Massachusetts Eye and Ear

HARVARD MEDICAL SCHOOL | Department of Otolaryngology Head and Neck Surgery

Gene Therapy Breakthrough Allows Congenitally Deaf Children to Hear

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Future Forum "Turning Point in Biotechnology: Gene Therapies, Tumor Vaccines, Antibody-Drug Combinations"
November 21, 2024, Berlin

Conflict of interest declaration

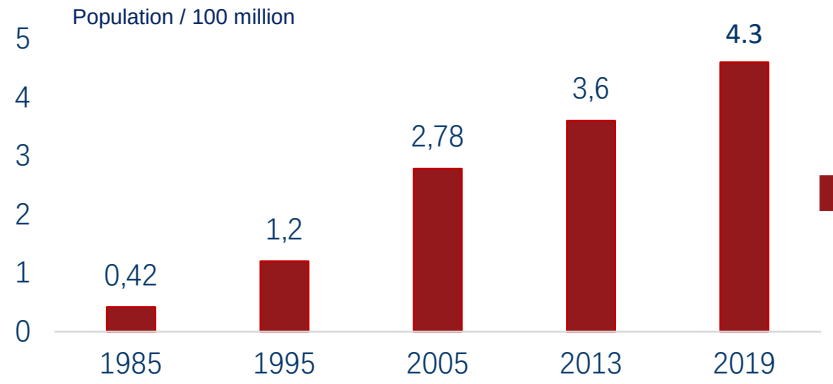
- Salubritas Therapeutics
- Regeneron

My interests were reviewed and are managed by Mass Eye and Ear and Mass General Brigham in accordance with their conflict of interest policies

Background

Over 1.5 billion now

Disabling hearing loss population



Number of people with hearing impairment worldwide (1985-2019)

430 million
Population with moderate or higher hearing impairment

34 million
children with hearing loss

2.5 billion
Population with hearing loss by 2050

The impact of unaddressed hearing loss

Infants & Children — Speech, language, and cognitive development

Adolescents & Adults — Education and Employment

Seniors — Social isolation and Mental health

Ludwig van Beethoven (1770-1827)

Thousand-hand Bodhisattva dance

The overall global cost of unaddressed hearing loss is greater than $ **980** billion annually

Hearing loss is the largest modifiable contributing factor (9%) to dementia and Alzheimer's disease (The Lancet International Commission).

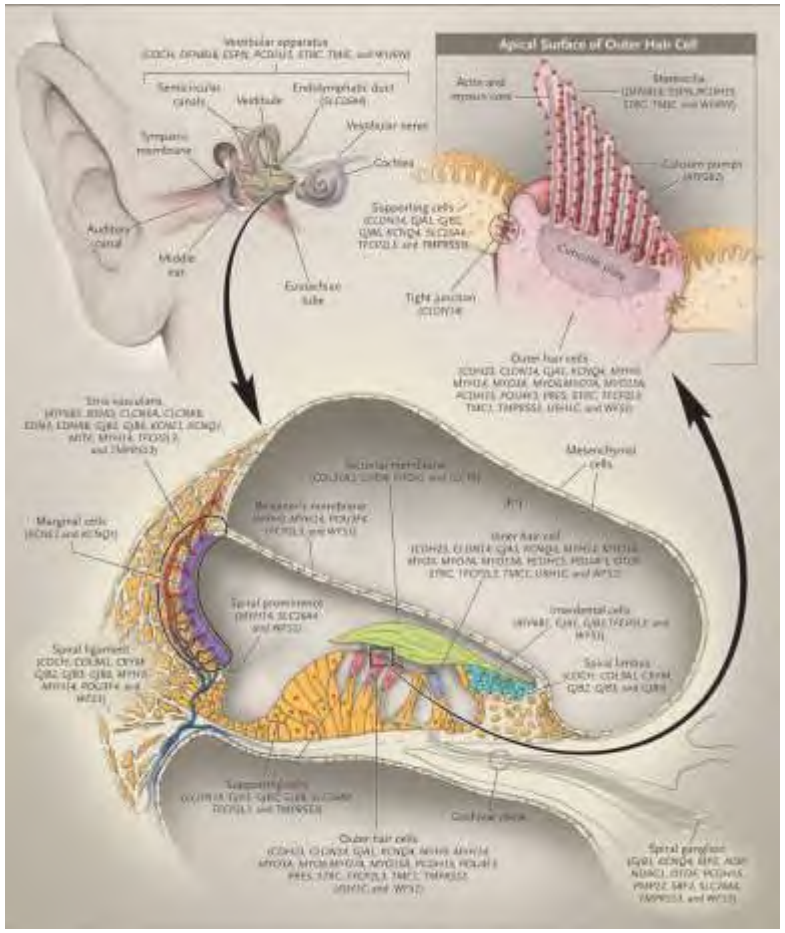
WHO, 2023.

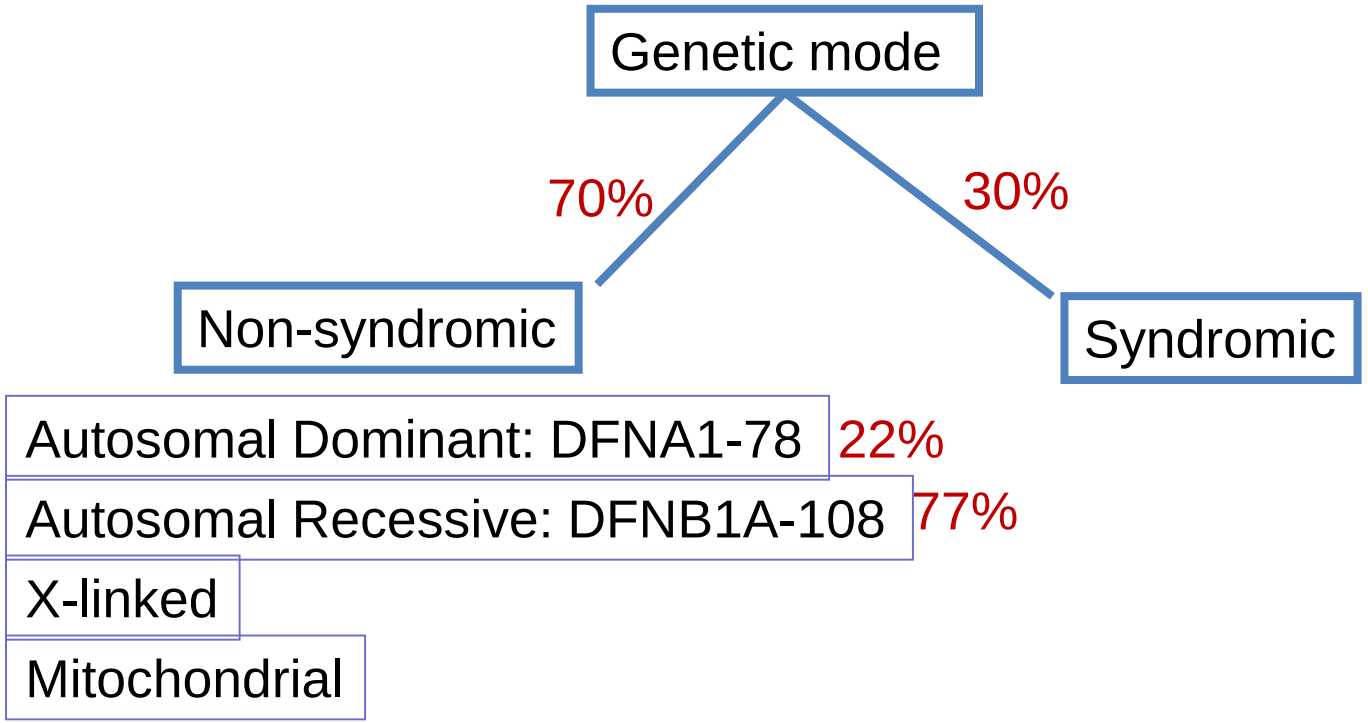
Background

Genetic Hearing Loss

One in ~500 newborns, ~26 millions worldwide

150 deafness genes identified



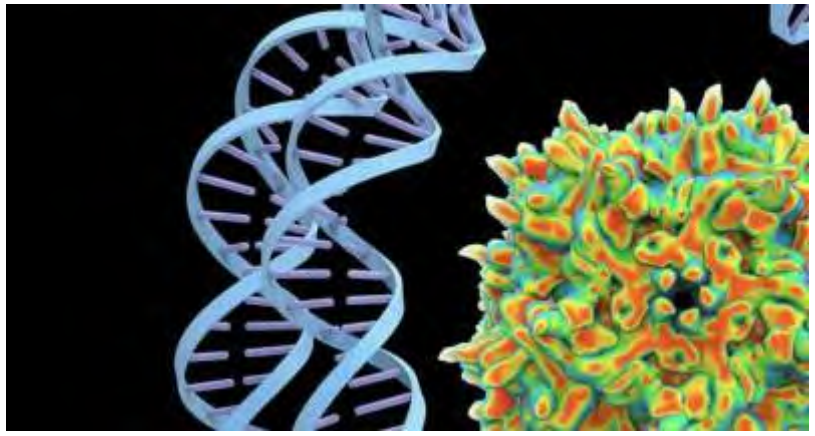


Background

No clinic drugs for genetic deafness

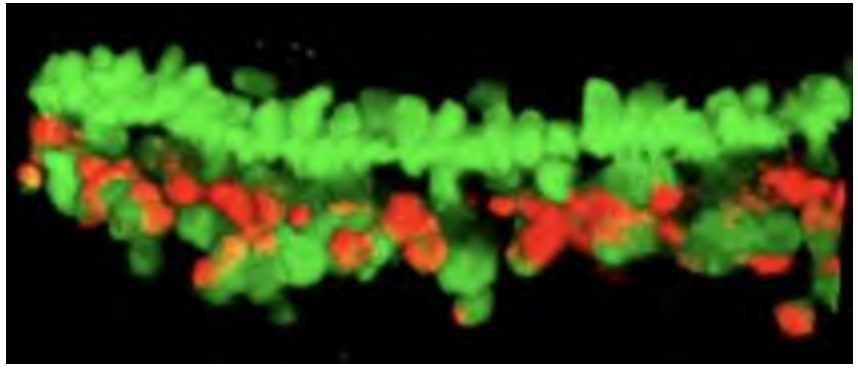
Current Treatment

Future Treatment



Gene therapy

Gene editing



Regeneration

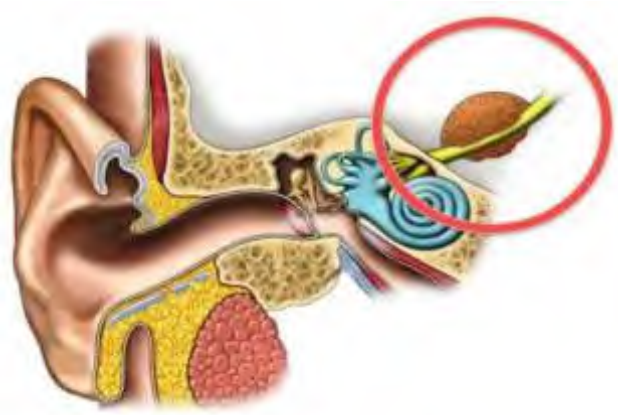
Drug therapy

OTOF Gene Therapy in Humans

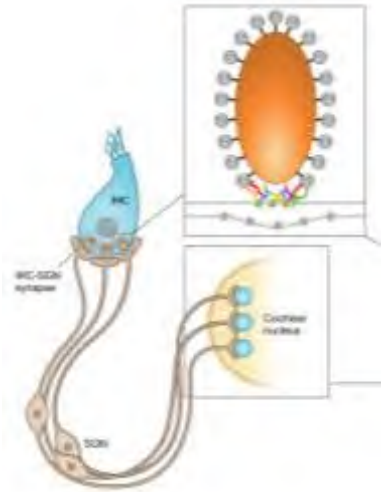
Background *OTOF* pathogenic mutation ➪ DFNB9

