

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	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 120 days, 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 identify. 6 female and 4 male were enrolled in this trail. No sex related analyses 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 trial 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