HARVARD MEDICAL SCHOOL | DEPARTMENT OF Otolaryngology

MEDIZINISCHE UNIVERSITÄT WIEN

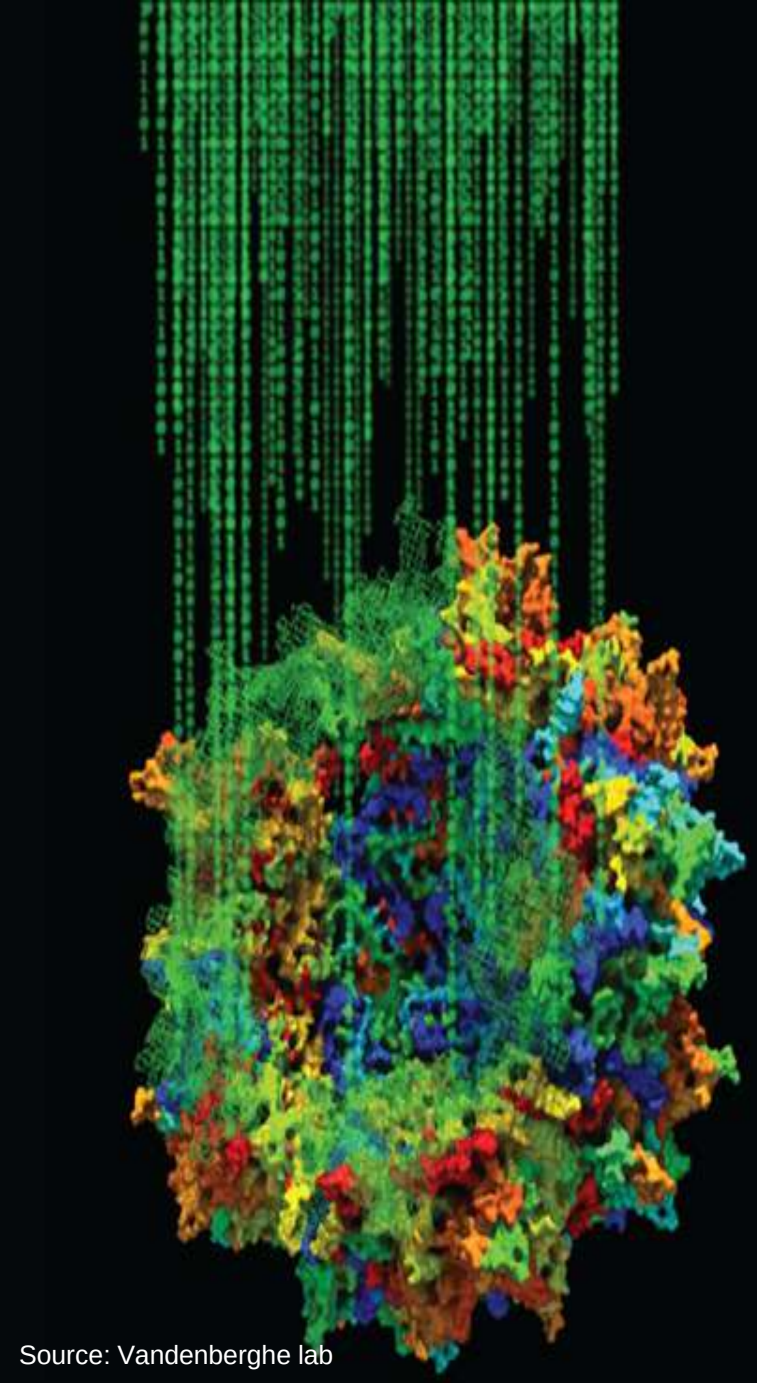
Inner Ear Gene Therapy
–
Recent Advances & Clinical Perspectives

Lukas Landegger, M.D., Ph.D.

Hearing Loss Association of America (HLAA) webinar

April 18, 2018

Massachusetts Eye and Ear • Beth Israel Deaconess Medical Center • Boston Children's Hospital
Brigham and Women's Hospital • Massachusetts General Hospital



Source: Vandenberghe lab

Overview

Hearing (loss) and current standard of care

(Inner ear) Gene therapy

Different approaches to target genes

- **(viral) vectors: common adeno-associated virus (AAV), Anc80, exo-AAV, adenovirus, etc.**
- **CRISPR-Cas9**

Rescue in mouse models of human inner ear disease

Stabilization vs. restoration (age-related?)

Hurdles on the way to the clinic?

Hearing loss

Most common sensory deficit in humans.

World Health Organization (WHO) fact sheet on deafness and hearing loss in March 2018:

- Over 5% of the world's population (466 million people) has disabling hearing loss. This number is expected to rise over 900 million people by 2050.

- 1.1 billion young people (aged between 12-35 years) at risk of hearing loss due to exposure to noise in recreational settings.

- Reasons for hearing loss include genetic causes, complications at birth, certain infectious diseases, chronic ear infections, the use of particular drugs, exposure to excessive noise, and ageing.