- DFNB9 is a congenital or prelingual, severe-to-complete, autosomal recessive deafness
- *OTOF* gene, influencing 2-8% of patients suffering from genetic hearing loss
- Otoferlin protein in the inner hair cells (IHCs)
- Exocytosis and vesicle replenishment of IHCs

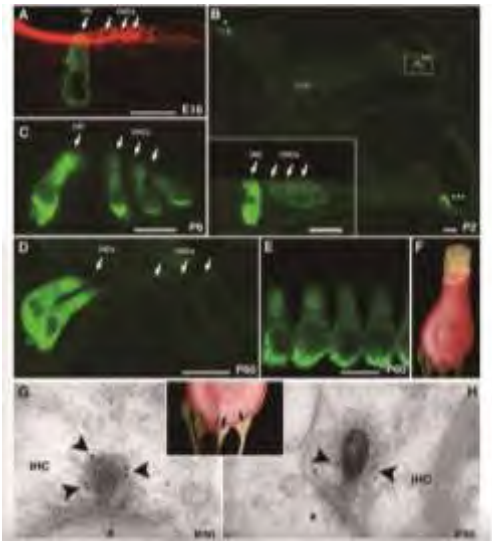
The mechanism of DFNB9 is clear



Auditory neuropathy



Exocytosis,
vesicle replenishment



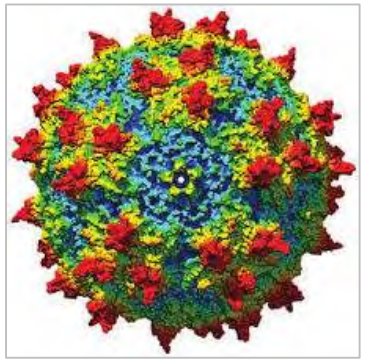
Otoferlin protein expressed in IHCs

MIM 601071
Physiological Reviews,2019

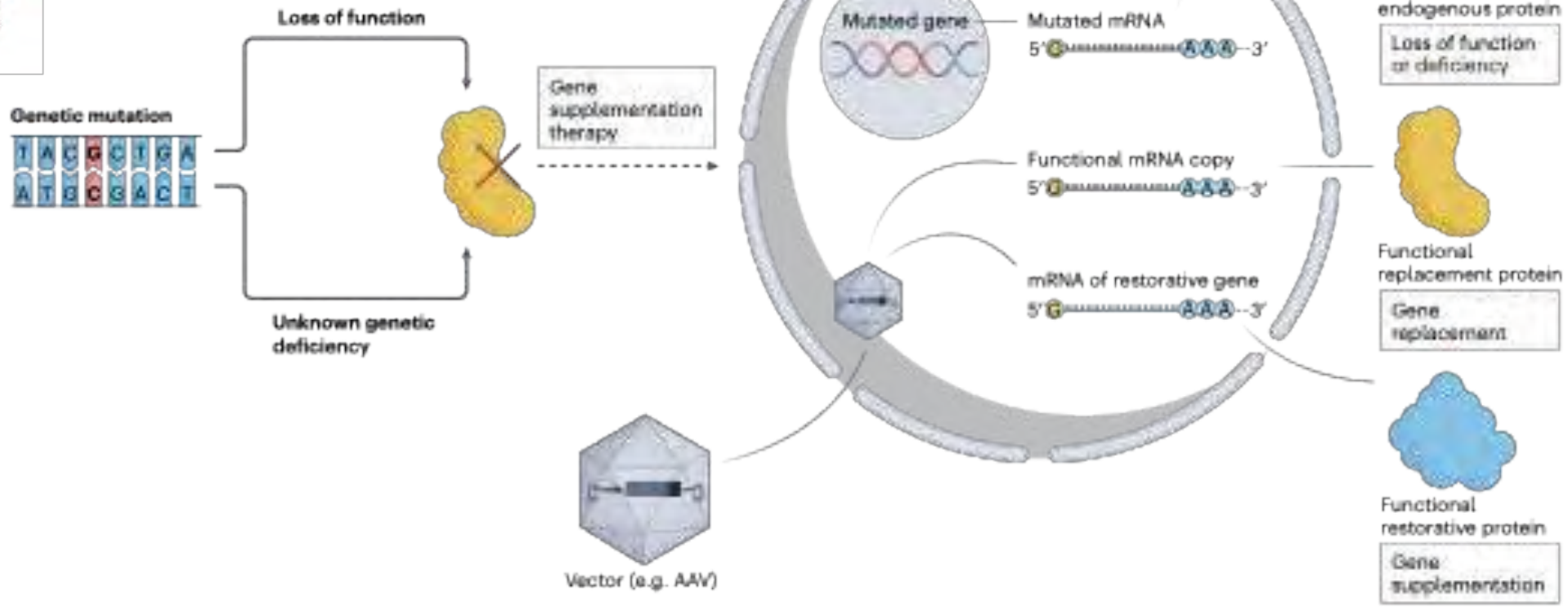
Background

AAV-gene replacement treatment for DFNB9

Adeno-Associated Virus (AAV) has been widely used for gene therapy to treat genetic diseases



AAV



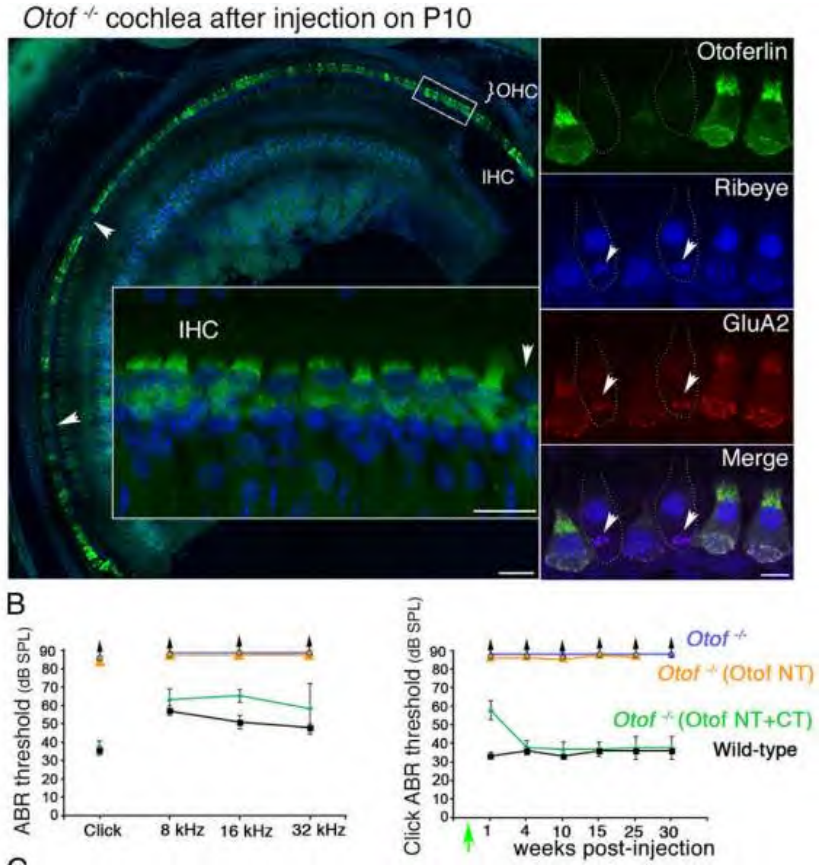
Ling et al., Nat Rev Drug Discov 2023

Background

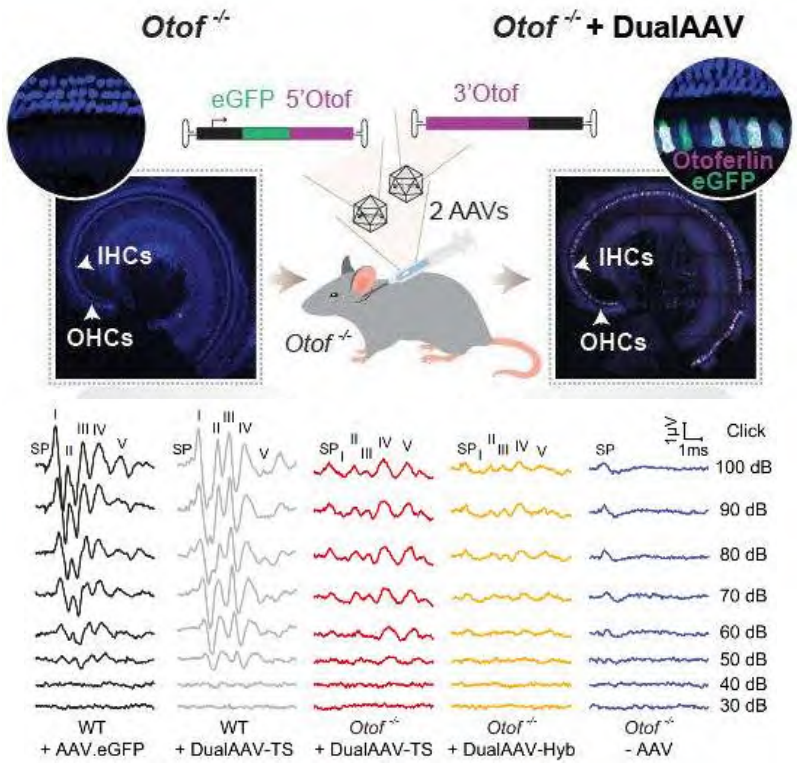
AAV-gene replacement treatment for DFNB9

- AAV is the most common vector for gene therapy in the inner ear
- *OTOF* size (~6kb) exceeds single AAV load (~4.7kb)

Dual-AAV mediated gene therapy restored hearing in *Otof*$^{-/-}$ mice



Proc Natl Acad Sci, 2019



EMBO Mol Med, 2019

Preclinical studies

Research strategy

Drug component

- Drug delivery system
- Promoter screening
- Recombination strategy

AAV vector production

- Optimization of manufacturing procession
- Small scale, medium scale, GMP

Safety and efficacy in mice

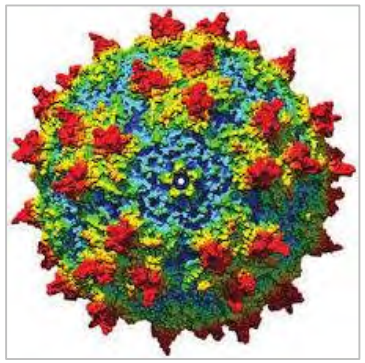
- Safety: vector biodistribution, behavior, pathology, routine blood test, serum biochemistry
- Efficacy: auditory function, immunohistochemistry

Safety in non-human primates

- Transfection, vector biodistribution, immunohistochemistry, routine blood test, serum biochemistry (hepatorenal function)

Results

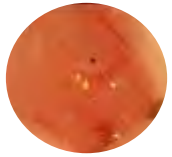
In vivo screen of delivery route and AAV vectors



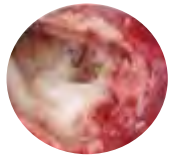
AAV

Optimization of the inner ear delivery route

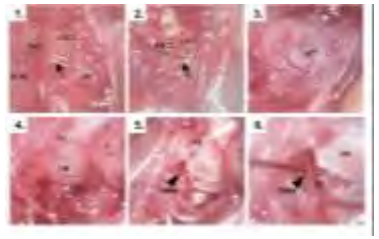
Injection route → Small animal → Large animals → Does not affect hearing



