurture factors.

The improved TB-ABR and behavioral audiograms also showed great individual variability. The TB-ABR audiogram exhibited low-frequency hearing loss in all participants, except for flat loss in participants 1 and 3 and high-frequency loss in participant 6 only. In contrast, the two previous studies showed that about one half of participants had flat loss and the other half had high-frequency loss[16,17]. The functionally more meaningful behavioral audiograms produced a similar pattern of results: of the 13 treated ears, eight had low-frequency hearing loss, two flat loss (7-L and 10-L), two had normal hearing (4 and 8) and only one had high-frequency loss (6). Because of the young age, the two previous studies collected behavioral threshold in only three participants, with one of them having low-frequency loss but the remaining two having high-frequency loss (see Figure in Lv et al.)[16]. We consider the low-frequency loss a more likely outcome than other audiogram types because low-frequency loss is characteristic of known patients of auditory neuropathy[22]. High-frequency loss is probably due to damaged outer hair cells in typical sensory hearing loss. The reasons underlying the difference between low and high-frequency loss are not clear but may be related to the different AAV serotypes and different surgical procedures used between the present and previous studies.

Perhaps the most important, as well as the most puzzling, result of the present study is the age dependence of gene therapy, with the best outcome being achieved for participants between 5 and 8 years old. In both our trial and the study by Wang et al.[17], younger participants, 1.5-, 1.6- and 1.2-year-old toddlers, all had slower and poorer hearing improvement. The poor outcome in these toddlers is counterintuitive, as younger ages should be associated with better outcomes because 'young' inner ears presumably have more complete cellular structure and better-preserved functions. Several possibilities might have contributed to this counterintuitive finding. One possibility is that the added volume of fluid during drug delivery may impede hearing function by inserting higher mechanical pressure on the hair cells in infants than adults. We consider this possibility unlikely for the following three reasons. First, there is no difference in cochlear size between infants and adults because the inner ear is fully mature at birth[23]. Second, based on two previous studies[24,25], we estimated the theoretically safe volume as 71 µl, which far exceeded the 30–40 µl added volume in the protocol. Third, other studies injected the drug through the round window with stapes fenestration for pressure release, but still obtained relatively poor hearing improvement in some participants[16,17]. Another possibility is related to surgical procedure, which may be delivered more consistently in adults than infants. We consider this possibility possible but unlikely because round windows have great individual variability form, size and features but are fully mature at birth[26]. Furthermore, the surgeons are all experienced in both pediatric and adult cochlear implantation. A third possibility is that hair cells are still proliferating in infants so that the episomal AAV vector genomes (vg) are diluted out over time, decreasing efficacy. We consider this possibility unlikely because there is no evidence for continuous hair cell proliferation after birth. A more likely cause for this age dependency is difference in transduction efficiency of AAV between adults and infants. However, no data exist to falsify this hypothesis.

As an early attempt using gene therapy to restore hearing, the present study lacks the capacity to probe mechanisms underlying the observed results such as the rapid improvement, large individual variability and age dependence. Nevertheless, the present result has fundamentally important implications. For example, even if the age of 5–8 years old turns out to be optimal for gene therapy, we do not want to wait for that long for these congenitally deafened children because of the critical period of language development. A possible strategy is to get a shot at an early age and another shot at ages from 5 to 8 years old.

The present result is also limited by the small sample size, including only one adult participant. The small sample size also limits our ability to evaluate the sensitivity of gene therapy to other potentially important biological variables such as ethnicity and sex. Future studies with a larger and more diverse sample size as well as longer follow-ups are needed to delineate the dosage and biological variable interactions and determine the optimal therapeutic window and strategies.

In conclusion, we found that a single injection of AAV-*OTOF* is well tolerated and safe, effectively improving hearing in DFNB9 patients of various ages. The effect of gene therapy is rapid, taking 1 month to achieve 62% in PTA and 78% in TB-ABR of the total improved hearing at 6 months. The present result has not only expanded the therapeutic window to include adolescents and adults, but also indicated an optimal age range of 5–8 years for the therapy. Future larger trials are needed to validate these findings.

Online content

Any methods, additional references, Nature Portfolio reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at https://doi.org/10.1038/s41591-025-03773-w.

References

1. Chang, Q. et al. 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.* **7**, 1077–1086 (2015).
2. Iizuka, T. et al. Perinatal Gjb2 gene transfer rescues hearing in a mouse model of hereditary deafness. *Hum. Mol. Genet.* **24**, 3651–3661 (2015).
3. Askew, C. et al. Tmc gene therapy restores auditory function in deaf mice. *Sci. Transl. Med.* **7**, 295ra108 (2015).
4. Isgrig, K. et al. Gene therapy restores balance and auditory functions in a mouse model of Usher syndrome. *Mol. Ther.* **25**, 780–791 (2017).
5. Pan, B. et al. Gene therapy restores auditory and vestibular function in a mouse model of Usher syndrome type 1c. *Nat. Biotechnol.* **35**, 264–272 (2017).
6. Dulon, D. et al. Clarin-1 gene transfer rescues auditory synaptopathy in model of Usher syndrome. *J. Clin. Invest.* **128**, 3382–3401 (2018).
7. Nist-Lund, C. A. et al. Improved TMC1 gene therapy restores hearing and balance in mice with genetic inner ear disorders. *Nat. Commun.* **10**, 236 (2019).
8. Roux, I. et al. Otoferlin, defective in a human deafness form, is essential for exocytosis at the auditory ribbon synapse. *Cell* **127**, 277–289 (2006).
9. Vona, B., Rad, A. & Reisinger, E. The many faces of DFNB9: relating OTOF variants to hearing impairment. *Genes (Basel)* **11**, 1411 (2020).
10. Iwasa, Y. I. et al. Detailed clinical features and genotype-phenotype correlation in an OTOF-related hearing loss cohort in Japan. *Hum. Genet.* **141**, 865–875 (2022).
11. Ford, C. L. et al. The natural history, clinical outcomes, and genotype-phenotype relationship of otoferlin-related hearing loss: a systematic, quantitative literature review. *Hum. Genet.* **142**, 1429–1449 (2023).
12. Landegger, L. D. et al. A synthetic AAV vector enables safe and efficient gene transfer to the mammalian inner ear. *Nat. Biotechnol.* **35**, 280–284 (2017).
13. Zinn, E. et al. In silico reconstruction of the viral evolutionary lineage yields a potent gene therapy vector. *Cell Rep.* **12**, 1056–1068 (2015).
14. Qi, J. et al. Preclinical efficacy and safety evaluation of AAV-OTOF in DFNB9 mouse model and nonhuman primate. *Adv. Sci. (Weinh.)* **11**, e2306201 (2024).
15. Qi, J. et al. AAV-mediated gene therapy restores hearing in patients with DFNB9 deafness. *Adv. Sci. (Weinh.)* **11**, e2306788 (2024).
16. Lv, J. et al. AAV1-hOTOF gene therapy for autosomal recessive deafness 9: a single-arm trial. *Lancet* **403**, 2317–2325 (2024).
17. Wang, H. et al. Bilateral gene therapy in children with autosomal recessive deafness 9: single-arm trial results. *Nat. Med.* **30**, 1898–1904 (2024).
18. Andres-Mateos, E. et al. Choice of vector and surgical approach enables efficient cochlear gene transfer in nonhuman primate. *Nat. Commun.* **13**, 1359 (2022).
19. Starr, A. et al. Pathology and physiology of auditory neuropathy with a novel mutation in the MPZ gene (Tyr145->Ser). *Brain* **126**, 1604–1619 (2003).
20. Zeng, F. G., Kong, Y. Y., Michalewski, H. J. & Starr, A. Perceptual consequences of disrupted auditory nerve activity. *J. Neurophysiol.* **93**, 3050–3063 (2005).
21. Chambers, A. R. et al. Central gain restores auditory processing following near-complete cochlear denervation. *Neuron* **89**, 867–879 (2016).
22. Zeng, F. G., Oba, S., Garde, S., Sininger, Y. & Starr, A. Temporal and speech processing deficits in auditory neuropathy. *Neuroreport* **10**, 3429–3435 (1999).
23. Atalay, B., Eser, M. B., Kalcioglu, M. T. & Ankarali, H. Comprehensive analysis of factors affecting cochlear size: a systematic review and meta-analysis. *Laryngoscope* **132**, 188–197 (2022).
24. Dai, C. et al. Rhesus cochlear and vestibular functions are preserved after inner ear injection of saline volume sufficient for gene therapy delivery. *J. Assoc. Res. Otolaryngol.* **18**, 601–617 (2017).
25. Ekdale, E. G. Comparative anatomy of the bony labyrinth (inner ear) of placental mammals. *PLoS ONE* **8**, e66624 (2013).
26. Toth, M., Alpar, A., Patonay, L. & Olah, I. Development and surgical anatomy of the round window niche. *Ann. Anat.* **188**, 93–101 (2006).