0.2%

0.4%

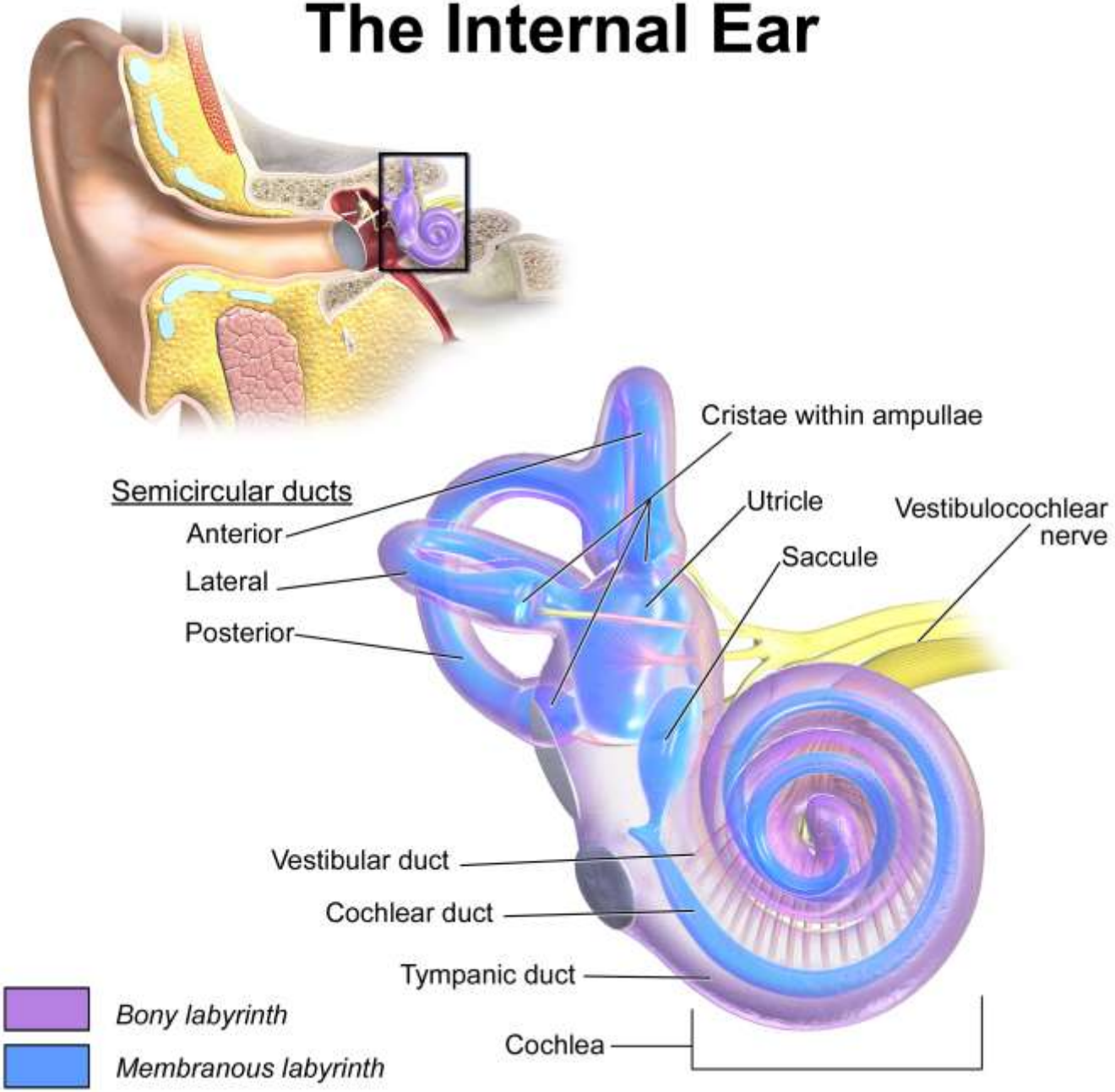
16%
>18 y/o

34%
65-69 y/o

72%
85-90 y/o

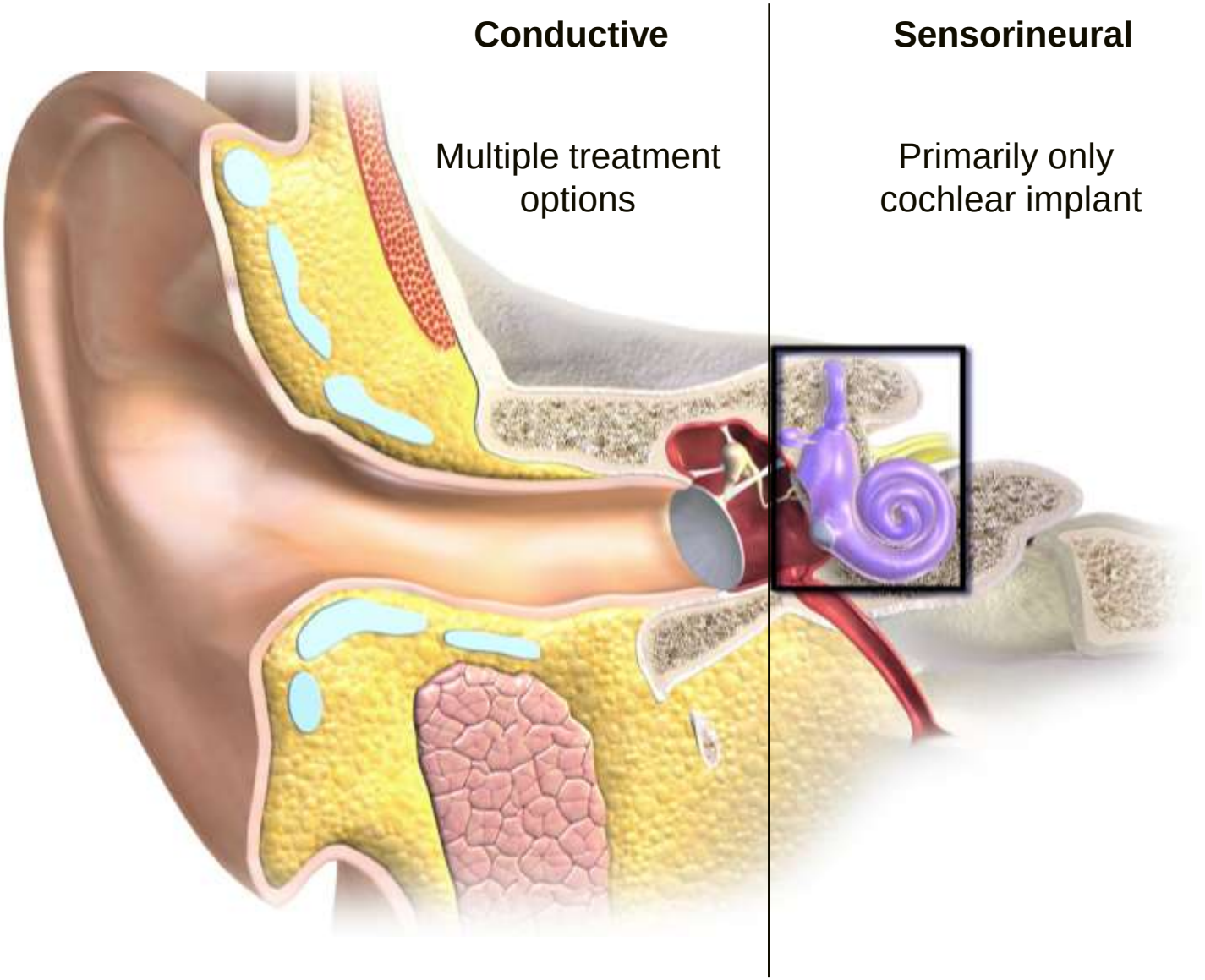
Source: https://en.wikipedia.org/wiki/World_Health_Organization#/media/File:Flag_of_WHO.svg
http://www.who.int/mediacentre/factsheets/fs300/en/
http://www.pexels.com

The Internal Ear



Source: https://en.wikipedia.org/wiki/Ear#/media/File:Blausen_0329_EarAnatomy_InternalEar.png