LW



RWM



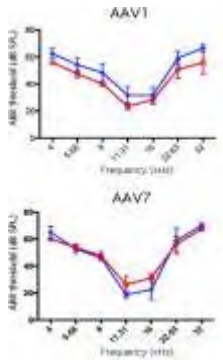
PSCC

Neonatal mice

Adult mice

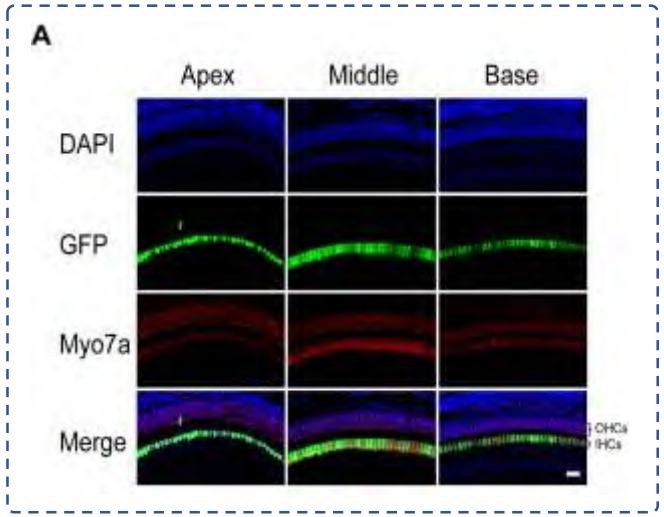
Guinea pig

Monkey



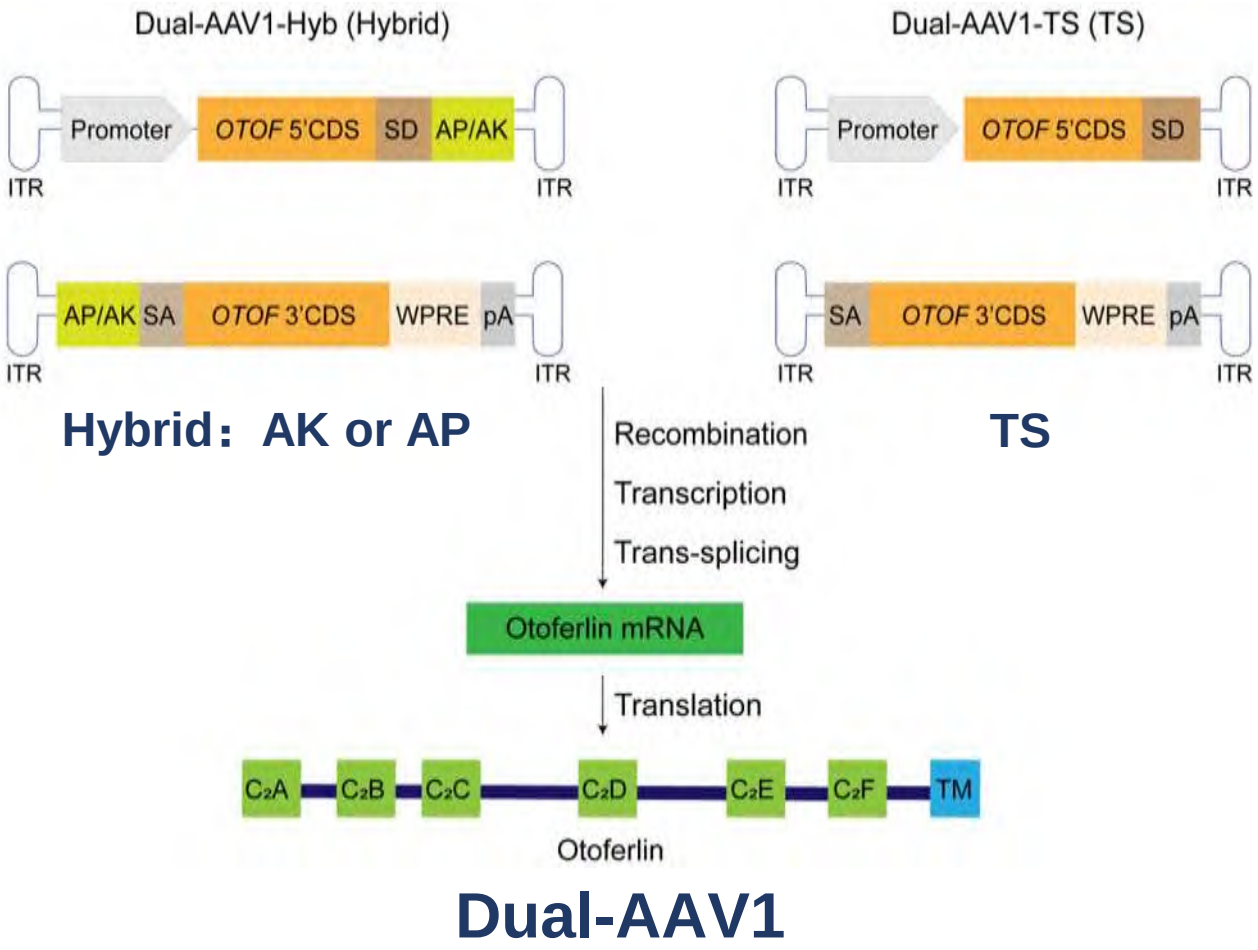
AAV1

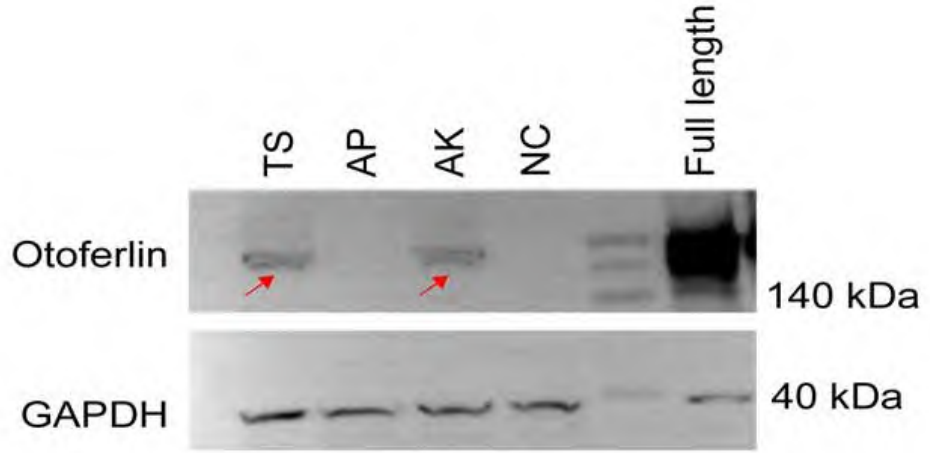
Efficient transduction in IHCs (>90%)



Results Recombination strategy for dual-AAV delivery

Dual-AAV-AK was selected.



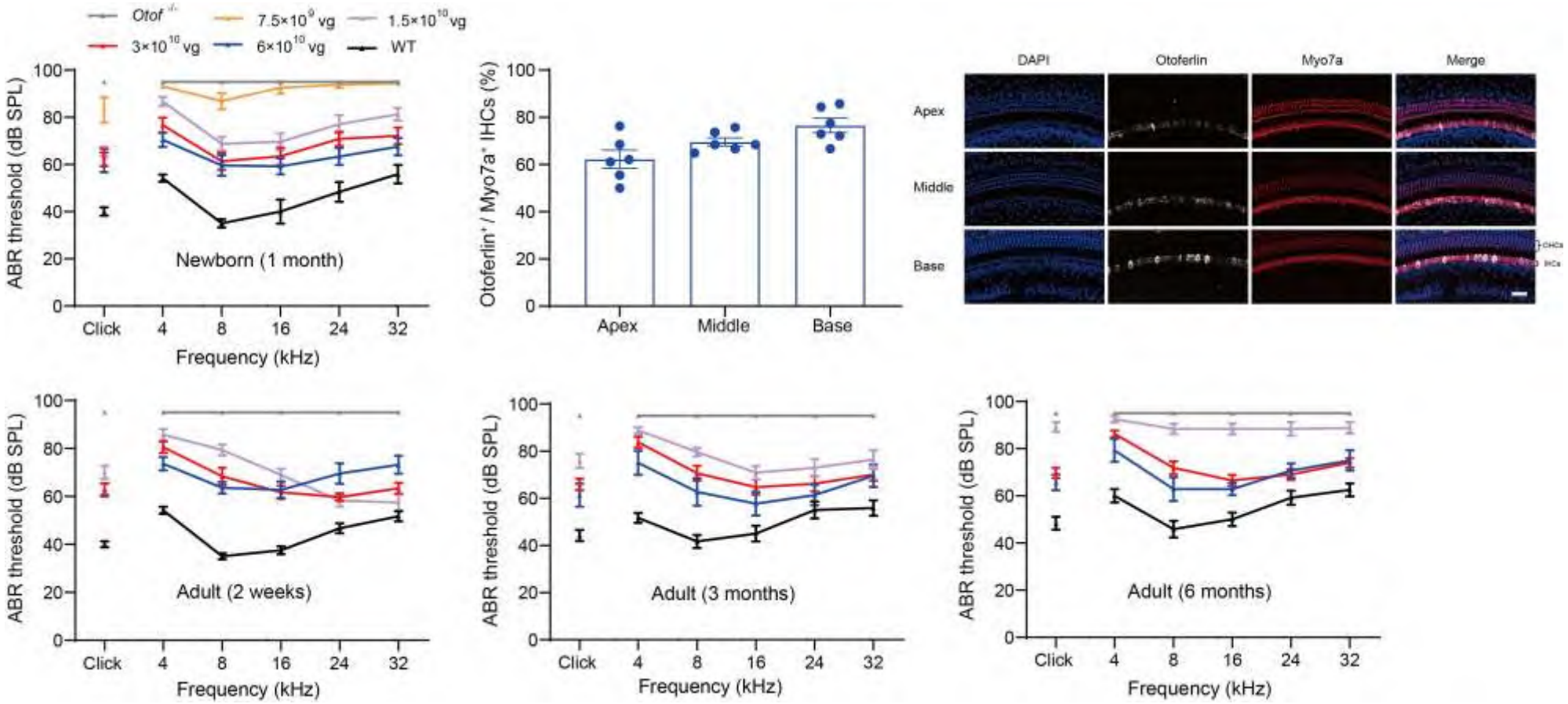


Western blot

Zhang et al., *Mol Ther Meth Clin Dev, 2023*

Efficacy of AAV1-hOTOF

- AAV1-hOTOF was produced under GMP conditions
- Dose-dependent efficacy
- Improved auditory function in both neonatal and adult *Otof*$^{-/-}$ mice



Zhang et al., *Mol Ther Meth Clin Dev, 2023*

AAV1-hOTOF Clinical Trial

Clinical study The first-in-human trial of gene therapy for deafness

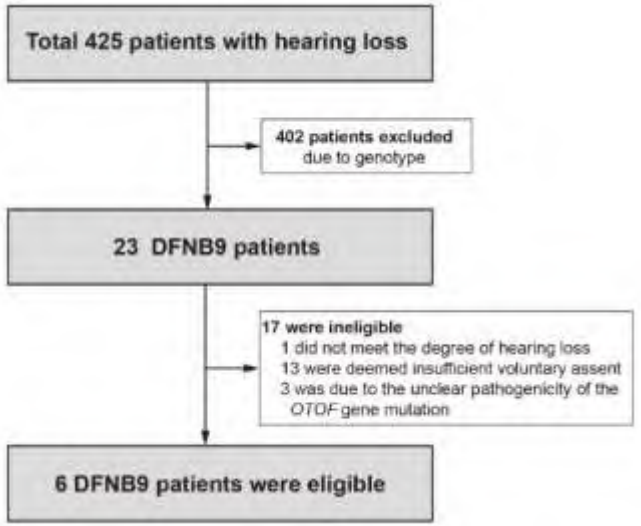
GMP drug

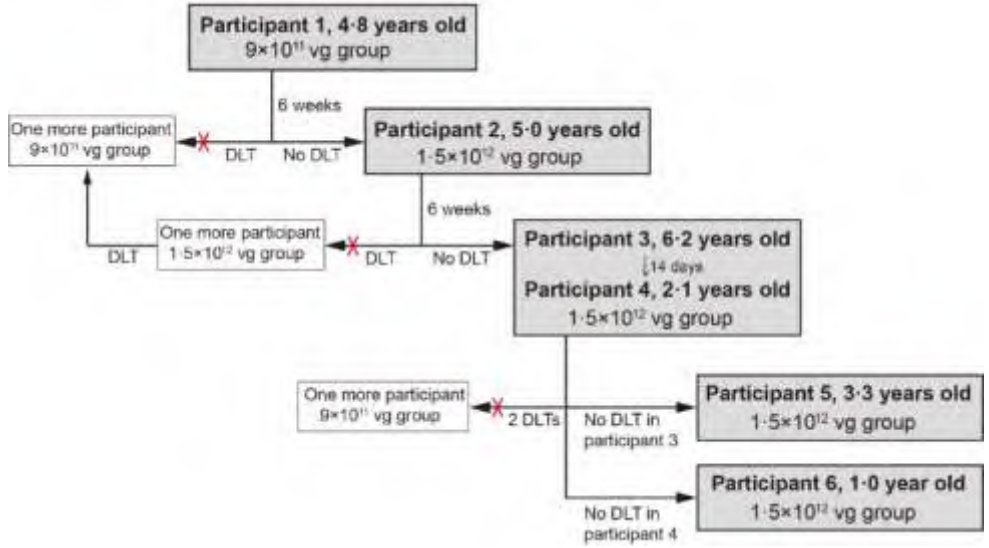
Ethical approval

Number: 2022085

Recruitment

- Age:1–18 years old
- General principal: from older to younger children
- Dose: 1 in 9×10^{11} vg (Low)
  5 in 1.5×10^{12} vg (High)
- Ethical approval: June 2022
- First recruit: Oct 2022