Jieyu Qi[1,2,3,4,19], Liyan Zhang[1,19], Ling Lu[1,5,19], Fangzhi Tan[1,19], Cheng Cheng[5,19], Yicheng Lu[1,19], Wenxiu Dong[6], Yinyi Zhou[2], Xiaolong Fu[2,7], Lulu Jiang[6], Chang Tan[6], Shanzhong Zhang[6], Sijie Sun[6,8], Huaien Song[8], Maoli Duan[9,10,11] ✉, Dingjun Zha[12] ✉, Yu Sun[13,14] ✉, Xia Gao[5] ✉, Lei Xu[15] ✉, Fan-Gang Zeng[16] ✉ & Renjie Chai[1,2,3,17,18] ✉

[1]Department of Otolaryngology Head and Neck Surgery, Zhongda Hospital, State Key Laboratory of Digital Medical Engineering, Jiangsu Provincial Key Laboratory of Critical Care Medicine, School of Life Sciences and Technology, School of Medicine, Advanced Institute for Life and Health, Southeast University, Nanjing, China. [2]Department of Radiology, Zhuhai People's Hospital, The Affiliated Hospital of Beijing Institute of Technology, Advanced Technology Research Institute, School of Life Science, Beijing Institute of Technology, Beijing, China. [3]Co-Innovation Center of Neuroregeneration, Nantong University, Nantong, China. [4]State Key Laboratory of Hearing and Balance Science, Beijing Institute of Technology, Beijing, China. [5]Department of Otolaryngology Head and Neck Surgery, Nanjing Drum Tower Hospital, Affiliated Hospital of Medical School, Nanjing University, Jiangsu Provincial Key Medical Discipline (Laboratory), Nanjing, China. [6]Otovia Therapeutics Inc., Suzhou, China. [7]Medical Science and Technology Innovation Center, Shandong

Provincial Hospital, College of Clinical and Basic Medicine, Shandong First Medical University and Shandong Academy of Medical Sciences, Jinan, China. [8]Fosun Health Capital, Shanghai, China. [9]Department of Otolaryngology, The First Affiliated Hospital of Wenzhou Medical University, Wenzhou, China. [10]Department of Otolaryngology Head and Neck Surgery and Audiology and Neurotology, Karolinska University Hospital, Stockholm, Sweden. [11]ENT Division, Department of Clinical Science Intervention and Technology, Karolinska Institute, Stockholm, Sweden. [12]Department of Otorhinolaryngology Head and Neck Surgery, Xijing Hospital, The Fourth Military Medical University, Xi'an, China. [13]Department of Otorhinolaryngology, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China. [14]Hubei Province Clinical Research Center for Deafness and Vertigo, Wuhan, China. [15]Department of Otolaryngology—Head and Neck Surgery, Shandong Provincial ENT Hospital, Shandong University, Jinan, China. [16]Center for Hearing Research, Departments of Anatomy and Neurobiology, Biomedical Engineering, Cognitive Sciences, Otolaryngology-Head and Neck Surgery, University of California Irvine, Irvine, CA, USA. [17]Department of Otolaryngology Head and Neck Surgery, Sichuan Provincial People's Hospital, University of Electronic Science and Technology of China, Chengdu, China. [18]Southeast University Shenzhen Research Institute, Shenzhen, China. [19]These authors contributed equally: Jieyu Qi, Liyan Zhang, Ling Lu, Fangzhi Tan, Cheng Cheng, Yicheng Lu. ✉e-mail: maoli.duan@ki.se; zhadjun@fmmu.edu.cn; sunyu@hust.edu.cn; gaoxia@nju.edu.cn; sdphxl@email.sdu.edu.cn; fzeng@uci.edu; renjiec@seu.edu.cn

Methods

Trial design

This is a multicenter, open-label, single-arm, controlled-intervention clinical trial conducted at five hospitals in China. The trial aims to determine the effect of Anc80L65-*OTOF* gene therapy in recovering hearing in DFNB9 participants, with planned 5-year follow-up. The trial has been conducted in strict accordance with the Declaration of Helsinki, GCP principles, ICH-GCP guidelines and the established clinical research protocol, which was approved by the Ethics Committee of all five participating sites (Shandong Second Provincial General Hospital; Union Hospital Tongji Medical College, Huazhong University of Science and Technology; Nanjing Drum Tower Hospital, Affiliated Hospital of Medical School, Nanjing University; Xijing Hospital, The Fourth Military Medical University; Ningbo No.2 Hospital), and subsequently revised under the auspices of the same committees. Informed consent forms were obtained from the participants or parents before the patients' enrollment in the trial, with additional explicit consent secured specifically for the sharing of patient video materials. The detail protocol was provided in supplementary information (Final Protocol). No sex-related analyses were conducted. This trial was registered (ClinicalTrials.gov registration: NCT05901480) on 26 June 2023 and is currently ongoing, with a protocol-defined target enrollment of 25 participants.

Participants

Ten participants, aged 1.5 to 23.9 years old, were enrolled based on whole exome sequencing, with identified homozygous or compound heterozygous pathogenic or likely pathogenic mutations in the *OTOF* gene, as well as confirmed bilateral hearing thresholds ≥65 dB. Participants who received previous gene therapy, had anti-AAV NAb >1:128 or had bilateral cochlear implants were excluded. Detailed inclusion and exclusion criteria are provided here and in the protocol in supplementary information.

Participant inclusion criteria

Subjects must meet all of the following inclusion criteria to be enrolled in the study:

(1) Age ≥1 year at the time of signing the informed consent form (ICF); male or female.
(2) Diagnosis of *OTOF*-related deafness is established as per the following criteria:
   (a) Hearing loss confirmed by auditory testing and ABR test (reports within 1 month before the signing of ICF are acceptable);
   (b) Presence of homozygous or compound heterozygous mutations in the *OTOF* gene confirmed by genetic testing.
(3) Auditory testing: bilateral severe (65 dB ≤ hearing threshold < 80 dB) or profound (80 dB ≤ hearing threshold < 95 dB) or complete (hearing threshold ≥ 95 dB) hearing loss; (if ABR test results are 'not produced,' subjects with bilateral hearing threshold <65 dB may be enrolled as judged by the investigator).
(4) Test results of vital signs, physical examination, laboratory tests (blood routine examination, blood biochemistry and urine routine examination, coagulation function, and so on), 12-lead electrocardiograms (ECGs) and other tests are all normal, or the abnormality is judged by the investigators as having no clinical significance.
(5) Subjects and their guardians sign the ICF.

Participant exclusion criteria

Subjects meeting any of the following criteria should not be enrolled:

(1) Patients who have previously experienced serious allergic reactions to any drug or its components in this study (≥Grade 3 SAE as per NCI-CTCAE v.5.0).
(2) Any previous gene therapy or blood neutralizing antibody level greater than 1:128.
(3) Patients with systemic diseases or having received relevant treatment that may affect hearing or surgical operation.
(4) Patients intolerant to general anesthesia.
(5) Patients with inner ear malformation.
(6) Bilateral cochlear implantation, or history of major inner ear surgery (as judged by the investigator) (except unilateral cochlear implantation).
(7) Patients with other mutations in deafness gene that affect judgment of *OTOF* gene therapy effect.
(8) Patients with Meniere's disease.
(9) Patients who are routinely treated with ototoxic drugs for other diseases.
(10) Patients with congenital deafness due to the presence of other nongenetic factors associated with hearing loss at birth.
(11) Subjects who are receiving, or are likely to receive, immunosuppressive therapy other than the study drug of this study.
(12) Subjects with hypersensitivity or intolerance to glucocorticoids.
(13) History of malignancy or meningitis.
(14) Subjects with persistent or active infection, positive HBsAg results and HBV DNA titer in peripheral blood higher than the lower limit of detection, positive HCV antibody test results and HCV RNA titer in peripheral blood higher than the lower limit of detection, positive HIV antibody test results or other immunodeficiency diseases, and positive syphilis test results.
(15) Subjects of childbearing potential who refuse to use effective contraception (hormonal or barrier contraception or abstinence) from signing the informed consent to 12 months after injection of AAV.
(16) Female subjects of childbearing potential with positive blood pregnancy test results or who are pregnant or lactating.
(17) Subjects who have participated in any other clinical trial within 4 weeks before the first dose and have received administration or treatment (including drug and device clinical trials, excluding noninterventional studies).
(18) Subjects who are unwilling or unable to comply with this protocol.
(19) Subjects who, in the opinion of the investigators, are unable to participate in the study or to complete the subsequent study due to any medical conditions.

All participants had normal vital signs, physical examinations, weight, laboratory tests (complete blood count, blood biochemistry, urine analysis, coagulation function, 12-lead ECG, blood pregnancy test and virology screening tests). Any abnormalities, if present, were deemed clinically insignificant by the investigators.

The hearing assessments included DPOAE, Click-ABR, TB-ABR, pure-tone audiometry, ASSR, CM and tympanogram examination. In the previous article, we published the raw data including the primary and secondary outcomes for participant 2 at 3 months and participant 3 at 2-month follow-up, including serum neutralizing antibodies and whole blood AAV, audiological examination data[15]. At present, participants 2 and 3 have been followed for 1 year. In this paper, we reused the resource data mentioned and added the original data of primary outcomes and secondary outcomes from longer follow-up, and also conducted further correlation analyses for all data.

Protocol amendment

This protocol was approved by the Ethics Committee of all participating sites, including the Shandong Second Provincial General Hospital (leading site), the Union Hospital Tongji Medical College, the Huazhong University of Science and Technology Nanjing Drum Tower Hospital, the Affiliated Hospital of Medical School, Nanjing University, the Xijing

Hospital, The Fourth Military Medical University, the Ningbo No. 2 Hospital. Detailed protocol amendments are summarized here and in Supplementary Information. The reasons are also shown in Supplementary Information together with the amendments.