Conductive or sensorineural hearing loss

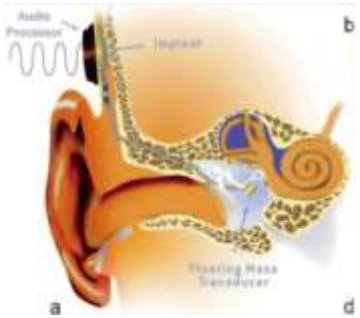


Source: https://en.wikipedia.org/wiki/Ear#/media/File:Blausen_0329_EarAnatomy_InternalEar.png

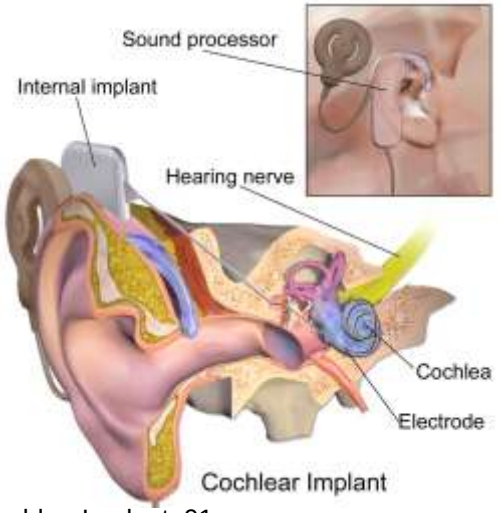
Current treatment options

Hearing aids (> sound amplification)

Enhanced sound transmission (> middle ear prostheses/middle ear active implants)



Direct neuronal stimulation (> cochlear implants)



