

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 (<i>n</i>) 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 <i>Only common tests should be described solely by name; describe more complex techniques in the Methods section.</i>
☐	☒ 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. <i>F</i> , <i>t</i> , <i>r</i>) with confidence intervals, effect sizes, degrees of freedom and <i>P</i> value noted <i>Give P values as exact values whenever suitable.</i>
☒	☐ 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 <i>d</i> , Pearson's <i>r</i>), 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	Speech perception was also evaluated by Mandarin Speech perception (Version 5.04.01).
Data analysis	Figures were made using Graphpad Prism (Version 10.4.2) and Adobe illustrator 2025 (version 29.7.1). The data statistical analysis was conducted using stata/MP 15 and Graphpad Prism.

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](#)

Participant data are de-identified and available in the text, figures, and tables. The statistical analysis plan is included in the clinical trial protocol, which is available in Supplementary Information. Requests for more information should be communicated to the corresponding author Y.S. Source data are provided with this paper.

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	We used male and female to report sex. The findings apply to both male and female. Twenty males and twenty-two females were enrolled in this trial, and the consents have been obtained. No sex-based analysis was performed.
Reporting on race, ethnicity, or other socially relevant groupings	The participants were all Asian. Patient ethnicity is not specifically reported in the study.
Population characteristics	The age of subjects: 0.8-32.3 years. Genotype: biallelic OTOF gene mutations. Treatment: AAV1-hOTOF gene therapy.
Recruitment	Patients could access the enrollment information through advertisements posted in the hospital, inquires during the consultation and multimedia platforms. Patients were screened and enrolled based on the genotypes, audiometric tests and related inclusion/exclusion criteria at baseline. Written informed consent was obtained from patients or legal guardians of the children before enrollment. Forty-two DFNB9 subjects with biallelic OTOF gene mutations were enrolled at the study sites. Full inclusion/exclusion criteria were provided in the trial protocol. No self-selection bias and other biases.
Ethics oversight	The study protocol was approved by the Ethics Committee of 8 trial centers. Patients or legal guardians of all patients provided written informed consents before participation. Before initiating the trial at center sites, protocols were approved by the Ethics Committees of the following center sites: Eye & ENT hospital of Fudan University, Peking Union Medical College Hospital, the Second Xiangya Hospital of Central South University, Chongqing General Hospital, West China Hospital of Sichuan University, the First Affiliated Hospital of Nanchang University, the First Affiliated Hospital of Zhengzhou University, and the Second Hospital of University of South China.

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](https://www.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	Because DFNB9 is rare disease, the number of DFNB9 patients is limited. The sample size of the study was based on enrollment feasibility and not based on statistical considerations.
Data exclusions	All enrolled participants (n=42) were included in the safety analyses. For efficacy analyses, only participants with hearing recovery were included (n=38).
Replication	We replicated related experiments on a total of 42 patients. AAV1-hOTOF were injected into 42 patients. The primary and secondary endpoints were evaluated by biochemical analyses, audiometric testing, and related questionnaires and tests in patients.
Randomization	The trial is non-randomized. Data analysis was performed between before and after gene therapy. Covariates were controlled using linear mixed-effects models, with participants (or ears) included as a random effect to account for within-subject correlations. Covariates, including age group, number of present DPOAEs, OTOF variants, and treatment side, were included.
Blinding	This trial was a single-arm trial. Blinding is not applicable for this study.

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

Antibodies

Antibodies used	Anti-human CD3 mAb(CD3-2)(MABTECH, Code:3605-1S); Anti-human IFN-r mAb (7-B6-1), ALP (MABTECH, Code:3420-9A).
Validation	ELISpot Pro: Human IFN-r (ALP) (MABTECH,Code:3420-2AST-10) was purchased from commercial vendor and validated by commercial vendor. The antibodies were included in the ELISpot Pro kit.

Eukaryotic cell lines

Policy information about [cell lines and Sex and Gender in Research](#)

Cell line source(s)	HEK293FT cells (NANJING COBIOER BIOSCIENCES CO.,LTD.)
Authentication	Authentication for HEK293FT cell line was validated by STR profiling.
Mycoplasma contamination	Cells were confirmed negative for mycoplasma.
Commonly misidentified lines (See ICLAC register)	No commonly misidentified lines were used in this study.

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	Chinese Clinical Trial Registry, ChiCTR2200063181.
Study protocol	The full Trial protocol was provided.
Data collection	Between Oct 19, 2022, and May 23, 2025, we screened 3193 participants for eligibility and enrolled 42 participants from 8 trial centers in China. The clinical trial was a multi-center trial, conducted at 8 sites in China, including: Eye & ENT Hospital of Fudan University (Shanghai, China), Peking Union Medical College Hospital (Beijing, China), The Second Xiangya Hospital of Central South University (Hunan, China), Chongqing General Hospital (Chongqing, China), West China Hospital of Sichuan University (Sichuan, China), The first Affiliated Hospital of Nanchang University (Jiangxi, China), The First Affiliated Hospital of Zhengzhou University (Henan, China), The Second Hospital of University of South China (Hunan, China).
Outcomes	The primary endpoint was dose-limiting toxicity within 6 weeks, defined as hematologic toxicity $\geq$ grade 4, non-hematologic toxicity $\geq$ grade 3, or aural toxicity $\geq$ grade 2 within 6 weeks. The grade was assessed according to CTCAE V5.0. The secondary endpoint assessed efficacy (auditory function and speech perception) and safety (adverse events). The primary and secondary outcomes were measured by biochemical analyses, audiometric testing, and related questionnaires and tests.

Plants

Seed stocks	<i>Report on the source of all seed stocks or other plant material used. If applicable, state the seed stock centre and catalogue number. If plant specimens were collected from the field, describe the collection location, date and sampling procedures.</i>
Novel plant genotypes	<i>Describe the methods by which all novel plant genotypes were produced. This includes those generated by transgenic approaches, gene editing, chemical/radiation-based mutagenesis and hybridization. For transgenic lines, describe the transformation method, the number of independent lines analyzed and the generation upon which experiments were performed. For gene-edited lines, describe the editor used, the endogenous sequence targeted for editing, the targeting guide RNA sequence (if applicable) and how the editor was applied.</i>
Authentication	<i>Describe any authentication procedures for each seed stock used or novel genotype generated. Describe any experiments used to assess the effect of a mutation and, where applicable, how potential secondary effects (e.g. second site T-DNA insertions, mosaicism, off-target gene editing) were examined.</i>