(1) Deleted 'brain MRI' from safety evaluation indicators; brain magnetic resonance imaging (MRI)/computed tomography (CT) should be performed only during the screening period, and whether to perform MRI/CT during subsequent follow-up should be decided at the discretion of the investigator; patients where MRI or CT should be performed are made clear.
(2) Auditory testing: revised auditory test items, deleted acoustic reflex, added tympanogram and added behavioral audiometry and ASSR test; deleted auditory test in week 3; added auditory test visit at the discretion of the investigator, added or removed auditory test items at the discretion of the investigator.
(3) AAV testing: added AAV testing on postoperative day 1.
(4) Route of administration: deleted single injection; the investigators will decide whether the second intracochlear injection should be conducted based on dose of the first injection by considering anatomical structure of artificial cochlea and drug loss. The timing of the second injection will be decided by recovery status of the first injection.
(5) Increased the screening period to 60 days.
(6) Genetic testing: added pedigree verification.
(7) Added bodyweight measurement on 3 days before (D−3) to 1 day before (D−1) gene therapy.
(8) Changed from single center to multicenter.
(9) Changed unilateral injection to allow bilateral injection as assessed by investigators.
(10) Inclusion criteria 1: adjusted 'Age ≥ 3 years' to 'Age ≥ 1 year.'
(11) Inclusion criteria 2b: deleted '(test report within 1 month before informed consent is acceptable).'
(12) Changed the planned sample size from '5' to '25' subjects.
(13) Dosage regimen: adjust injection volume.
(14) Increased follow-up period to 5 years.
(15) NAb testing: conduct NAb testing until the results turn negative.

Procedures

Ears of participants received a single AAV-*OTOF* injection ranging from 8.4×10^{11} to 11.2×10^{11} vg (volume 30–40 µl) through round window membrane by the otologic surgeon from each site. Vital signs, AAV vectors in blood, NAb in serum, complete blood count, blood biochemistry and auditory tests, including pure-tone audiometry, DPOAE, CM, Click-ABR and TB-ABR, ASSR and tympanogram were performed at baseline, 1 day, 3 days, 1 week ± 1 day, 2 weeks ± 3 days, 1 month ± 3 days 2 months ± 3 days, 4 months ± 6 days, 6 months ± 6 days, 9 months ± 6 days and 12 months ± 6 days. Second injection might be considered, if the testing results from the first dosing still met the inclusion criteria. The timing of the second injection depended on recovery from the first injection. The detailed study flowchart can be found in the protocol in Supplementary Information.

AAV-*OTOF* design and manufacture

AAV-*OTOF* was used as a 1:1 genomic mixture of two AAV viruses (AAV-*OTOF*-N and AAV-*OTOF*-C). The expression of *OTOF* was driven by the mouse *Myo15* promoter—a gene specific to hair cells. The two AAV vectors carried different coding sequences of the human *OTOF* gene (NM_001287489.2), with the split site between exons 20 and 21. AAV-*OTOF*-N contained a splice donor (SD) signal sequence after the N-terminal sequence of *OTOF*; correspondingly, AAV-*OTOF*-C contained a splice acceptor (SA) signal sequence before the C-terminal sequence of *OTOF*. An AK sequence for homologous recombination was inserted between the SD and 3′-inverted terminal repeat of AAV-*OTOF*-N, and between the 5′-inverted terminal repeat and SA of AAV-*OTOF*-C. A woodchuck hepatitis virus posttranscriptional regulatory element was inserted after the *OTOF*-C sequence to enhance full-length *OTOF* expression. AAV-*OTOF*-N and AAV-*OTOF*-C were manufactured aseptically at a titer of 2.8×10^{13} vg ml^{-1} and individually filled in 150 µl and 75 µl canning volumes into sterile vials by PackGene Biotechnology Co., Ltd. When in use, 75 µl of AAV-*OTOF*-N was extracted aseptically and injected into sterile vials containing 75 µl of AAV-*OTOF*-C.

AAV-*OTOF* delivery

AAV-*OTOF* was delivered via round window membrane injection into the cochlea. In participants with unilateral cochlear implantation, AAV therapy was administered in the contralateral ear. In participants without a cochlear implant, both ears received a single injection of the AAV-*OTOF* simultaneously.

During AAV-*OTOF* injection, the round window membrane is exposed through the trans-mastoid facial recess, which had been fully proven to be safe and effective in cynomolgus monkeys and humans[14,15]. The exposure process was performed using a microscope and an endoscope jointly. In brief, after incising the skin and periosteum, mastoid was exposed. Important anatomical landmarks, the facial nerve and the vertical segment of chorda tympani nerve beneath the mastoid were exposed by using a high-speed otologic drill. The facial recess between the two nerves was drilled through to access the tympanic cavity and fully expose the round window membrane. A 33G needle was used to puncture the round window membrane and to deliver 30–40 µl of AAV-*OTOF* at a constant rate of 100 nl s^{-1} using a nanoliter-level injection pump under an endoscope.

Medical care for participants

To minimize potential inflammatory reactions, each participant received oral prednisone or equivalent corticosteroids at a dose of 0.5 mg kg^{-1} day^{-1} starting 3 days before surgery, and the dose was increased to 1 mg kg^{-1} day^{-1} on the day of surgery until week 1 and then reduced to 0.5 mg kg^{-1} day^{-1} in weeks 2–3, to 0.25 mg kg^{-1} day^{-1} in weeks 4–5, to 0.125 mg kg^{-1} day^{-1} in weeks 6–8, and then discontinued. The maximum dose of prednisone did not exceed 40 mg day^{-1}. Actual dosing was assessed by the researcher based on the circumstances of the individual participant. At 3 days before to 1 day before gene therapy (D−3 to D−1), participants underwent preoperative checks, including vital signs, physical examination, ECG, laboratory indicators and pregnancy blood.

Endpoints

The primary endpoints were safety and tolerability. Safety analysis included the incidence and severity of AEs and their correlation with the study drug, and physical examination (including cranial nerve testing), the laboratory test indicators (blood routine examination, urine routine examination, blood biochemistry, coagulation function and so on), vital signs, ECG examination, neutralizing antibodies detection and AAV testing in peripheral blood. AEs were defined as any untoward medical occurrences in a participant administered the drug, even if these did not necessarily have a causal relationship with the treatment. The secondary endpoints measured the degree of hearing recovery of each participant according to the required follow-up timepoints, and the content of the test mainly includes behavioral audiometry, ABR, ASSR, DPOAE, CM and tympanogram examination. Data of CM and tympanogram examination were not included here. In addition to data at individual frequencies, the thresholds at 0.5, 1, 2 and 4 kHz were used to calculate the PTA, average TB-ABR and ASSR thresholds as a global measure of hearing improvement.

AEs recording and determination

AEs were recorded from the time of the subject's first exposure to the drug until 5 years follow-up or commencement of another treatment regimen (whichever occurred earlier). AEs were assessed and graded

in terms of type and frequency according to NCI-CTCAE v.5.0. Tolerability referred to the participant's tolerance to the injected dose of AAV-*OTOF* in this trial. In this paper, AEs from ten participants with up to 1-year follow-up were summarized in Table 2.

NAb detection

Participant serum was collected according to the protocol, then stored frozen at −80 °C. Serum samples were transported under conditions of −65 °C to −85 °C to Shanghai Jollin Lab Co., Ltd. for NAb detection. Each individual sample in each screening test batch is tested in duplicate wells. During detection, in the biosafety cabinet, HEK293T cells were diluted to 1.25×10^6 cells ml^{-1} using analytical medium (DMEM + 3% FBS). Subsequently, the cell dilution was added to the culture plate at 80 µl per well and incubated in a 5% CO_2 incubator for 5.5–6.5 h. The AAV pseudovirus carrying the luciferase reporter gene (AAV-Luc) was diluted to 1.0×10^{11} GC ml^{-1} using the analytical medium. Human serum samples were diluted with the negative matrix (mixed human negative serum). Serial dilutions of human samples, the positive control (AAV NAb) and the negative control (negative matrix) were respectively mixed with the pseudovirus dilution in equal volumes and incubated at room temperature for 30–60 min. Thereafter, serum sample–pseudovirus mixtures were added to the culture plate at 20 µl per well and incubated in a 5% CO_2 incubator for 23.5–24.5 h. The cell culture plate was retrieved from the incubator and allowed to return to room temperature, followed by the addition of the luciferase detection reagent at 100 µl per well and incubation at room temperature away from light for 5 min. The chemiluminescent signal values were collected using a microplate reader, and the relative light units (RLU) were recorded. According to the average RLU value of the negative control, the signal inhibition rate (TI) of the samples was calculated. TI = (mean RLU (negative control) − mean RLU (sample))/mean RLU (negative control). If TI ≥ 50%, it was regarded as a positive result and the sample was classified as positive. The titer value of the sample was calculated based on the method's minimum dilution factor (MRD = 10) multiplied by the dilution factor of the sample diluted to the last positive sample.

AAV-*OTOF* detection

According to the protocol, whole blood samples were collected from participants, stored frozen and transported at −65 °C to −85 °C. AAV detection was performed at Shanghai Jollin Lab Co., Ltd. Nucleic acid extraction was performed on all whole blood samples, and the optical density at 260 nm/280 nm ($OD_{260/280}$) and DNA concentration were measured. The extracted DNA was stored at −20 °C. This method is applicable for the droplet digital polymerase chain reaction (ddPCR)-based detection of the gene copy numbers of the AAV-*OTOF* in the human whole blood matrix. Genomic DNA was extracted from 200 µl of whole blood at the room temperature using the MagMAX DNA Multi-Sample Ultra v.2.0 Kit in accordance with the instructions, and DNA was eluted with 50 µl elution solution. The DNA concentration and absorbance $OD_{260/280}$ was determined using an ultramicro spectrophotometer. The ddPCR reaction mixture of 20 µl was prepared by combining the template, primers, probes, ddPCR reaction premix and deionized water. The reaction was conducted on the DropDx-2044 ddPCR instrument (RainSure Scientific). The reaction procedure was as follows: pre-denaturation at 95 °C for 10 min; denaturation at 95 °C for 30 s; extension at 60 °C for 60 s and 72 °C for 15 s; enzyme inactivation at 98 °C for 10 min; where denaturation and extension were carried out for 40 cycles. After the reaction, the ddPCR reaction chip was transferred to a PCR reader (RainSure Scientific) to output the raw data (copies $µl^{-1}$) to calculate the AAV concentration in 100 µl whole blood. The detection range was 1 to 1×10^6 copies per 20 µl reaction mixture.