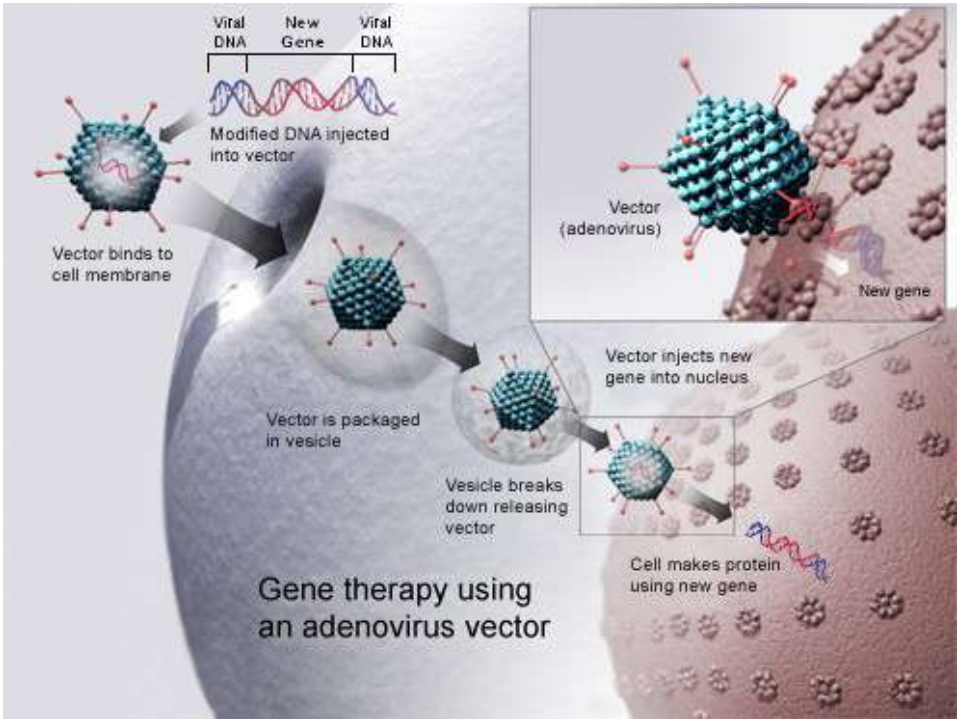
Source: https://en.wikipedia.org/wiki/Hearing_aid#/media/File:BTEhearingaids.png
https://en.wikipedia.org/wiki/Hearing_aid#/media/File:HearingAid_ITE.png
https://en.wikipedia.org/wiki/Hearing_aid#/media/File:Hearing_aid_cic.jpg
Beutner et al. GMS Curr Top Otorhinolaryngol Head Neck Surg. 2009
https://en.wikipedia.org/wiki/Cochlear_implant#/media/File:Blausen_0244_CochlearImplant_01.png

Gene therapy

The transplantation of normal genes into cells in place of missing or defective ones in order to correct genetic disorders.

Around 100 genes that cause non-syndromic hearing loss are known.

Ca. 30 gene therapy trials for ten diseases of the retina (December 2017: first FDA-approved gene therapy "Luxturna" to treat RPE65 mutation-associated retinal dystrophy), but only one for severe-to-profound hearing loss and vestibular dysfunction.



Source: https://upload.wikimedia.org/wikipedia/commons/3/3d/Gene_therapy.jpg

Genetics and implications for therapy

Usually, every human has two copies of a certain gene (one set of 22 chromosomes inherited from mother, another set from father; exception: sex chromosomes)

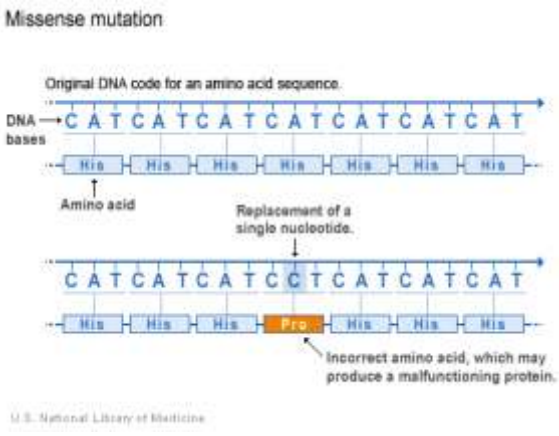
Simplified statement:
If it is enough to have one affected gene to suffer from the condition = dominant disease
If both genes have to be affected to suffer from condition = recessive disease

Simple gene addition (recessive) > no functioning gene; delivery of copy of normal gene sufficient (most animal studies so far)

Gene disruption (dominant) > by targeting the disease-causing dominant gene, the remaining normal gene can take over

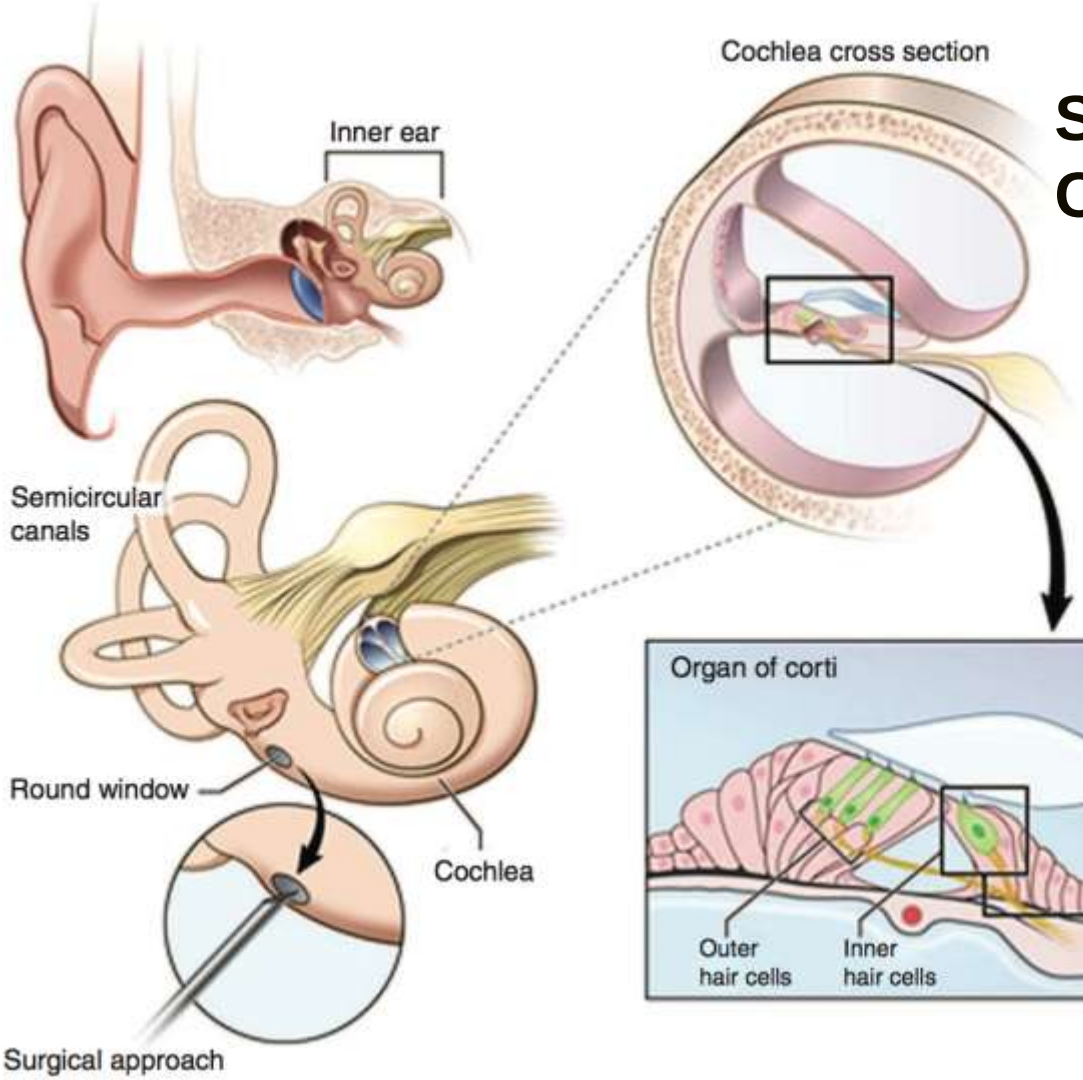
Gene editing (recessive or dominant missense) > CRISPR-Cas9