425 Patients were registered and six were enrolled

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

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

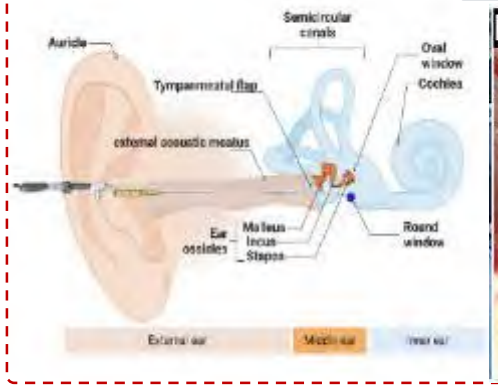
Table 1: **Baseline characteristics, genotype, and vector dose for each participant**

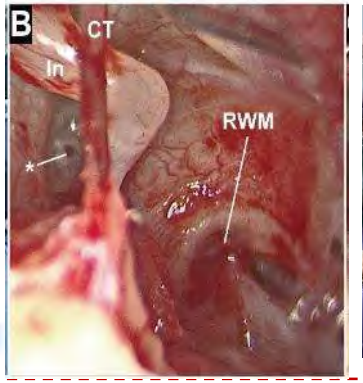
The Lancet, 2024

Clinical study

Safety evaluation on unilateral gene therapy

Drug administration





(Trans-External auditory canal) Endoscopic

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

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

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

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

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

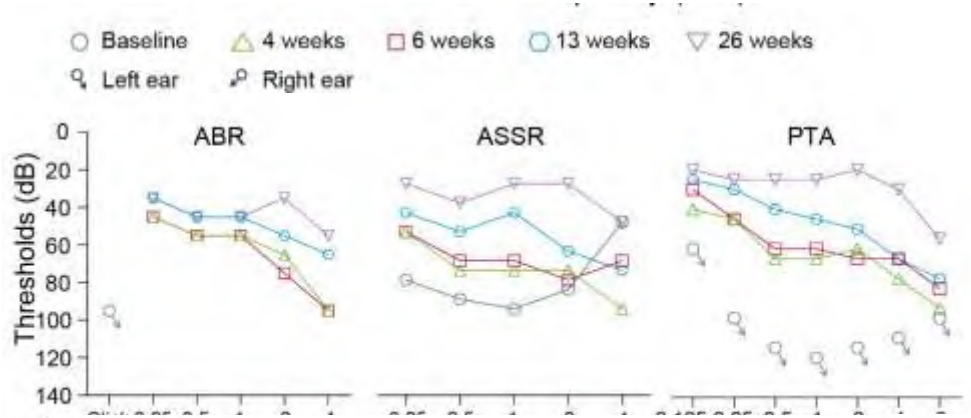
Table 2: **Summary of adverse events**

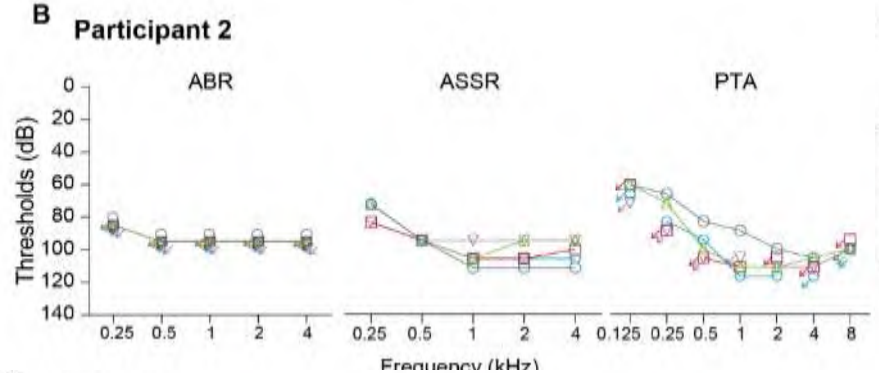
- **No dose-limiting toxicity (DLT) and serious adverse event**
- **Increased of neutralizing antibodies to AAV**
- **No T cells response to AAV**

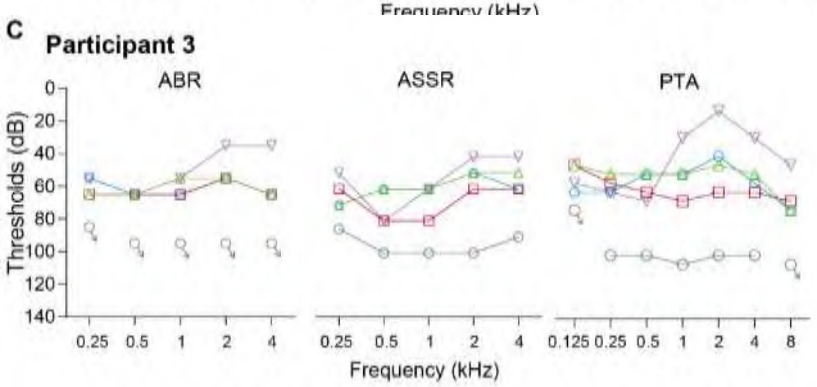
Clinical study

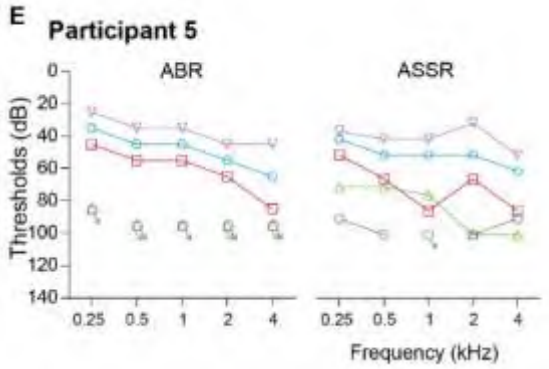
Hearing restoration by AAV-hOTOF

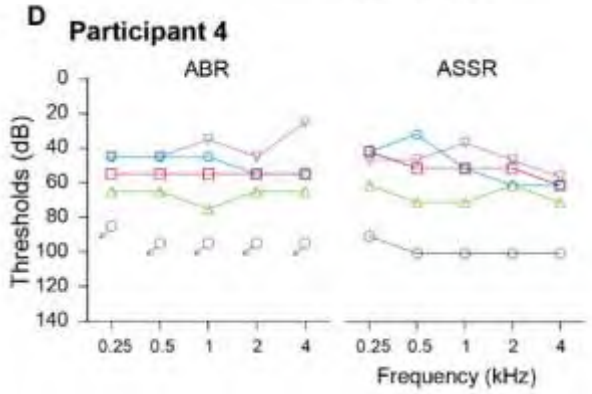
- Define efficacy: 10 dB reduction in the average ABR threshold (0.5–4 kHz)
- Baseline before treatment：ABR from >95 dB in all patients
- After treatment: Hearing was restored to 38 dB-55 dB