Auditory brainstem response

All auditory tests were conducted in a sound insulation shielding room. Under sedative hypnosis, ABR thresholds are recorded, including Click-ABR and TB-ABR. The stimulus sound intensity starts from 80 dB nHL, and the threshold was determined with an accuracy of 5 dB. The stimulus sound frequencies for TB-ABR included 0.25, 0.5, 1, 2 and 4 kHz. Three electrodes (recording electrode, reference electrode and ground electrode) were used to record the hearing thresholds, and placed on the top of the forehead, the mastoid behind the ears and flat between the brows, respectively. Responses were averaged 1,024 times for Click-ABR and 2,048 times for TB-ABR. The time width was 12.5 ms. The threshold was determined based on a reproducible and visually detectable wave V. Threshold corresponding to each sound intensity was recorded at least twice to distinguish true responses from artifacts.

Auditory steady-state response

In a sound insulation shielding room, ASSR thresholds of air conduction stimulus were recorded. The stimulus frequencies included 0.5, 1, 2 and 4 kHz. The modulated wave is a pure tone of 75–110 Hz. ASSR was elicited by increasing or decreasing the stimulus sound intensity in 5 dB steps within the maximum sound intensity range of the instrument. Three electrodes (recording electrode, reference electrode and ground electrode) were used to record the hearing thresholds, and placed on the top of the forehead, the mastoid behind the ears and flat between the brows, respectively. Electrode paste was applied to ensure that the impedance of each electrode did not exceed 5 ohms. Each signal epoch was recorded for a duration of approximately 5–6 min, and the average was computed from a total of 30–35 epochs. The allowed error range for ASSR values was within 1%, and the results were determined by a computer.

Pure-tone audiometry

Pure-tone audiometry measurements were conducted separately for each ear at frequencies of 0.125, 0.25, 0.5, 1, 2, 4, 6 and 8 kHz. For participants younger than 1 year, behavioral observation audiometry was administered. For participants aged 1–2.5 years, visual reinforcement audiometry was used. This test involved several repetitions beforehand to establish accurate conditioned responses. For participants over 2.5 years old, play audiometry was employed, with a similar need to establish good conditioned responses before the test. During the formal test, pauses were introduced between continuous sound stimuli, with intervals of several seconds. The stimulus sound intensity started from the maximum output of the instrument, decreasing in 10 dB steps until there was no response from the participant. The intensity was then increased in 5 dB steps until the participant responded to the sound. Assessments were conducted using insert earphones (IP30, Radioear) placed in the test ears of younger patients, regardless of the age-based testing protocol employed. The insert earphones provide an inter-aural attenuation of 60 dB, reducing the likelihood of the nontest ear detecting the test signals[27].

Distortion product otoacoustic emission

Before the measurement, the participant's ear canal was cleaned. The speaker in the probe inputted two initial pure tones, f1 and f2, into the outer ear canal. The microphone in the probe simultaneously collected the response signals. The input sound intensities of the two initial pure tones were set at 65/55 dB SPL, with a frequency ratio of f2/f1 = 1.22. The f2 tone was presented at frequencies of 556; 684; 988; 1,481; 2,222; 2,963; 4,444; 5,714 and 8,000 Hz or 500; 750; 1,000; 1,500; 2,000; 3,000; 4,000; 6,000 and 8,000 Hz. The highest amplitude of DPOAE response occurs at the frequency of 2f1−f2. The root-mean-square value of the sound pressure at the five continuous frequencies above and the five continuous frequencies below the 2f1−f2 frequency component is taken as the background noise for each f2 frequency. The presence of DPOAE was determined if the output signal is greater than −10dB SPL, at the same time greater than the background noise by 6 dB.

Minigene assay

Wild-type DNA fragments encompassing partial intron 17 (75 bp)−exon 18 (121 bp)−partial intron 18 (547 bp) of the OTOF gene were

amplified using NEST-PCR. Site-directed mutagenesis was performed to introduce the mutation utilizing the PrimeStar Mutagenesis Basal Kit (Takara). The wild-type and mutated PCR products were subsequently cloned into the pcMINI expression vector and transfected into HEK293T and HeLa cells via the Lipo2000 transfection reagent (Invitrogen). Reverse transcription was performed with the PrimeScript RT Reagent Kit and gDNA Eraser (cat. no. RR047A, Takara). The resulting cDNA was amplified by PCR, and the PCR products were analyzed through electrophoresis on a 1.5% agarose gel followed by Sanger sequencing. Primers used in this experiment were shown in the Supplementary Information.

Statistical analysis

No statistical method was used to predetermine sample size due to the limited number of patients with DFNB9—a rare disease. The experiments were not randomized. The investigators were not blinded to allocation during experiments and outcome assessment.

For continuous variables of baseline data, the statistical description of mean, s.d., quartiles, maximum and minimum values were performed; for categorical variables, frequency and corresponding percentage were described. Safety analysis were performed in the Safety Analysis Set and summarized by clinical visit. Safety analyses included otologic examinations, laboratory tests and other reported otologic or systemic AEs. AEs were summarized by type and frequency. For efficacy analyses, quantitative indicators were summarized by calculating change trends from baseline, including means, s.d. and 95% confidence intervals for changes; qualitative indicators were summarized by describing trends in change from baseline by clinical visit. Among them, 95% confidence intervals for changes were not included, which will be shown in the summary report of this clinical trial. This study was an exploratory study and no formal hypothesis testing was performed.

For post hoc analyses of the hearing threshold, no response was recorded as '> the maximum sound intensity' tested. When analyzing pure-tone audiometry, TB-ABR and ASSR data, the threshold was represented as the maximum sound intensity +1 dB. For Click-ABR, if a patient did not respond to clicks at 99 or 100 dB nHL, the threshold was represented as 101 dB nHL. The timecourse of gene therapy efficacy was analyzed up to 6 months by including all participants. The improved thresholds were fitted by exponential growth functions within 6 months with the R^2 being calculated. Age dependence of the therapeutic efficacy was analyzed by including all participants with data from 0.5–1 month after surgery. Correlation in thresholds between different audiometric methods was also analyzed across all participants, with R^2 being calculated to quantify the amount of variability in one threshold explained by the other threshold. All data analyses were conducted using Microsoft Excel (v.16.54 21101001). Excel and GraphPad Prism (v.9) were used for graph preparation.

Reporting summary

Further information on research design is available in the Nature Portfolio Reporting Summary linked to this article.

Data availability

At the outset of the trial, we omitted a data sharing provision from the consent documents signed by participants. As a result, in accordance with our Ethics Committee policies, we are not authorized to release the raw data to the public. Furthermore, the study is still in progress. Instead, de-identified patient characteristics and hearing threshold improvements from raw datasets generated in this study are already included in the paper. Request for more information about the raw data is subject to a confidentiality agreement with Southeast University and Otovia Therapeutics Inc., and must comply with applicable legal and regulatory requirements. Qualified researchers may request access to the trial information by contacting renjiec@seu.edu.cn. The requests will be fulfilled within 120 days, and data transfer agreement may be required. Source data are provided with this paper.

References

27. Katz, J., Chasin, M., English, K., Hood, L. J. & Tillery, K. L. *Handbook of Clinical Audiology* 7th edn (Lippincott Williams & Wilkins, 2015).

Acknowledgements

We would like to express our gratitude to all participants and their families for their active support and cooperation, which have made the study possible. We also extend our thanks to the medical workers at the five hospitals, who provided professional medical service and daily care for the participants during their hospitalization. We thank the editors for their comments and suggestions, especially on the results interpretation and discussion, which have improved the accuracy, clarity and readability of the reported study. This work was supported by the National Key Research and Development Program of China (2021YFA1101300 to R.C., 2021YFA1101800 to R.C., 2020YFA0113600 to J.Q., 2020YFA0112503 to R.C. and 2024YFC2511100/1103 to L.L.), the National Natural Science Foundation of China (82330033 to R.C., 82030029 to R.C., 92468302 to R.C., 92149304 to R.C., 82371162 to J.Q., U23A200440 to J.Q., 82371161 to F.T., 82471176 to L.X., 82401369 to L.Z., 82192862 to X.G., 82071059 to L.L. and 82471185 to L.L), the STI2030-Major Projects (2022ZD0205400 to J.Q.), the Key Program of Jiangsu Natural Science Foundation (BG2024037 to F.T.), the Shenzhen Science and Technology Program (JCYJ20240813161801003 to R.C.), the China Postdoctoral Science Foundation (GZB20240145 to L.Z., 2024M750455 to L.Z.), Taishan Scholars Project-Young Experts Program of Shandong Province (tsqn202408320 to J.Q., tsqn202211357 to L.X.), Shandong Province Outstanding Youth Science Foundation (ZR2024YQ049 to J.Q.), the Natural Science Foundation of Jiangsu Province (BK20232007 to R.C., BK20241692 to L.Z.), the 2022 Open Project Fund of Guangdong Academy of Medical Sciences (YKY-KF202201 to R.C.), the Jiangsu Provincial Scientific Research Center of Applied Mathematics (BK20233002 to R.C.), the Nanjing Medical Science and Technology Development Project (YKK19072 to L.L.) and Research Personnel Cultivation Programme of Zhongda Hospital Southeast University (CZXM-GSP-RC164 to L.L.). This study also was funded by Otovia Therapeutics Inc. The commercial sponsor (Otovia Therapeutics Inc.) participated in study design, protocol revisions, paper revisions and submission decisions. The other funders had no role in study design, data collection and analysis, decision to publish or preparation of the paper. The authors are responsible for the accuracy and completeness of data collection and analysis, fidelity of the trial and this report.