Functional gene addition (recessive or dominant; vector usually persists outside of chromosome; non-hereditary)



Source: https://en.wikipedia.org/wiki/Missense_mutation#/media/File:Missense_Mutation_Example.jpg

Gene therapy in the (human) inner ear



Success story: Cochlear implant

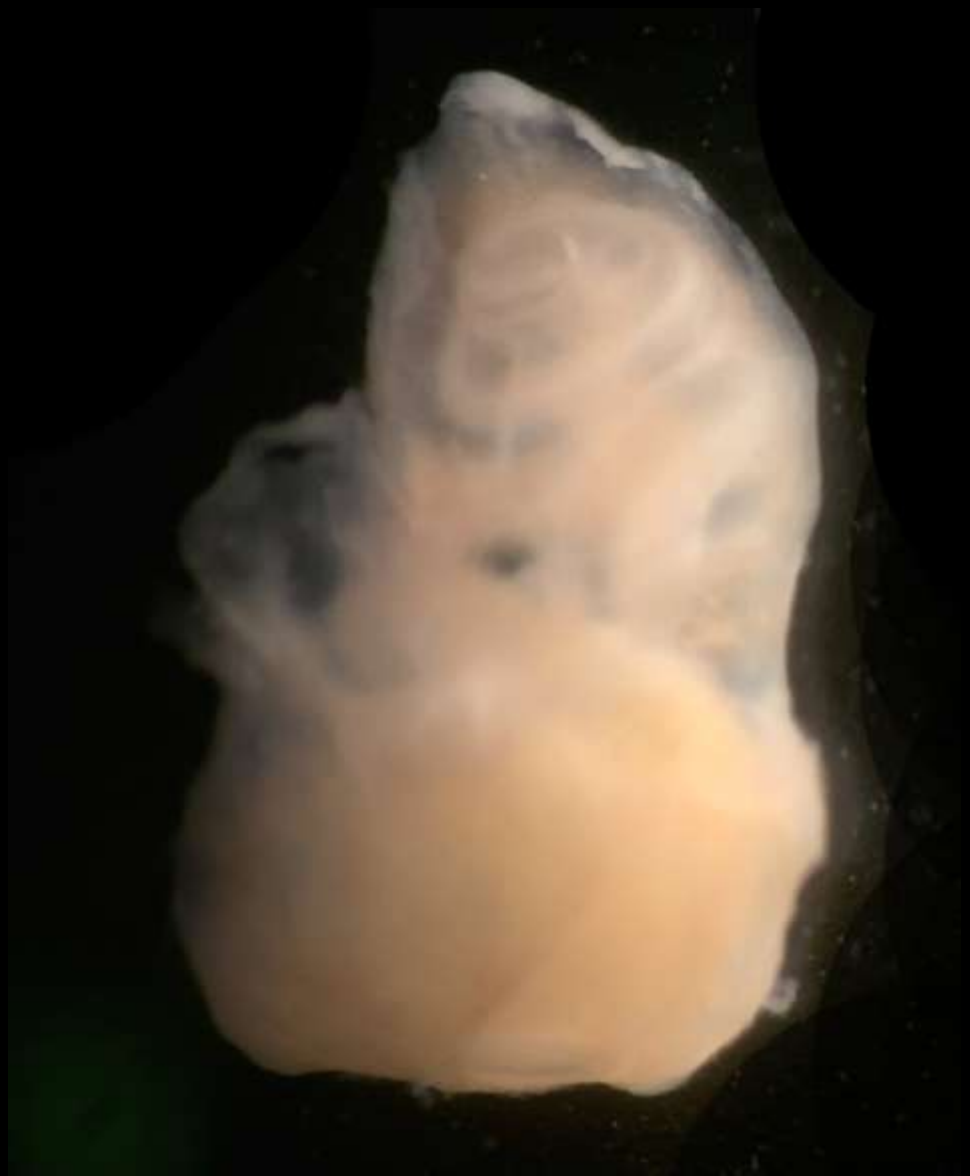
- Natural sound perception?
- Frequency sensitivity?
- Speech discrimination in noisy environments?