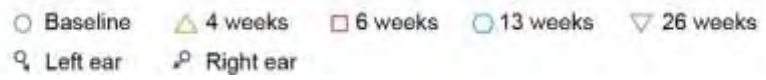


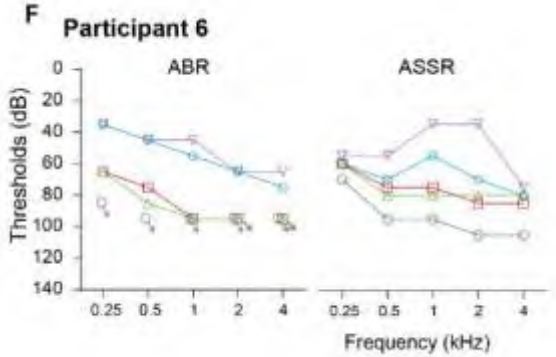




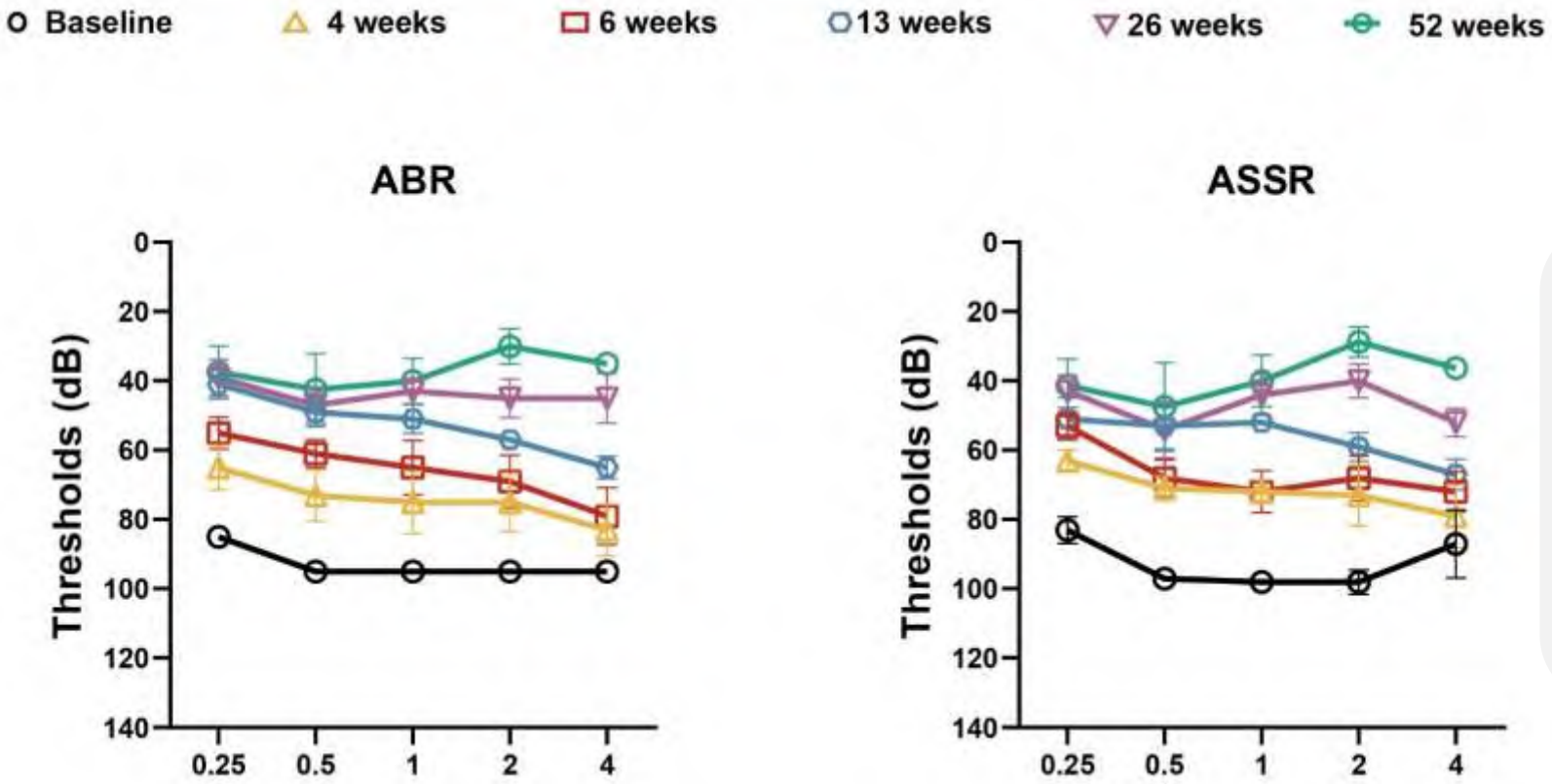








Clinical trial Hearing recovery in a time-dependent manner



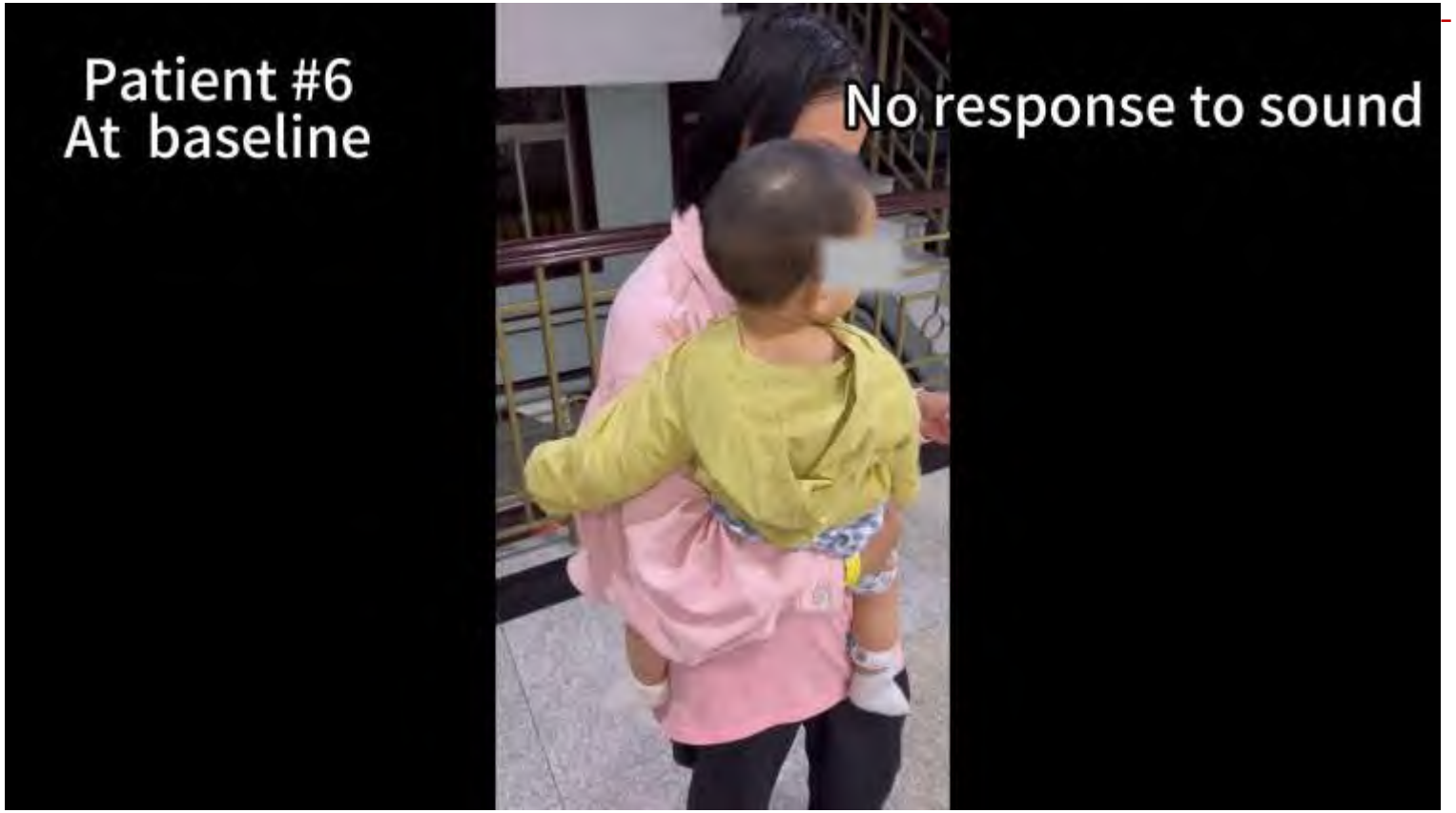
- 52 weeks, n=4, patients #1, 3, 4 & 5
- 4-26 weeks, n=5, patients #1, 3, 4, 5, & 6

Clinical study

Improvement of hearing and speech perception in 5 patients

Speech test



Before gene therapy:

No response to sound

After gene therapy: improve speech perception and communicate with parents.

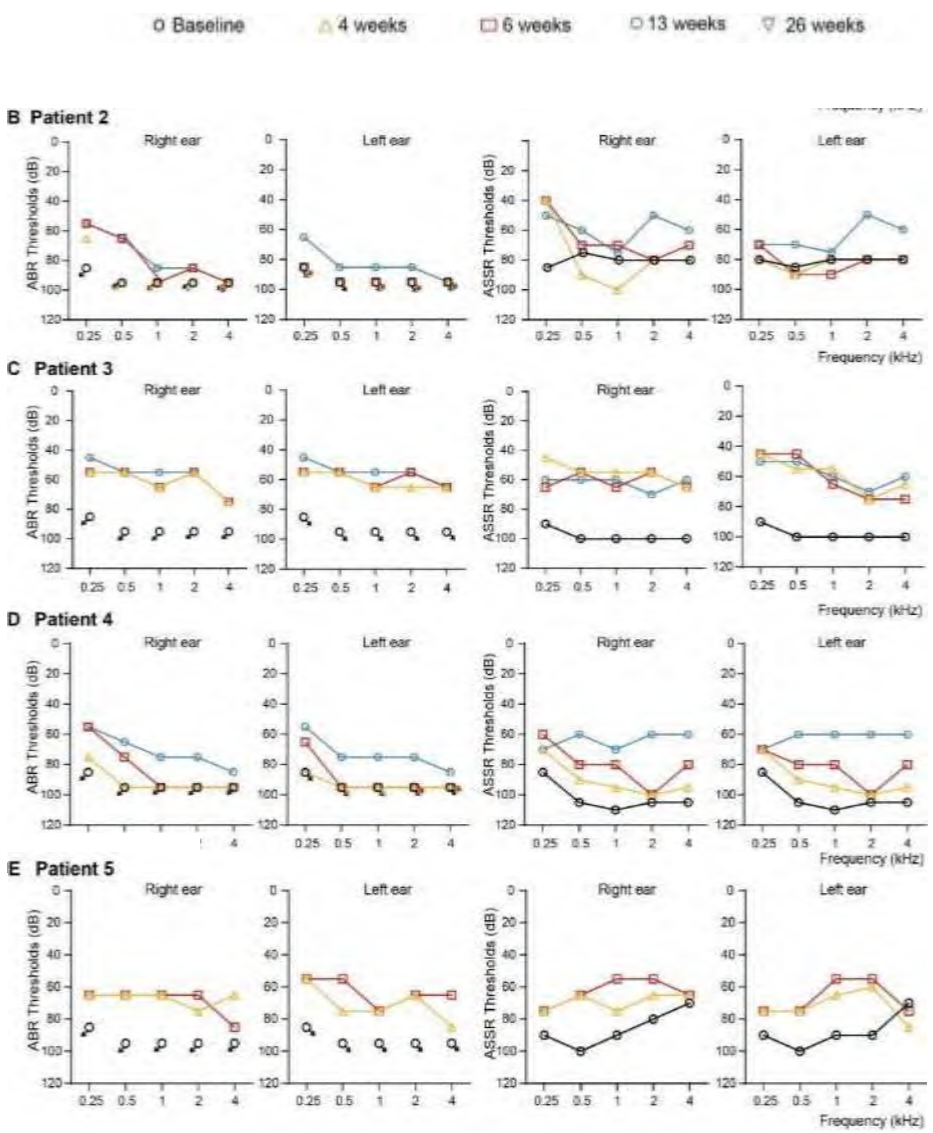
Lv et al., Lancet 2024

Clinical trial

Bilateral AAV1-hOTOF gene therapy for participants with DFNB9

Clinical study

Hearing recovery after bilateral gene therapy



Beyond hearing restoration, bilaterial injection recovered the capacity of sound source localization and improved speech perception in noisy environments.

Wang et al., Nat Med, 2024

Clinical trial

Sound source localization ability and speech perception were improved

Before gene therapy: no response to sound.

- **2.6 years old, born deaf.**
- **Response to sound from at 3 weeks.**
- **Dance to music at 13 weeks.**
- **Say some simple words at 26 weeks, such as Baba (Father).**