Author contributions

J.Q., F.T., L.Z. and R.C. had full access to all the data in the study. R.C., F.-G.Z., L.X., X.G., Y.S., D.Z. and M.D. were responsible for the concept and design of the study. J.Q., L.Z., L.L., F.T., C.C., Y.L., W.D., Y.Z., X.F., L.J., S.Z., S.S. and H.S. acquired, analyzed and interpreted the data. F.-G.Z., R.C., J.Q., F.T. and L.Z. drafted the paper. R.C., F.-G.Z., J.Q., F.T., L.Z., W.D. and S.Z. reviewed the paper critically for important intellectual content. F.-G.Z., R.C. and J.Q. performed statistical analysis. R.C., J.Q., L.X., F.T., L.Z., L.L., X.G. and Otovia Therapeutics Inc. obtained funding. C.T. and S.Z. provided administrative, technical or material support. R.C., F.-G.Z., L.X., Y.S., D.Z., X.G. and M.D. supervised the study. W.D., S.Z. and J.Q. were involved in study implementation, recruitment and oversight of staff. L.X., Y.S., D.Z., G.X. and K.W. were the lead physicians at the study sites.

Competing interests

W.D., S.Z. and C.T. were paid employees of Otovia Therapeutics Inc. L.J. is a paid employee of Otovia Therapeutics Inc. S.S. and H.S. are

paid employees of Otovia Therapeutics Inc. and Fosun Health Capital. R.C. is the unpaid chief scientist of Otovia Therapeutics Inc. The other authors declare no competing interests.

Additional information

Extended data is available for this paper at https://doi.org/10.1038/s41591-025-03773-w.

Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s41591-025-03773-w.

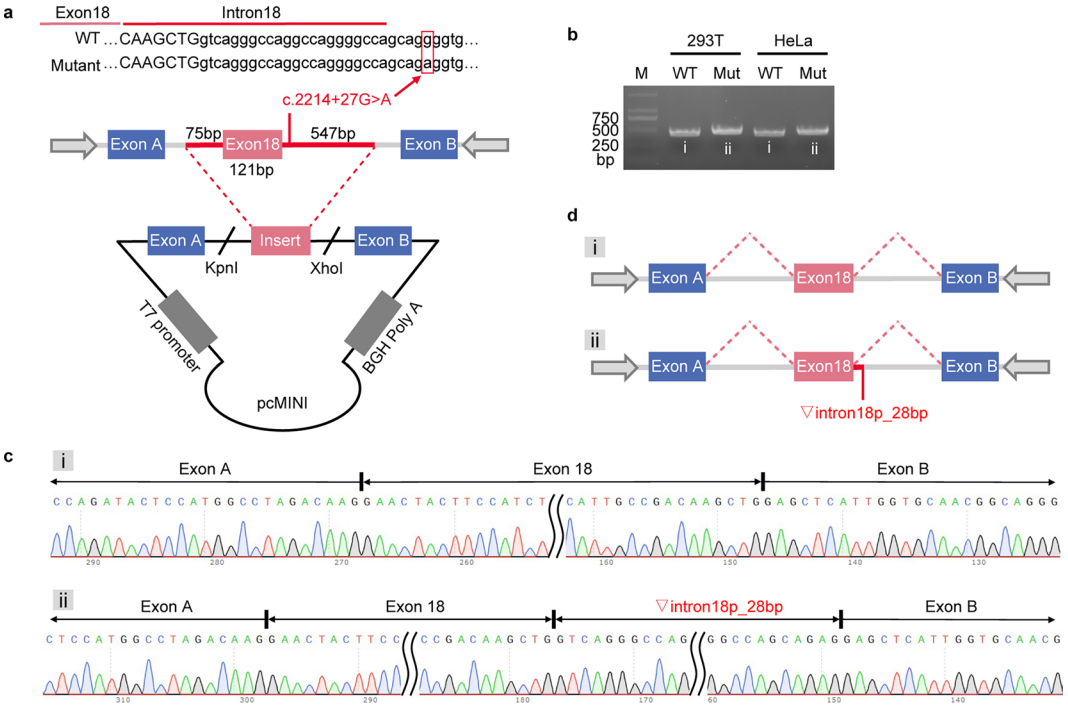
Correspondence and requests for materials should be addressed to Maoli Duan, Dingjun Zha, Yu Sun, Xia Gao, Lei Xu, Fan-Gang Zeng or Renjie Chai.

Peer review information *Nature Medicine* thanks Terence Flotte, Mustafa Tekin and Hidekane Yoshimura for their contribution to the peer review of this work. Primary Handling Editor: Anna Maria Ranzoni, in collaboration with the *Nature Medicine* team.

https://doi.org/10.1038/s41591-025-03773-w

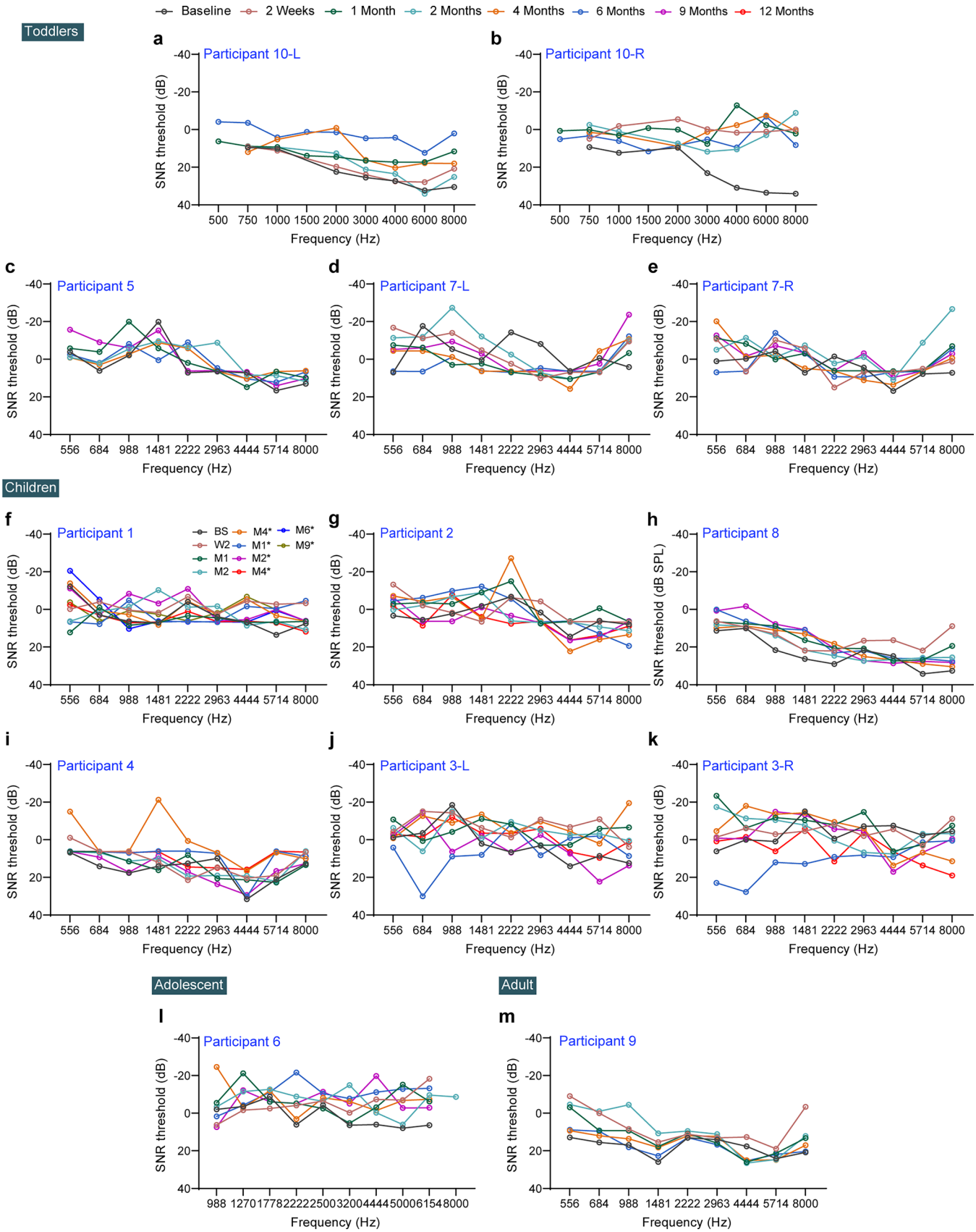
Extended Data Fig. 1 | Sanger sequencing results of the participants and their parents. Arrows indicated the variants in *OTOF* alleles. Two variants inherited from the same parent are located on the same allele in participants 4 and 8, which represents a cis variant situation. Variants in Participants 1, 6 and 10 were homozygous in biallelic *OTOF*. Mutation c.1927G>A in participant 8 was confirmed by the sequencing result of antisense chain, so as the c.5108delinsTCTT in participant 9's mother.