Ca. 15,000 hair cells in one human ear – same cell type in vestibular system; stereocilia on top; do not regenerate in mammals*

Goal: Restoration of “natural hearing”

Source:
- Chien et al. Gene Therapy Restores Hair Cell Stereocilia Morphology in Inner Ears of Deaf Whirler Mice. Molecular Therapy (open access) 2016.
-* Cox et al. Spontaneous hair cell regeneration in the neonatal mouse cochlea in vivo. Development (open access) 2014.

Mouse cochlea



Source: © Lukas Landegger

Functional rescue in mouse models

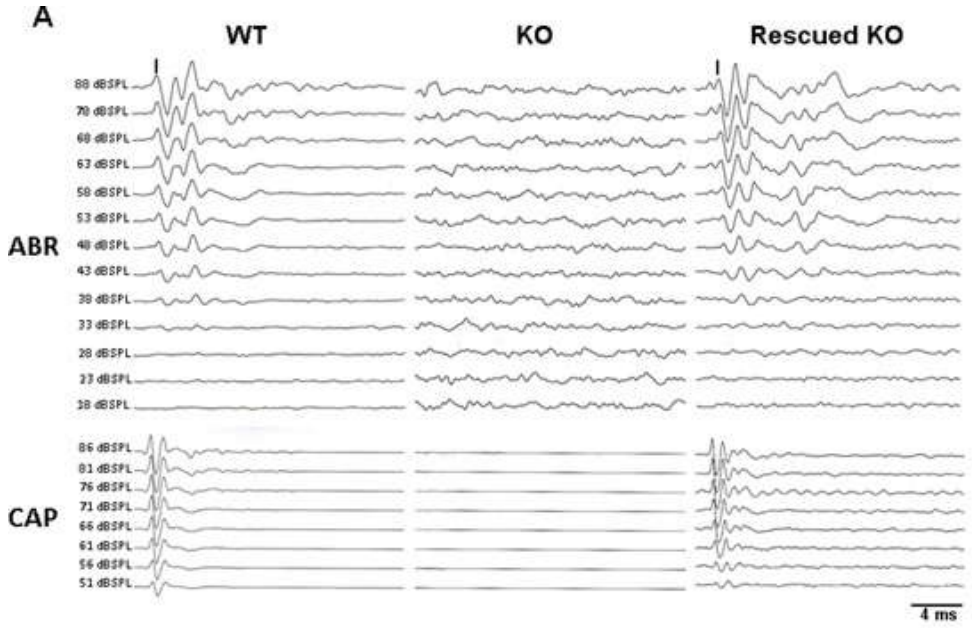
VGLUT3 (vesicular glutamate transporter-3) important for communication between inner hair cell and auditory nerve

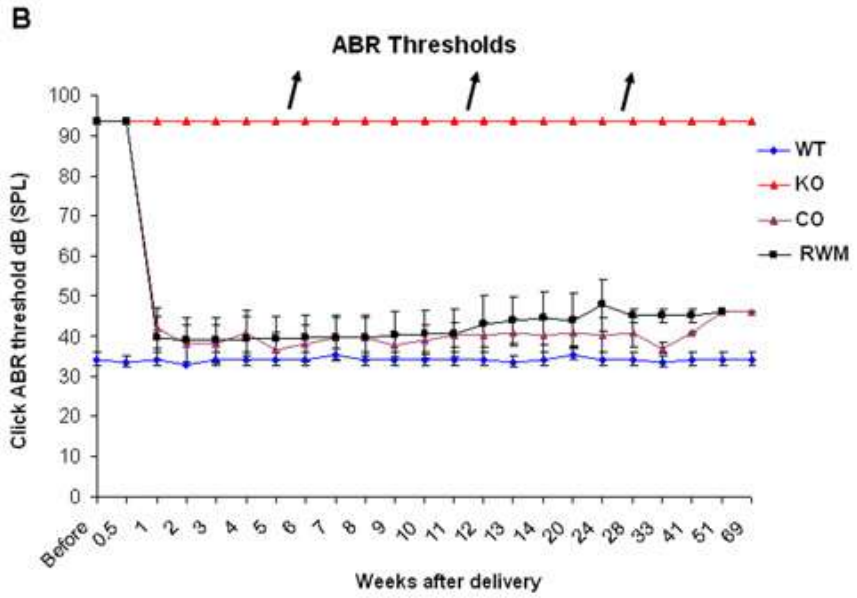
Mice lacking this transporter are deaf (only relevant in few human patients)

(Relevant fact: Mice usually develop hearing around two weeks after birth)

AAVs (in this case AAV1) target inner hair cells > exactly what is needed here

Mouse pups lacking the transporter injected with VGLUT3 > did not go deaf





Source: Akil et al. Restoration of hearing in the VGLUT3 knockout mouse using virally mediated gene therapy. Neuron (open access) 2012.