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Wang et al., Nat Med, 2024


THE LANCET
Volume 403 · Number 10441 · Pages 2263-2368 · May 25-31, 2024
www.thelancet.com
"The breakthrough study by Lv and colleagues provides a paradigm shift in the treatment of hearing impairment in children and offers hope for treating other genetic forms of hearing loss."
See Comment page 2352
Comment
Collective action and legal mobilisation for the right to health in the climate crisis
Articles
Contribution of vaccination to improved survival and health: modelling 50 years of the Expanded Programme on Immunisation
Articles
AAV9-NSTIM gene therapy for autosomal recessive deafness 9
Seminar
Systemic lupus erythematosus
Seminar
Chronic suppurative otitis media
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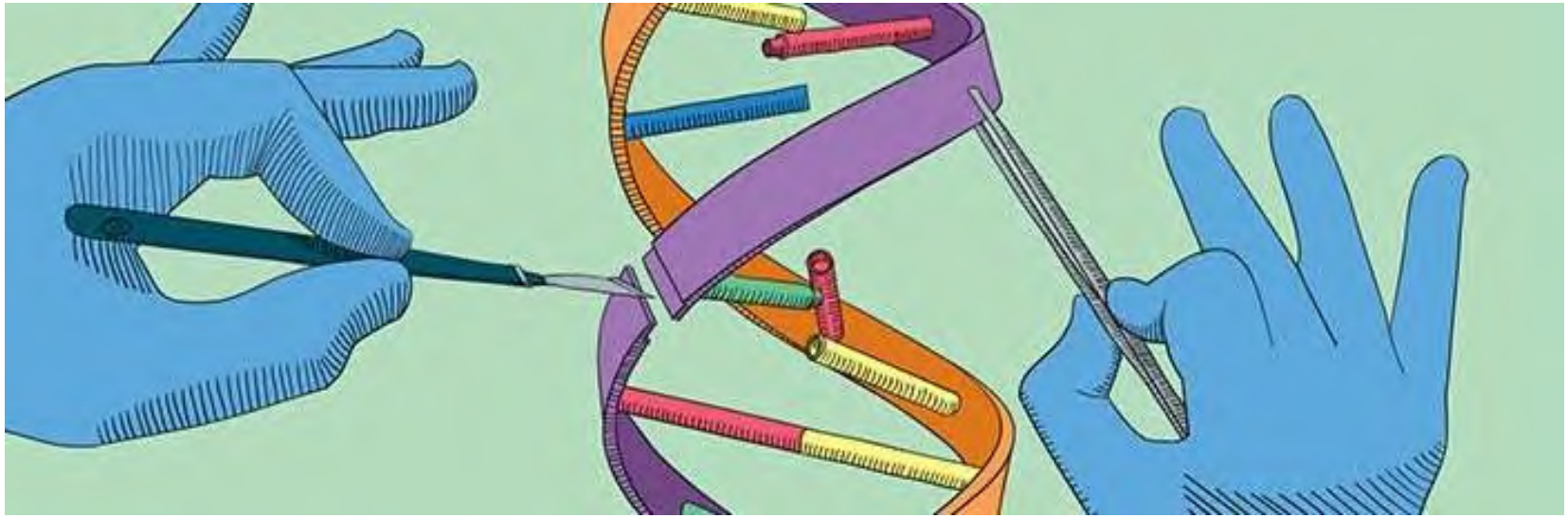
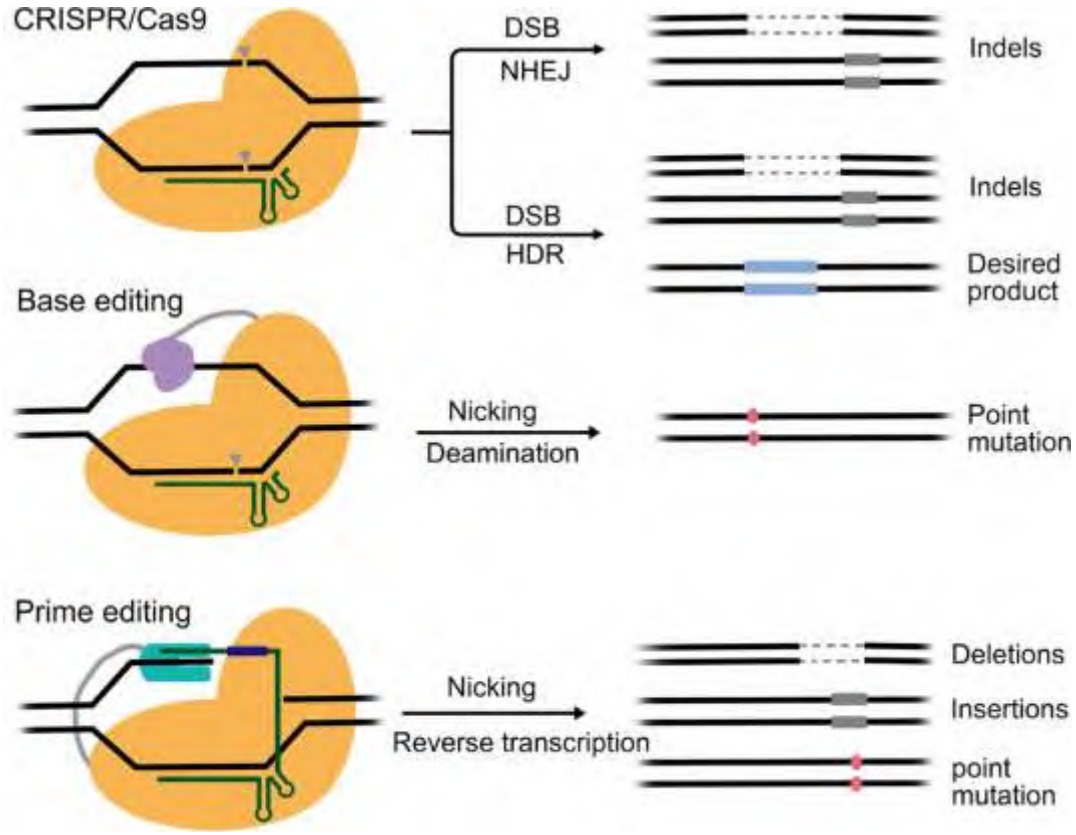
Editing Therapy for Genetic Hearing Loss

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DSB
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Base editing
Nicking
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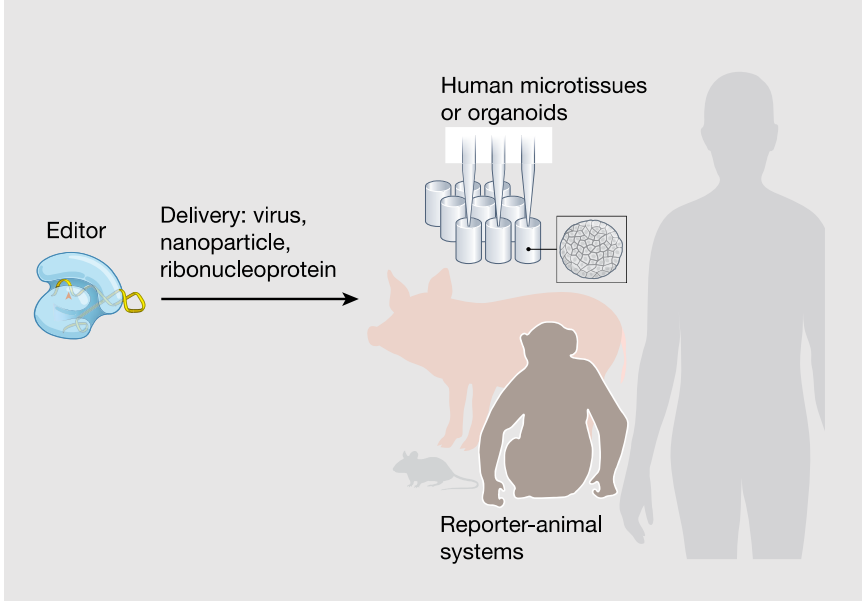
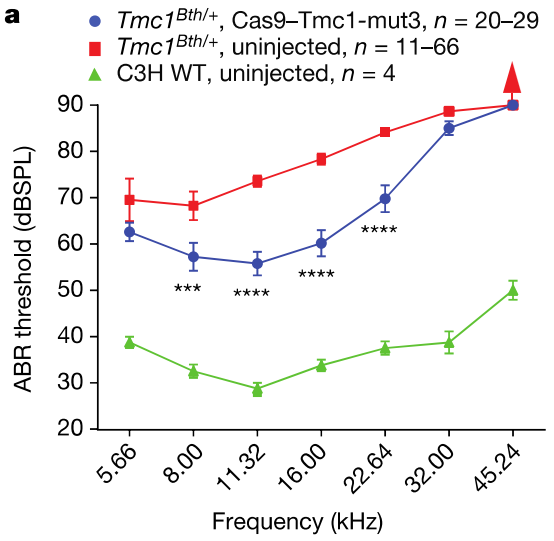
Prime editing
Nicking
Reverse transcription
Deletions
Insertions
point
mutation


Human microtissues
or organoids
Editor
Delivery: virus,
nanoparticle,
ribonucleoprotein
Reporter-animal
systems

doi:10.1038/nature25164

Treatment of autosomal dominant hearing loss by *in vivo* delivery of genome editing agents

Xue Gao[1,2,3]†*, Yong Tao[4,5]†*, Veronica Lamas[4], Mingqian Huang[4], Wei-Hsi Yeh[1,2,3,6], Bifeng Pan[7], Yu-Juan Hu[4,5], Johnny H. Hu[1,2,3], David B. Thompson[1,2], Yilai Shu[4,8], Yamin Li[9], Hongyang Wang[4,10], Shiming Yang[10], Qiaobing Xu[9], Daniel B. Polley[4], M. Charles Liberman[4], Wei-Jia Kong[5], Jeffrey R. Holt[7], Zheng-Yi Chen[4]§ & David R. Liu[1,2,3]§



Article

https://doi.org/10.1038/s41467-023-40476-7

Treatment of monogenic and digenic dominant genetic hearing loss by CRISPR-Cas9 ribonucleoprotein delivery in vivo

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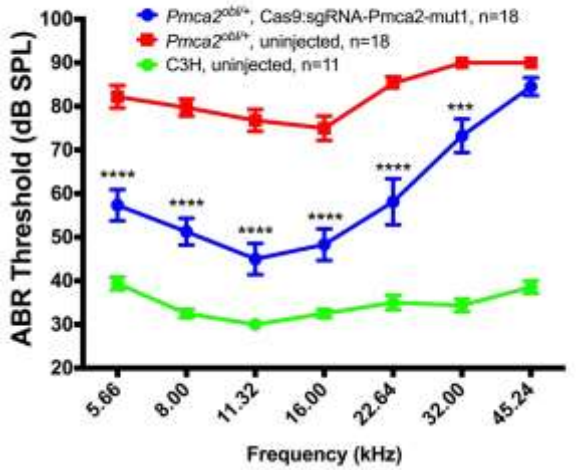
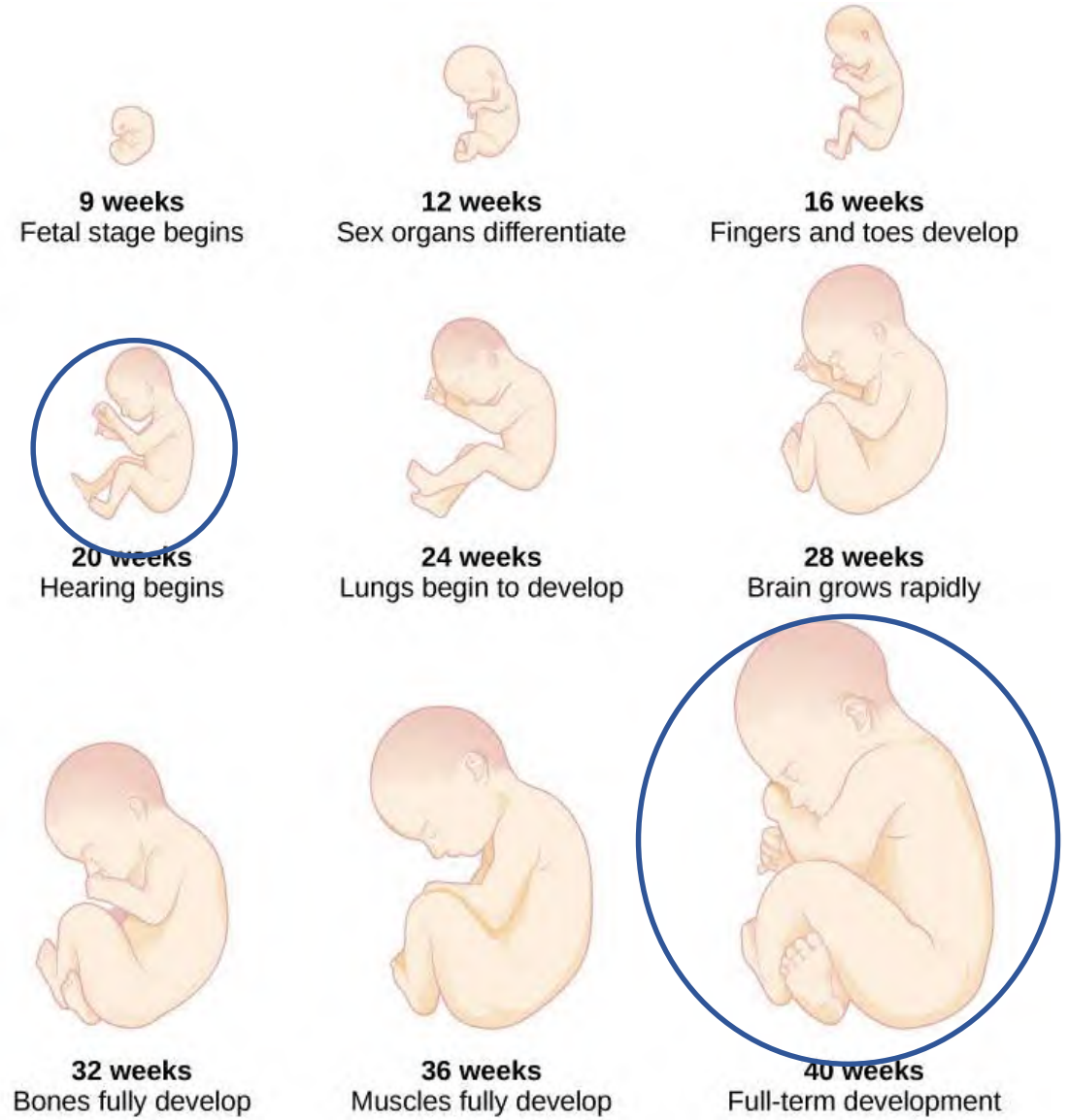
Check for updates