https://doi.org/10.1038/s41591-025-03773-w



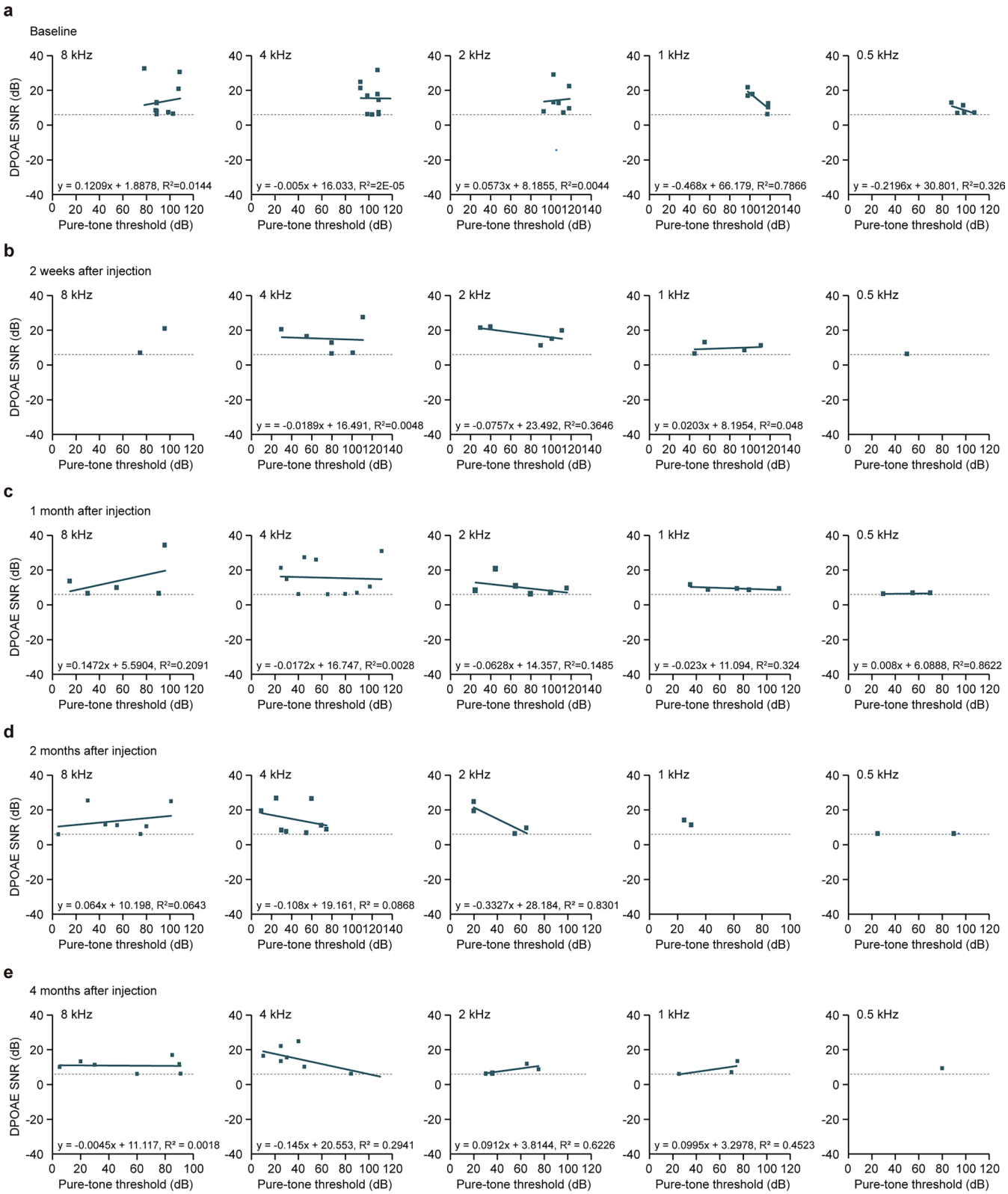
Extended Data Fig. 2 | Minigene-based characterization of c.2214+27 G > A mutation demonstrates pathogenic splicing defects in Participant 4. **a**, Schematic illustration of cloned vectors. **b**, Gel electrophoresis of the RT-PCR products in both HEK293T and HeLa cell lines. In the mutant groups, abnormal splicing bands named "ii" were discovered in both HeLa and HEK293T cells. **c**, The Sanger sequences of RT-PCR products showed the alternative splicing affected by the variation c.2214+27 G > A in OTOF (ii) compared to WT allele (i). **d**, The schematic diagrams indicated that the variant c.2214+27 G > A affected the normal splicing of *OTOF* mRNA, resulting in 28 bp base retention at the left of intron 18.

https://doi.org/10.1038/s41591-025-03773-w



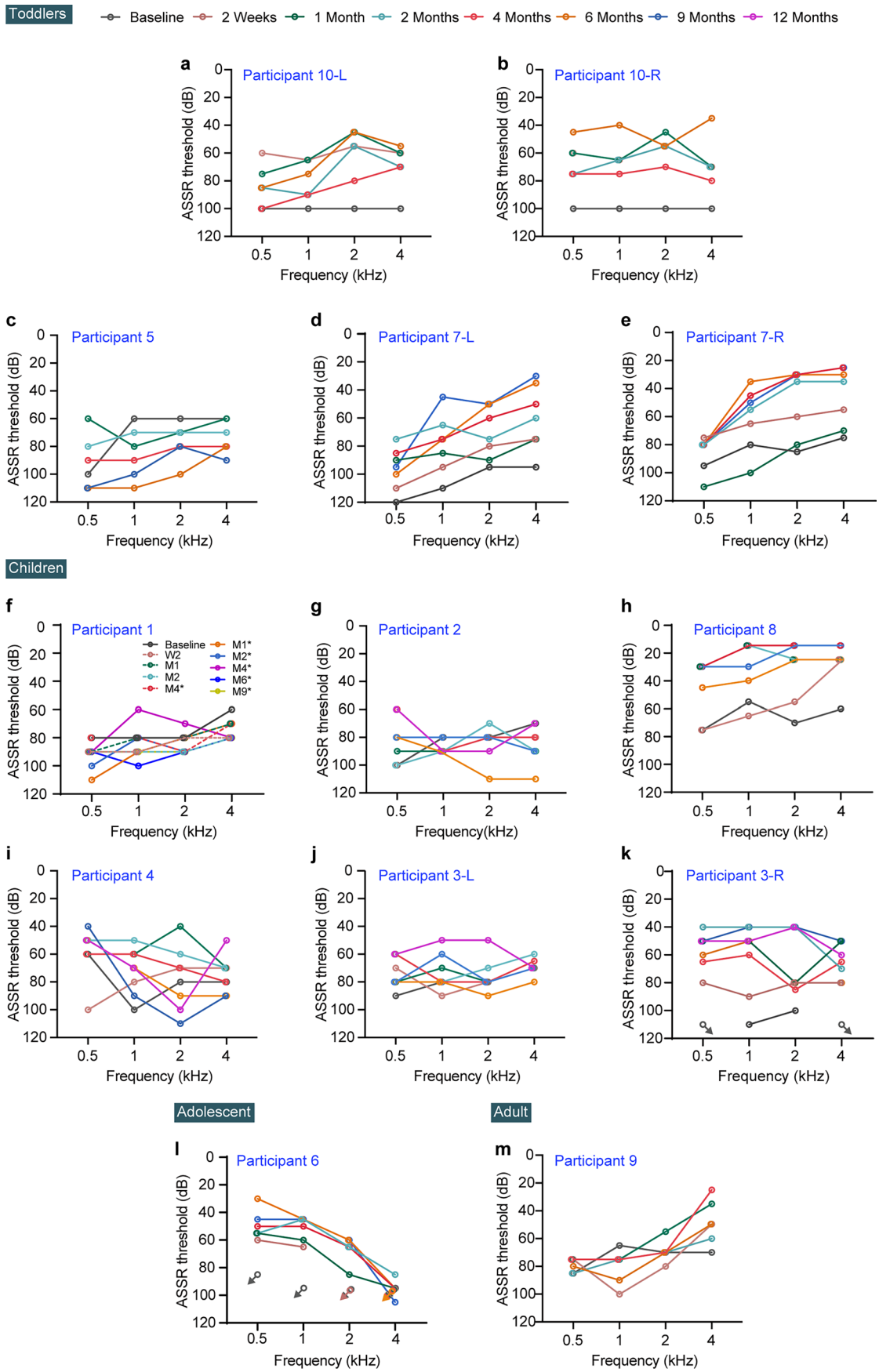
Extended Data Fig. 3 | The signal-to-noise ratio (SNR) of the DPOAE test results. SNR thresholds of DPOAE for Participant 10 **(a-b)**, 5 **(c)**, 7 **(d-e)**, 1 **(f)**, 2 **(g)**, 8 **(h)**, 4 **(i)**, 3 **(j-k)**, 6 **(l)**, and 9 **(m)** in order of age. In panel f, the dashed lines represent data from the first injection, while solid lines and * represent data from the second injection for Participant 1.

https://doi.org/10.1038/s41591-025-03773-w



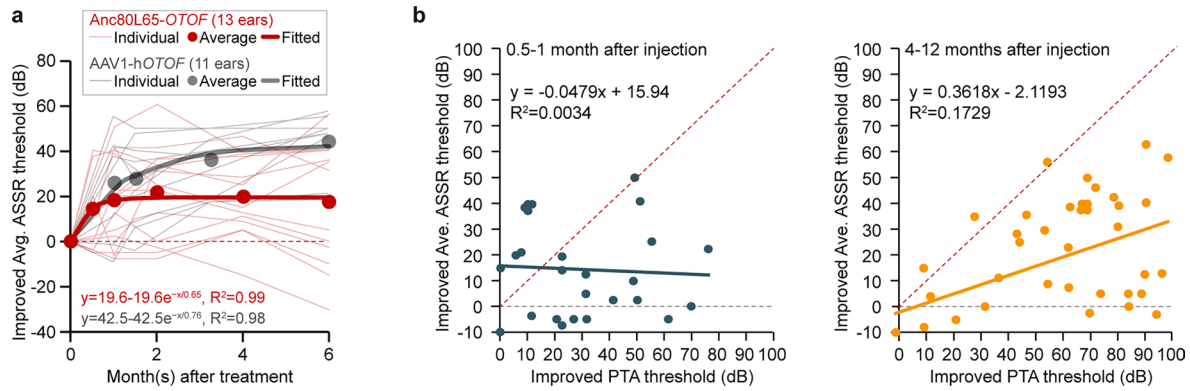
Extended Data Fig. 4 | Correlation analyses between pure-tone thresholds and SNR of DPOAE at the corresponding frequency. Correlation between SNR of DPOAE and Pure-tone thresholds at 0.5, 1, 2, 4, 8 kHz at baseline **(a)** and at 2 weeks **(b)**, 1month **(c)**, 2 months **(d)** and 4 months **(e)** after injection. The solid green lines represent a linear fit to the data, with the regression equation and R^2 being shown in the panel. The dashed grey lines represent the SNR threshold = 6 dB.