Functional rescue in mouse models

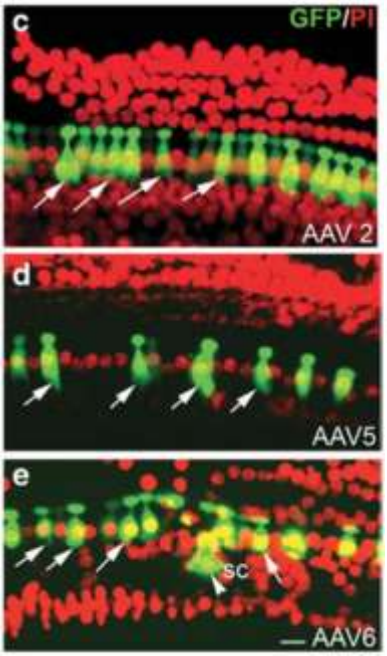
“...driving the expression of exogenous Tmc1 in inner hair cells in vivo.”

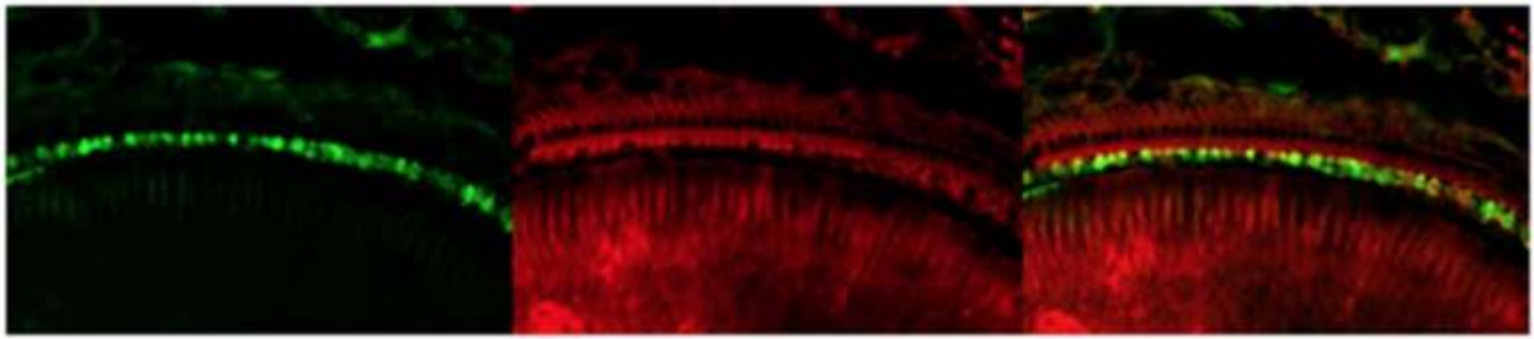
“Whirlin gene therapy also increased inner hair cell survival in the treated ears compared to the contralateral nontreated ears.”

“The AAV8-whirlin-treated whirler ears had more surviving inner hair cells (IHCs)…”

Source:
- Askew et al. Tmc gene therapy restores auditory function in deaf mice. Science Translational Medicine © 2015.
- Chien et al. Gene Therapy Restores Hair Cell Stereocilia Morphology in Inner Ears of Deaf Whirler Mice. Molecular Therapy (open access) 2016.
- Isgrig et al. Gene Therapy Restores Balance and Auditory Functions in a Mouse Model of Usher syndrome. Molecular Therapy (open access) 2017.

Mouse models (AAV-GFP vectors in vivo)





Chien et al. Laryngoscope 2015

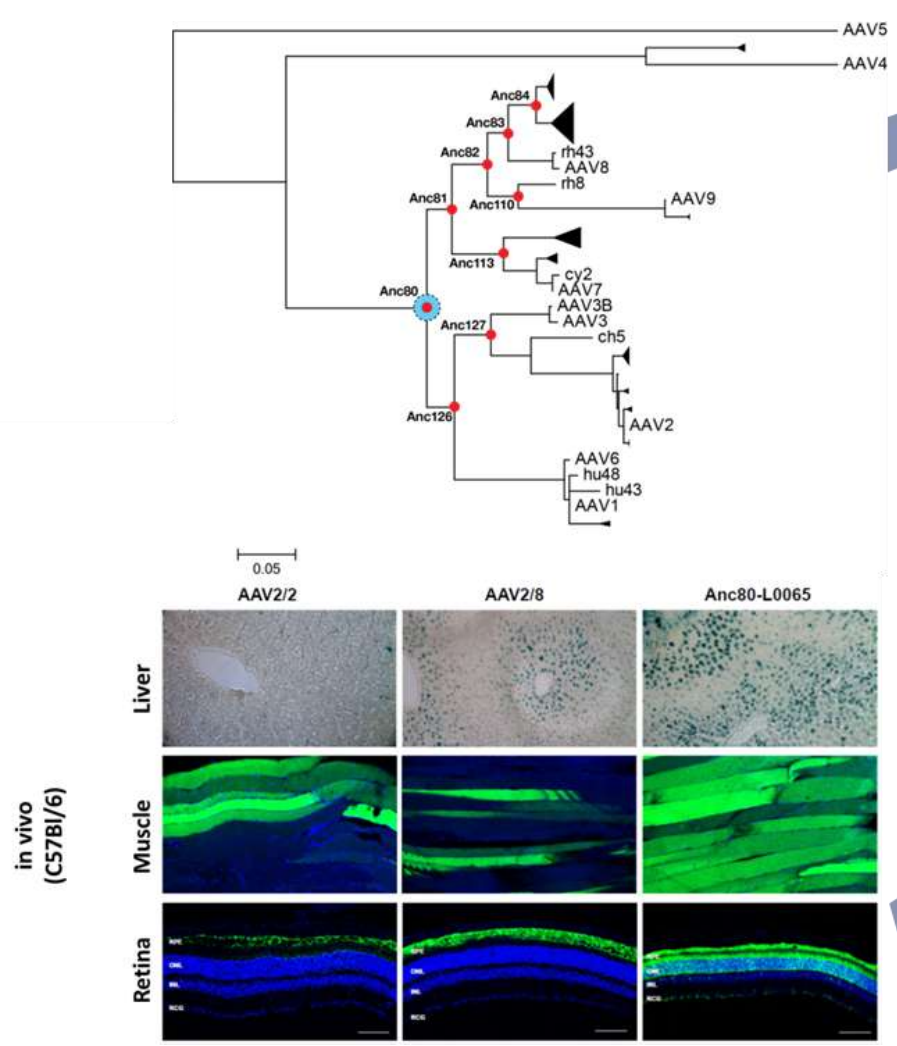
Kilpatrick et al. Gene Ther. 2011 > **“Among all five serotypes, inner hair cells were the most effectively transduced cochlear cell type.”**