Yong Tao[1,2,3,15], Veronica Lamas[1,2,13,15], Wan Du[1,2,15], Wenliang Zhu[1,2,15], Yiran Li[1,2,14], Madelynn N. Whittaker[4,5], John A. Zuris[6,7,8], David B. Thompson[6,7,8], Arun Prabhu Rameshbabu[1,2], Yilai Shu[1,2,9], Xue Gao[6,7,8], Johnny H. Hu[6,7,8], Charles Pei[2], Wei-Jia Kong[10], Xuezhong Liu[11], Hao Wu[3], Benjamin P. Kleinstiver[4,5,12], David R. Liu[6,7,8] ✉ & Zheng-Yi Chen[1,2] ✉



Human Fetal Hearing Begins at the 2nd Trimester



Iowan State Univ Press; Lahlou et al., JCI Insight 2024

Editing Rescues Hearing in Adult Mouse Model of Mir96 Dominant Hearing Loss DFNA50

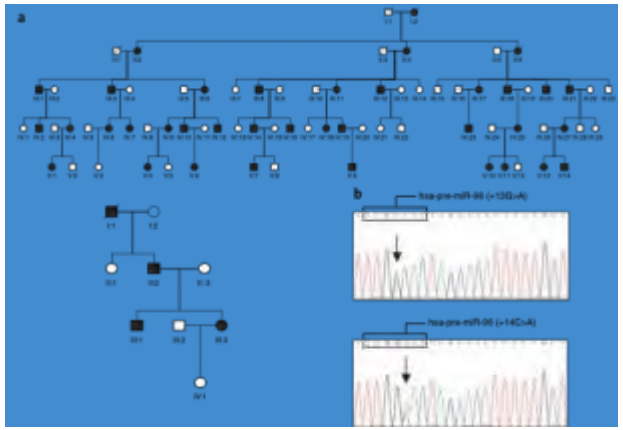
2024 Nobel Prize in Physiology or Medicine: Discovery of MicroRNA

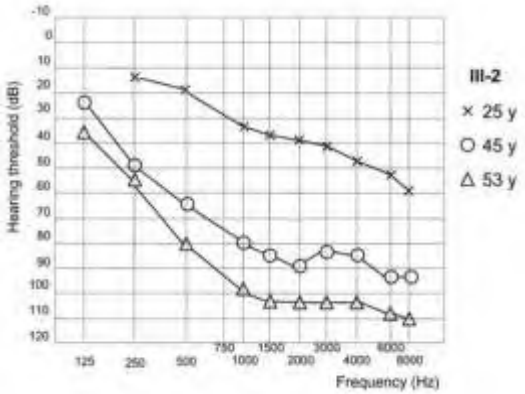
Victor Ambros and Gary Ruvkun

MicroRNA 96 Mutations Cause Delayed Onset Progressive Hearing Loss in Humans

Mutations in the seed region of human miR-96 are responsible for nonsyndromic progressive hearing loss

Ángeles Mencía[1,2], Silvia Modamio-Høybjør[1,2], Nick Redshaw[3], Matías Morín[1,2], Fernando Mayo-Merino[1,2], Leticia Olavarrieta[1,2], Luis A Aguirre[1,2], Ignacio del Castillo[1,2], Karen P Steel[4], Tamas Dalmay[3], Felipe Moreno[1,2] & Miguel Ángel Moreno-Pelayo[1,2]



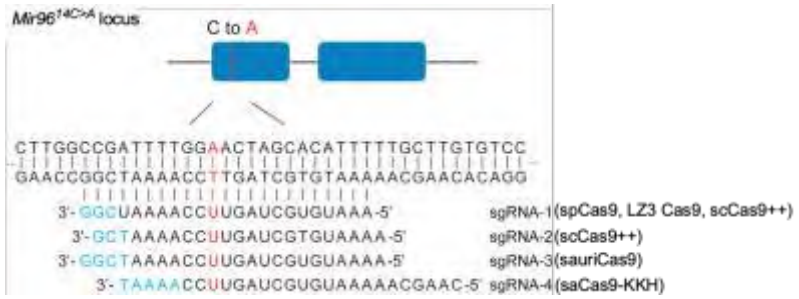


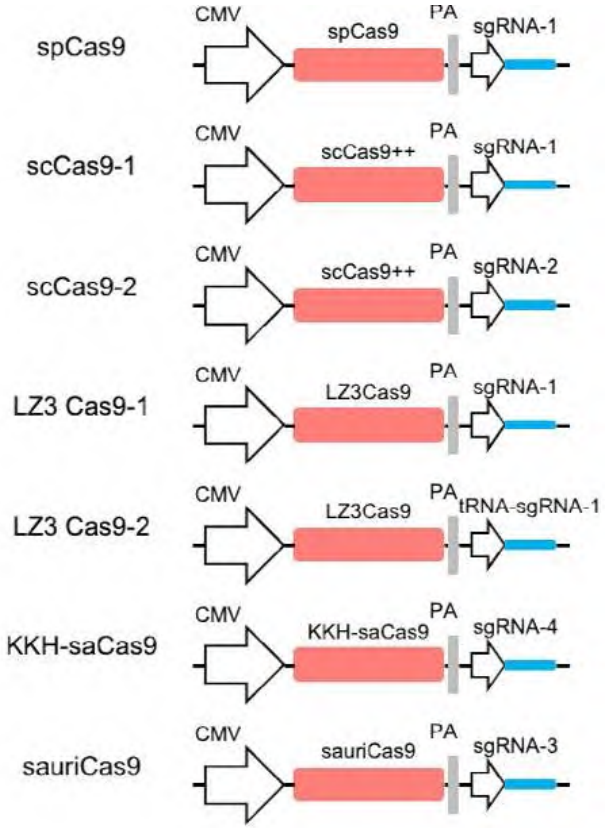
An ENU-induced mutation of miR-96 associated with progressive hearing loss in mice

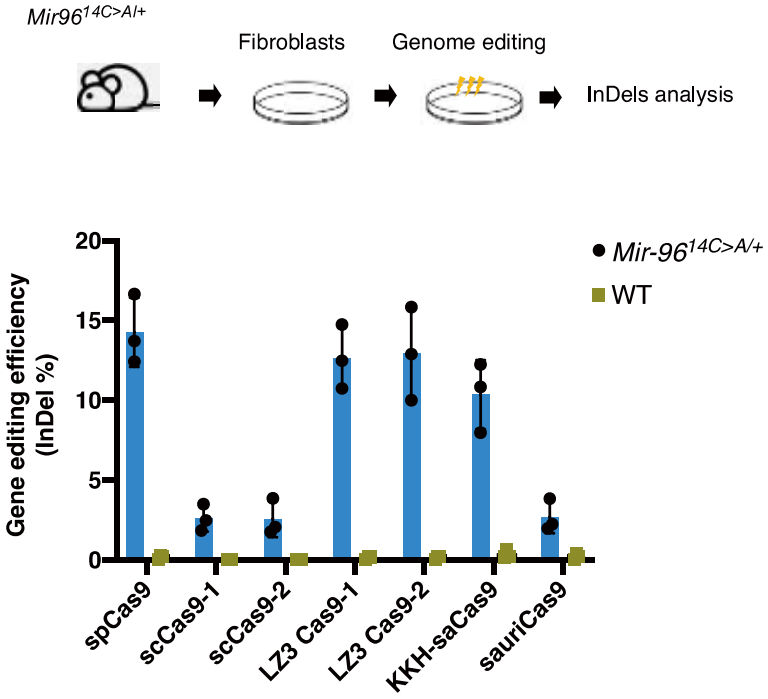
Morag A Lewis[1], Elizabeth Quint[2], Anne M Glazier[1], Helmut Fuchs[3], Martin Hrabé De Angelis[3], Cordelia Langford[1], Stijn van Dongen[1], Cei Abreu-Goodger[1], Matias Piipari[1], Nick Redshaw[4], Tamas Dalmay[4], Miguel Angel Moreno-Pelayo[5,6], Anton J Enright[1] & Karen P Steel[1,2]

Mencia et al., Nat Genet 2009; Lewis et al., Nat Genet, 2009; Kuhn et al., PNAS 2010

Screening of Editors for Efficient Editing







MiR-96$^{s1334/+}$ fibroblast

spCas9/sgRNA-1 Total Reads: 49112

		Reads
	5'- TGGCCGATTTTGGCACTAGCACATTTTTGCTTGT -3'	Reference
WT	5'- TGGCCGATTTTGGCACTAGCACATTTTTGCTTGT -3'	25687
+14C>A	5'- TGGCCGATTTTGGAACTAGCACATTTTTGCTTGT -3'	14021
+1 bp	5'- TGGCCGATTTTTGGAACTAGCACATTTTTGCTTGT -3'	2542
-1 bp	5'- TGGCCGATT-TGGAACTAGCACATTTTTGCTTGT -3'	2285
-2 bp	5'- TGGCCGATT-- GGAACTAGCACATTTTTGCTTGT -3'	1129
-10 bp	5'- TGG - - - - - - - - - - ACTAGCACATTTTTGCTTGT -3'	873
+2 bp	5'- TGGCCGATTTTTTGGAACTAGCACATTTTTGCTTGT -3'	721

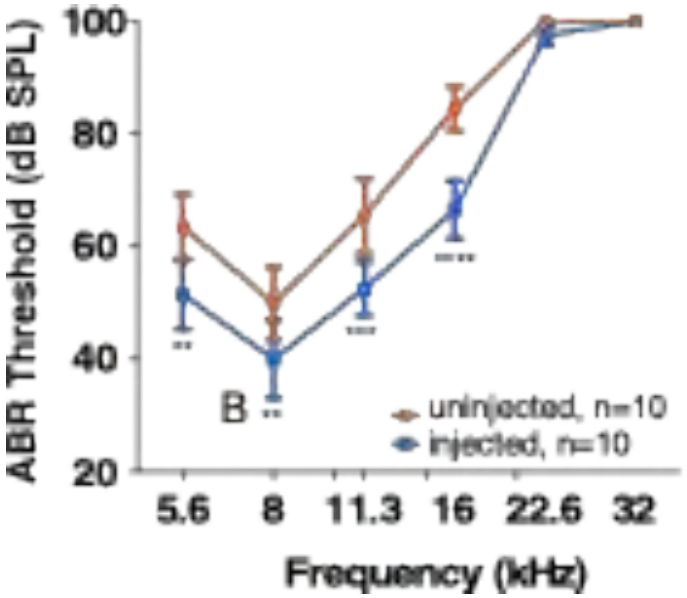
MiR-96$^{+/+}$ fibroblast

spCas9/sgRNA-1 Total Reads: 51450

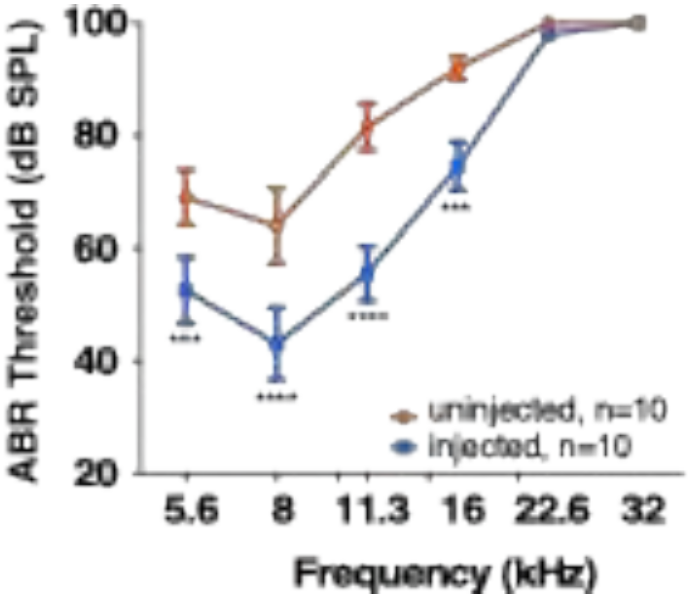
		Reads
	5'- TGGCCGATTTTGGCACTAGCACATTTTTGCTTGT -3'	Reference
WT	5'- TGGCCGATTTTGGCACTAGCACATTTTTGCTTGT -3'	51312
-1 bp	5'- TGGCCGATT-TGGCACTAGCACATTTTTGCTTGT -3'	138