https://doi.org/10.1038/s41591-025-03773-w



Extended Data Fig. 5 | The ASSR threshold test results. ASSR thresholds for Participant 10 **(a-b)**, 5 **(c)**, 7 **(d-e)**, 1 **(f)**, 2 **(g)**, 8 **(h)**, 4 **(i)**, 3 **(j-k)**, 6 **(l)**, and 9 **(m)** in order of age. Arrows indicate no response at the tested maximum sound intensity, with the direction of the arrow representing either the left or right ear, respectively. In panel **f**, the dashed lines represent data from the first injection, while solid lines and * represent data from the second injection for Participant 1.

https://doi.org/10.1038/s41591-025-03773-w



Extended Data Fig. 6 | ASSR data analysis on the efficacy of gene therapy. **a**, Time course of gene therapy shown as the improved thresholds (y-axis) by the ASSR. The red and grey dots represent the average data, while other slight lines represent the individual data. The red dots and lines represent the thresholds for participants in this trial, and the grey dots and lines represent the thresholds for 11 ears from the 8 patients in published articles. The thick red and grey lines showed the fitted exponential growth functions of the improved thresholds with the R^2 being shown in the panels. **b**, Correlation between improved PTA thresholds and improved ASSR thresholds and at 0.5-1 month (left panel) and 4-12 months (right panel) after injection. The solid green and yellow lines represent linear fits to the data, with the regression equations and R^2 being shown in the panels. The red dashed diagonal lines represent the theoretical perfect fit with $R^2 = 1$.

https://doi.org/10.1038/s41591-025-03773-w

Extended Data Table 1 | Interpretation of the OTOF variants

Participant	Position (hg38)	Nucleotide Change (NM_194248.3)	Amino Acid Change	Zygosity	ACMG criteria	ACMG classification	Segregation Analysis	
							Father	Mother
1	chr2:26465010	c.4819C>T	p.Arg1607Trp	Homo	PM3_Strong+PP3+PM2_Supporting	LP	Het	Het
2	chr2:26467186	c.4275G>A	p.Trp1425Ter	Het	PVS1+PM2_Supporting	LP	Het	WT
	chr2:26477421-26477445	c.2377_2401del	p.Glu793SfsTer16	Het	PVS1+PM2_Supporting	LP	WT	Het
3	chr2:26470629	c.3987G>A	p.Trp1329Ter	Het	PVS1+PM2_Supporting+PP4	P	Het	WT
	chr2:26484613	c.1066T>G	p.Trp356Gly	Het	PM3+PP3_Moderate+PM2_Supporting+PP4	LP	WT	Het
4	chr2:26484550	c.1129G>A	p.Val377Ile	Het	PM2_Supporting+PM4	VUS	Het	WT
	chr2:26473477	c.3498_3499delinsG	p.Val1167CysfsTer5	Het	PVS1+PM2_Supporting+PP4	P	Het	WT
	chr2:26479237	c.2214+27G>A	/	Het	PM3+PM2_Supporting+PS3_Moderate+PP4	LP	WT	Het
5	Chr2:26477445	c.2377G>T	p.Glu793Ter	Het	PVS1 +PM3_Strong+PM2_Supporting	P	WT	Het
	Chr2:26467367	c.4225A>T	p.Lys1409Ter	Het	PVS1 +PM3+PM2_Supporting	P	Het	WT
6	chr2:26463567	c.5108delinsTCTT	p.Arg1703delinsLeuPhe	Homo	PM2_Supporting+PM3_Moderate+PM4	LP	Het	Het
7	chr2:26476018	c.2887C>T	p.Arg963Ter	Het	PVS1+PM2_Supporting+PM3_Supporting +PP4	P	Het	WT
	chr2:26480892	c.1697G>C	p.Arg566Pro	Het	PM2_Supporting+PM3+PP3_Moderate+PP4	LP	WT	Het
8	chr2:26465794-26465802	c.4669_4677del	p.Leu1557_Val1559del	Het	PM3_Strong+PM2+PM4	LP	Het	WT
	chr2:26462160-26462162	c.5212_5214del	p.Ile1738del	Het	PM2+PM3+PM4	LP	WT	Het
	chr2:26479639	c.1927G>A	p.E643K	Het	PM2+PM3+BP4	VUS	WT	Het
9	chr2:26502301	c.709C>T	p.Arg237Ter	Het	PVS1+PM2_Supporting+PM3_Strong	P	Het	WT
	chr2:26463567	c.5108delinsTCTT	p.Arg1703delinsLeuPhe	Het	PM2_Supporting+PM3_Moderate+PM4	LP	WT	Het
10	chr2:26463969	c.5098G>C	p.Glu1700Gln	Homo	PS4+PM1+PP3_Moderate+PP4	LP	Het	Het

Het: heterozygous; Homo: homozygous; WT: wild type; LP: Likely pathogenic; P: Pathogenic; VUS: Variant of Uncertain Significance. Extended Data Table 1 includes the genomic location, amino acid changes, pathogenicity assessments, and segregation analyses for each variant. Pathogenicity evaluations were conducted manually in accordance with the American College of Medical Genetics and Genomics (ACMG) guidelines. For compound heterozygous cases, parental mutations were confirmed through Sanger sequencing to establish cis/trans configurations.

https://doi.org/10.1038/s41591-025-03773-w

Extended Data Table 2 | The number of frequencies with presented DPOAE

Participant	10L	10R	5	7L	7R	1	1*	2	8	4	3L	3R	6	9
Age in order (years)	1.5	1.5	1.6	1.8	1.8	3.9	3.9	5.3	6.7	7.1	8	8	14.5	23.9
Baseline	7	6	6	2	4	5	2	3	9	9	4	1	3	8
W2	7	0	ND	3	2	0	ND	4	6	7	0	0	1	4
M1	7	1	4	3	3	5	5	3	8	9	0	1	0	7
M2	7	2	3	3	1	4	2	5	8	9	1	2	0	5
M4	5	1	4	4	3	2	6	4	9	6	0	0	0	9
M6	0	1	3	6	6	ND	6	4	9	9	6	7	0	8
M9	—	—	5	3	2	ND	2	6	7	9	2	5	1	—
M12	—	—	—	—	—	ND	—	6	—	9	2	5	—	—

ND: Not detected —: The follow-up time has not yet arrived. *: Participant 1 received a second injection of AAV-OTOF.

https://doi.org/10.1038/s41591-025-03773-w

Extended Data Table 3 | Treatment and communication mode prior to gene therapy

Ear with gene therapy*	Age (years)	Treatment before gene therapy	Communication mode
10-L	1.5	No	N/A
10-R		No	N/A
5-L	1.6	No	N/A
7-L	1.8	No	N/A
7-R		No	N/A
1-L	3.9	No	Auditory-verbal
2-L	5.3	Hearing aid	Auditory-verbal
8-R	6.7	No	Auditory-verbal
4-R	7.1	Hearing aid	Auditory-verbal
3-L	8.0	Hearing aid	Gesture
3-R		Hearing aid	
6-L	14.5	Hearing aid	Auditory-verbal
9-L	23.9	Hearing aid	Auditory-verbal

* All participants with unilateral AAV-OTOF injection were undergone the cochlear implantation.

https://doi.org/10.1038/s41591-025-03773-w

Extended Data Table 4 | Average thresholds of ABR, PTA and ASSR using for post-hoc analyses