Sustained Hearing Rescue by Editing in Adult Mice

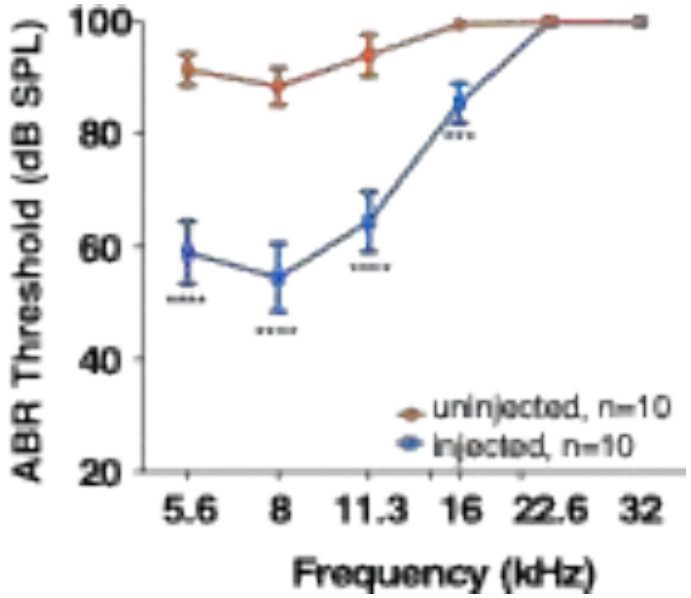
10 weeks



14 weeks



20 weeks

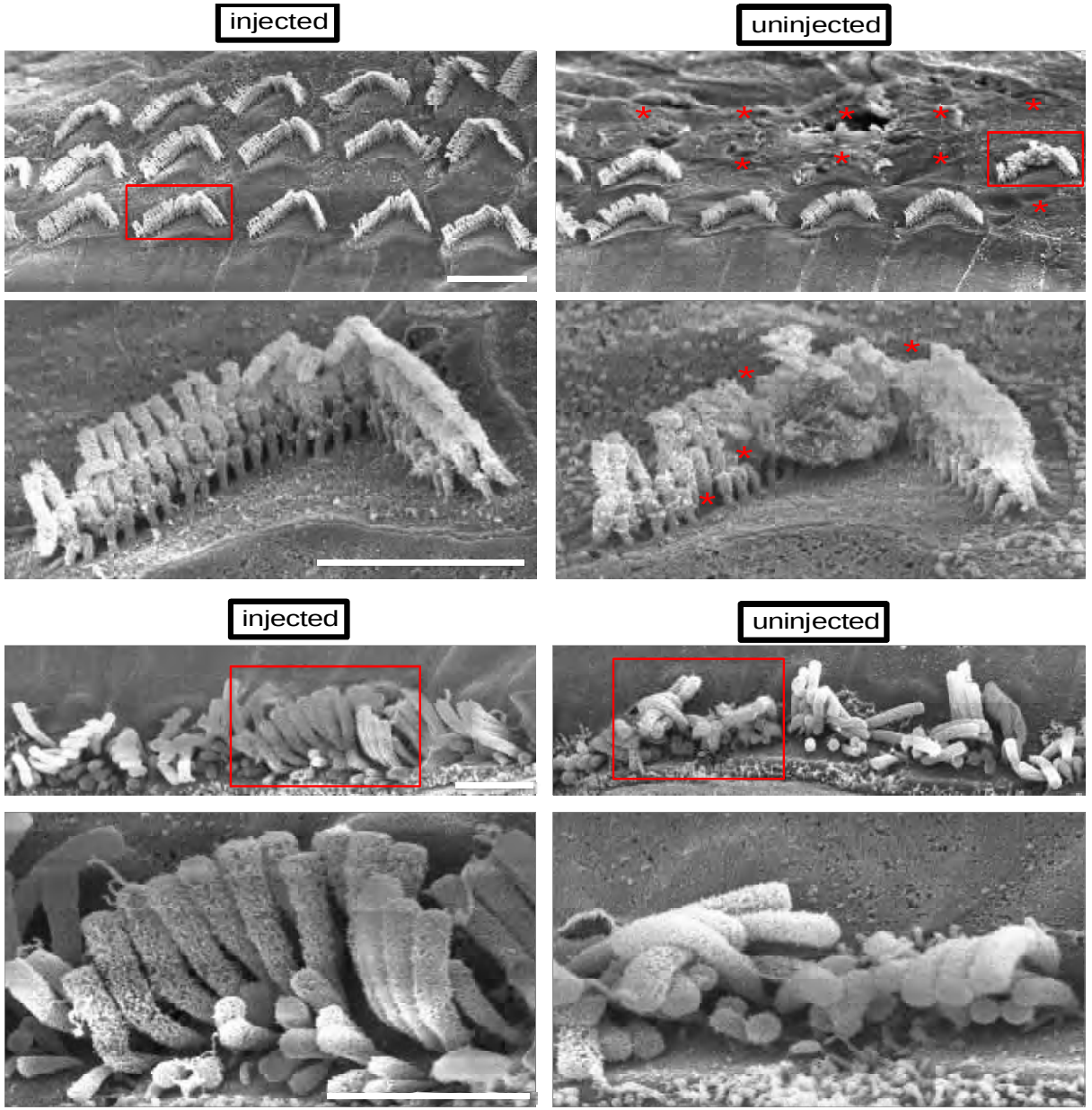


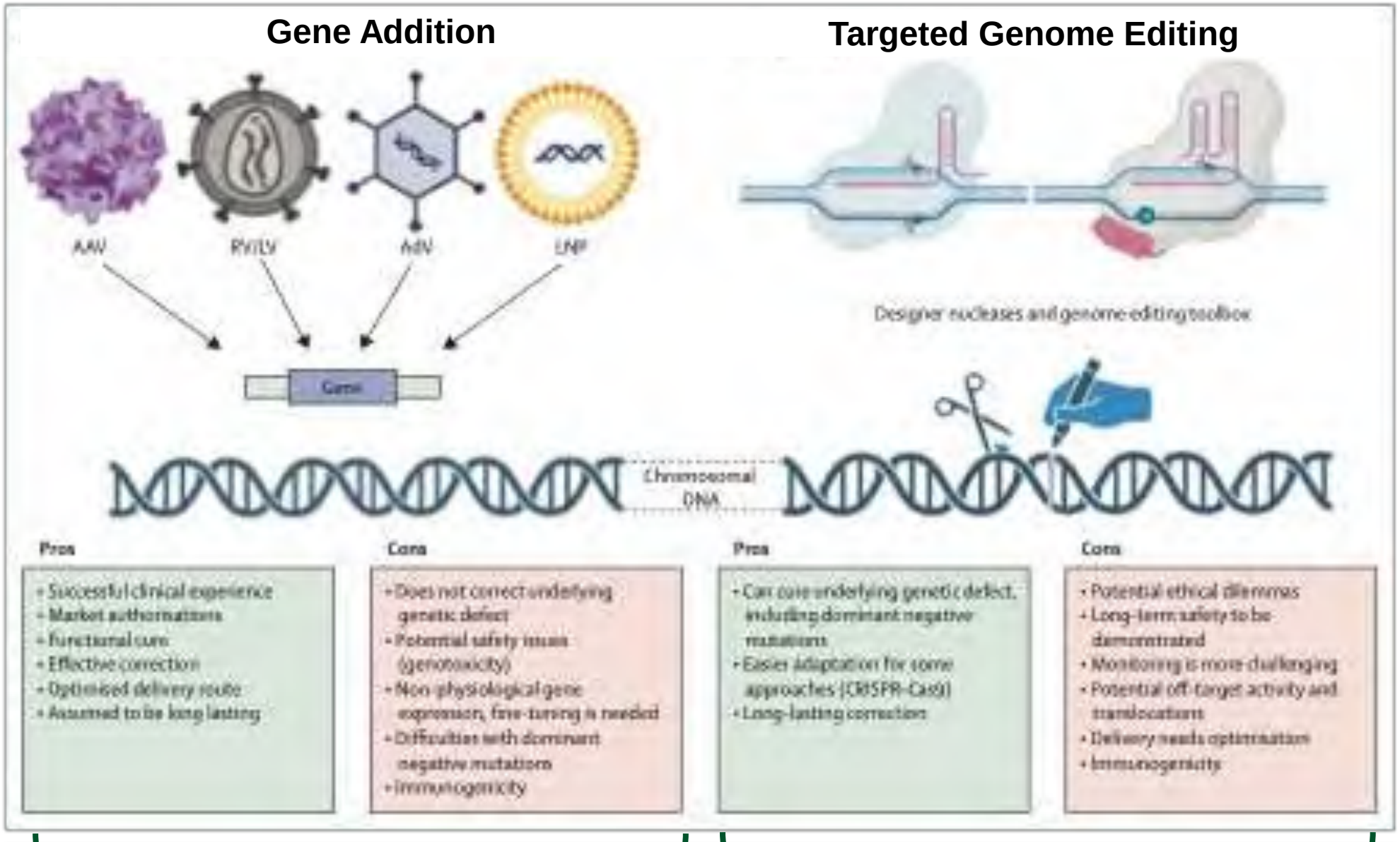
6x10^9 vg, AAV2-SaCas9-KKH-sgRNA4 Injection: 6 weeks of age

Mir96$^{s1334/+}$; uninjected

Mir96$^{s1334/+}$; injected

Editing Treatment Rescues Hair Cells





One product for one disease (OTOF)

30-200 products for one disease (USH2A)

Schambach et al., Lancet 2024

Streamline Regulatory Path for Somatic Gene Editing Therapy

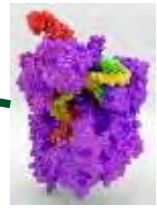
CGE
Somatic Cell Genome Editing

DFNA41 **DFNA2**

Delivery Vehicle

Delivery route

Editors



Cell targets

gRNA1 + **gRNA2**

Similar preclinical profiles

Shown to be safe in clinical trials

Regulatory path for other gRNAs-mediated treatment

Somatic Cell Genome Editing Program Phase 2

NIH National Institutes of Health
Office of Strategic Coordination—The Common Fund

Overarching Goal: Accelerate the translation of genome editing therapies into the clinic.

Develop Targeted Delivery Technologies

Develop and validate delivery systems that can target a substantial proportion of clinically relevant cells.

Advance Therapeutic Development Studies

Accelerate the clinical development and evaluation of novel genome editing therapeutics.

Establish New Regulatory Pathways to the Clinic

Lay the groundwork for clinical trials that assess the safety and efficacy of promising genome editing therapies to treat multiple diseases.

Disseminate Successful Strategies for Starting Clinical Trials

Share strategies, technologies, and protocols with the community through a publicly accessible platform.

The SCGE program aims to reduce the burden of diseases caused by genetic changes.

CGE


Establish New Regulatory Pathways to the Clinic
Lay the groundwork for clinical trials that assess the safety and efficacy of promising genome editing therapies to treat multiple diseases.

Acknowledgments

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Morag Lewis

Univ Miami

Xuezhong Liu
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