Click-ABR thresholds (dB)														
Participant	**10L**	**10R**	**5**	**7L**	**7R**	**1**	**1***	**2**	**8**	**4**	**3L**	**3R**	**6**	**9**
Age in order (years)	1.5	1.5	1.6	1.8	1.8	3.9	3.9	5.3	6.7	7.1	8	8	14.5	23.9
Baseline	101	101	101	101	101	101	99	101	101	101	101	101	101	101
W2	100	101	ND	101	101	101	ND	85	80	40	101	101	101	90
M1	90	60	101	60	50	101	95	30	30	25	80	75	90	70
M2	90	50	101	40	30	101	95	40	30	25	50	35	95	45
M4	80	40	101	50	30	99	101	25	30	25	45	35	55	40
M6	70	40	101	40	30	ND	80	25	30	25	35	25	80	40
M9	—	—	101	30	30	ND	99	25	30	25	45	25	75	—
M12	—	—	—	—	—	ND	—	30	—	25	45	25	—	—
Average TB-ABR thresholds (dB)														
Participant	**10L**	**10R**	**5**	**7L**	**7R**	**1**	**1***	**2**	**8**	**4**	**3L**	**3R**	**6**	**9**
Age in order (years)	1.5	1.5	1.6	1.8	1.8	3.9	3.9	5.3	6.7	7.1	8	8	14.5	23.9
Baseline	101	101	96	101	101	ND	88	ND	101	96	96	96	101	101
W2	101	98	ND	96	101	ND	ND	75	68	53	87	83	101	101
M1	88	78	96	ND	ND	ND	83	49	60	50	68	49	58	74
M2	83	63	96	53	48	96	83	44	55	44	59	43	74	78
M4	88	63	95	65	40	88	81	45	59	33	54	44	59	78
M6	88	58	96	53	43	ND	84	45	58	35	53	43	61	79
M9	—	—	96	40	40	ND	92	46	58	36	58	40	60	—
M12	—	—	—	—	—	ND	—	48	—	38	56	39	—	—
Average PTA thresholds (dB)														
Participant	**10L**	**10R**	**5**	**7L**	**7R**	**1**	**1***	**2**	**8**	**4**	**3L**	**3R**	**6**	**9**
Age in order (years)	1.5	1.5	1.6	1.8	1.8	3.9	3.9	5.3	6.7	7.1	8	8	14.5	23.9
Baseline	121	121	112	101	99	116	95	112	100	105	95	116	105	101
W2	111	111	ND	101	92	116	ND	80	51	35	54	60	83	90
M1	112	110	61	95	79	89	73	50	49	29	64	66	83	70
M2	101	93	84	63	79	83	68	51	25	21	54	43	73	73
M4	112	78	91	35	38	95	84	39	29	16	41	36	63	65
M6	94	68	95	33	34	ND	96	34	21	18	25	26	59	70
M9	—	—	64	33	31	ND	99	29	23	13	34	26	53	—
M12	—	—	—	—	—	ND	—	29	—	10	34	19	—	—
Average ASSR thresholds (dB)														
Participant	**10L**	**10R**	**5**	**7L**	**7R**	**1**	**1***	**2**	**8**	**4**	**3L**	**3R**	**6**	**9**
Age in order (years)	1.5	1.5	1.6	1.8	1.8	3.9	3.9	5.3	6.7	7.1	8	8	14.5	23.9
Baseline	100	100	70	105	84	75	80	83	65	80	80	108	94	73
W2	60	63	ND	90	64	85	ND	88	55	80	78	83	79	76
M1	61	60	68	85	90	80	88	88	24	58	75	58	74	60
M2	75	66	73	69	51	88	88	88	24	58	68	48	63	73
M4	85	75	85	68	45	80	75	78	19	68	71	69	65	61
M6	65	44	100	65	44	ND	90	98	34	75	83	68	58	73
M9	—	—	95	55	46	ND	88	83	23	83	73	45	64	—
M12	—	—	—	—	—	ND	—	78	—	68	58	50	—	—

ND: Not detected. —: The follow-up time has not yet arrived. *: Participant 1 received a second injection of AAV-OTOF.

nature portfolio

Corresponding author(s): Renjie Chai

Last updated by author(s): Apr 20, 2025

Reporting Summary

Nature Portfolio wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Portfolio policies, see our Editorial Policies and the Editorial Policy Checklist.

Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a	Confirmed
☐	☒ The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement
☐	☒ A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☒	☐ The statistical test(s) used AND whether they are one- or two-sided *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
☒	☐ A description of all covariates tested
☒	☐ A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
☐	☒ A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
☒	☐ For null hypothesis testing, the test statistic (e.g. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted *Give P values as exact values whenever suitable.*
☒	☐ For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☒	☐ For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
☒	☐ Estimates of effect sizes (e.g. Cohen's *d*, Pearson's *r*), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection	There is no custom algorithms or software used in this manuscript.
Data analysis	The following softwares were used in this study: Microsoft Excel (v16.54 21101001), Graphpad Prism (v9).

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Portfolio guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our policy

At the outset of the trial, we omitted a data sharing provision from the consent documents signed by participants. As a result, in accordance with our Ethics Committee policies, we are not authorized to release the raw data to the public. Furthermore, the study is still in progress. Instead, de-identified patient characteristics and hearing threshold improvements from raw datasets generated in this study are already included in the manuscript. Request for more information about the raw data is subject to a confidentiality agreement with Southeast University and Otovia Therapeutics Inc., and must comply with applicable

legal and regulatory requirements. Qualified researchers may request access to the trial information by contacting renjiec@seu.edu.cn. The requests will be fulfilled within 120days, and data transfer agreement may be required.

Research involving human participants, their data, or biological material

Policy information about studies with human participants or human data. See also policy information about sex, gender (identity/presentation), and sexual orientation and race, ethnicity and racism.

Reporting on sex and gender	Male and female were used for sex indentify. 6 female and 4 male were enrolled in this trail. No sex related ayalyses were reported.
Reporting on race, ethnicity, or other socially relevant groupings	All participants were Asia from China. Patient ethnicity is not specifically reported in the study.
Population characteristics	The age of participants: 1.5-23.9 years. Genotype: biallelic OTOF gene mutations. Treatment: Injection of AAV-OTOF into the inner ear through the round window membrane.
Recruitment	Thirty-one participants with OTOF mutations and hearing loss have been screened in the clinical trial from 26th July 2023 to 27th June 2024 at the five centers, based on genetic screening, neutralizing antibodies (NAb) testing, and audiologic examinations from a total of 393 candidates. Four participants were excluded due to the titer of blood NAb > 1:128, whilst the fifth excluded due to failed distortion-product otoacoustic emission (DPOAE). Ten participants enrolled and received the AAV-OTOF (Anc80L65 capsid) injection, in which center 1 did 5 participants, center 2 did 1 participant, center 3 did 1 participant, center 4 did 2 participants, and center 5 did 1 participant. The information regarding the 10 participants is detailed in Table 1. No self-selection bias and other biases.
Ethics oversight	The trial was approved by the Ethics Committee of all participating sites, including (1) Shandong Second Provincial General Hospital, (2) Union Hospital Tongji Medical College, Huazhong University of Science and Technology, (3) Nanjing Drum Tower Hospital, Affiliated Hospital of Medical School, Nanjing University, (4) Xijing Hospital, The Fourth Military Medical University, (5) Ningbo No.2 Hospital, and subsequently revised under the auspices of the same committees.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	10 participants were enrolled in this trails. This trail aimed to assess the tolerability and efficacy of AAV-OTOF. Due to the limited number of cases with DFNB9, a kind of rare diseases, we enrolled a small sample of participants in the first stage for concept validation.
Data exclusions	No data was excluded.
Replication	We replicated related experiments on a total of 10 patients. Anc80-OTOF were injected into 10 patients. The primary endpoints were safety and tolerability, including the incidence and severity of AEs and their correlation with the study drug, and physical examination (including cranial nerve testing), the laboratory test indicators (blood routine examination, urine routine examination, blood biochemistry, coagulation function, etc.), vital signs, ECG examination, neutralizing antibodies detection and AAV testing in peripheral blood. AEs were defined as any untoward medical occurrences in a participant administered the drug, even if these did not necessarily have a causal relationship with the treatment. The primary endpoints were analyzed in the Safety Analysis Set, every patient was included in this Analysis Set. The secondary endpoints measured the degree of hearing recovery of each participant according to the required follow-up time points, and the content of the test mainly include behavioral audiometry, ABR, ASSR, DPOAE, CM and tympanogram examination. Each sample analyzed was a unique sample. The experimental findings were replicated. Post-hoc analyses were performed in the average thresholds from all patient at different follow-up visits. Every patient at each time-points was considered as a replication.
Randomization	This is a single-arm clinical trial. The comparison of both primary and secondary outcomes was occurred at before and after surgery.The reason is that using a blank control for patients is not in line with ethical requirements, and there is no approved drug available to treat DFNB9. We control the covariables through very strict patient inclusion and exclusion criteria.
Blinding	This is a single-arm intervention trial. Blinding is not applicable.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☒	☐ Antibodies
☐	☒ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☒	☐ Animals and other organisms
☐	☒ Clinical data
☒	☐ Dual use research of concern
☒	☐ Plants

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)	HEK293T cells (BeNa Culture Collection, Cat: BNCC353535); HEK293T cells (CellCook, Cat: CC4003); Hela cells (CellCook, Cat: CC1101).
Authentication	HEK 293T cell lines are authenticated by STR profiling (BeNa Culture Collection and Guangzhou Cellcook Biotech Co.,Ltd.). Hela cell lines are authenticated by STR profiling (Guangzhou Cellcook Biotech Co.,Ltd.).
Mycoplasma contamination	All cells were confirmed negative for mycoplasma.
Commonly misidentified lines (See ICLAC register)	No commonly misidentified cell lines were used.

Clinical data

Policy information about clinical studies

All manuscripts should comply with the ICMJE guidelines for publication of clinical research and a completed CONSORT checklist must be included with all submissions.

Clinical trial registration	ClinicalTrials.gov, NCT05901480
Study protocol	The trial protocol and amendments were provided in the Methods Section and Supplementary information document.
Data collection	10 DFNB9 patients from 5 sites aged 1.5 to 23.9 years were enrolled in this trail between July, 2023 and July, 2024. Primary and secondary outcomes were safety within 5 years and corresponding auditory function assessments on the efficacy, respectively. The data were only collected at research centers that had obtained ethical approvals.
Outcomes	The primary endpoints were safety and tolerability. Safety analysis included the incidence and severity of AEs and their correlation with the study drug, and physical examination (including cranial nerve testing), the laboratory test indicators (blood routine examination, urine routine examination, blood biochemistry, coagulation function, etc.), vital signs, ECG examination, neutralizing antibodies detection and AAV testing in peripheral blood. AEs were defined as any untoward medical occurrences in a participant administered the drug, even if these did not necessarily have a causal relationship with the treatment. The secondary endpoints measured the degree of hearing recovery of each participant according to the required follow-up time points, and the content of the test mainly include behavioral audiometry, ABR, ASSR, DPOAE, CM and tympanogram examination. Data of CM and tympanogram examination were not included here. In addition to data at individual frequencies, the thresholds at 0.5, 1, 2, and 4 kHz were used to calculate the PTA, average TB-ABR and ASSR thresholds as a global measure of hearing improvement.

Plants

Seed stocks	None
Novel plant genotypes	None
Authentication	None