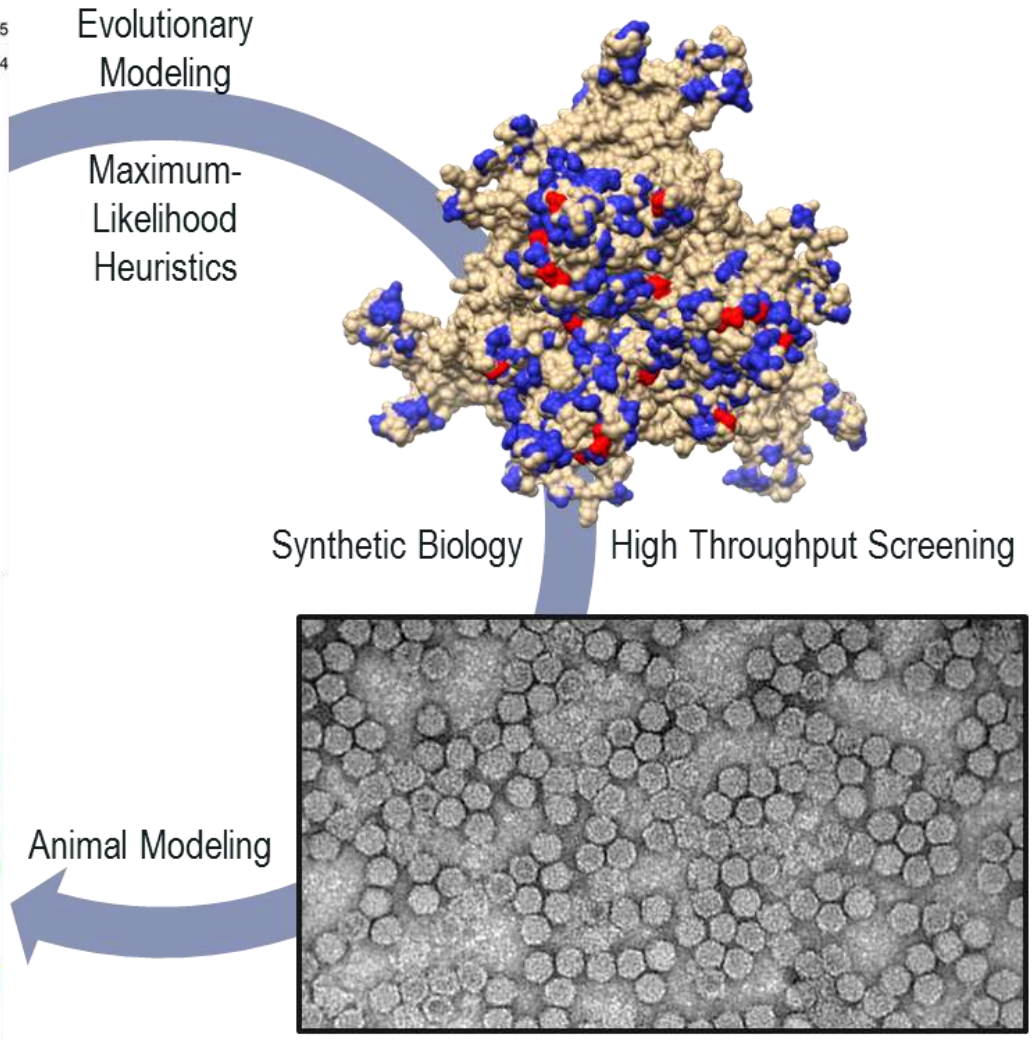
Difficulty: Outer hair cells

Source:
- Reprinted by permission from Springer Nature. Kilpatrick et al. Adeno-associated virus-mediated gene delivery into the scala media of the normal and deafened adult mouse ear. Gene Therapy © 2011.
- Reprinted by permission from Wiley. Chien et al. Cochlear gene transfer mediated by adeno-associated virus: Comparison of two surgical approaches. Laryngoscope © 2015.

Anc80: Computer-modeled synthetic AAV

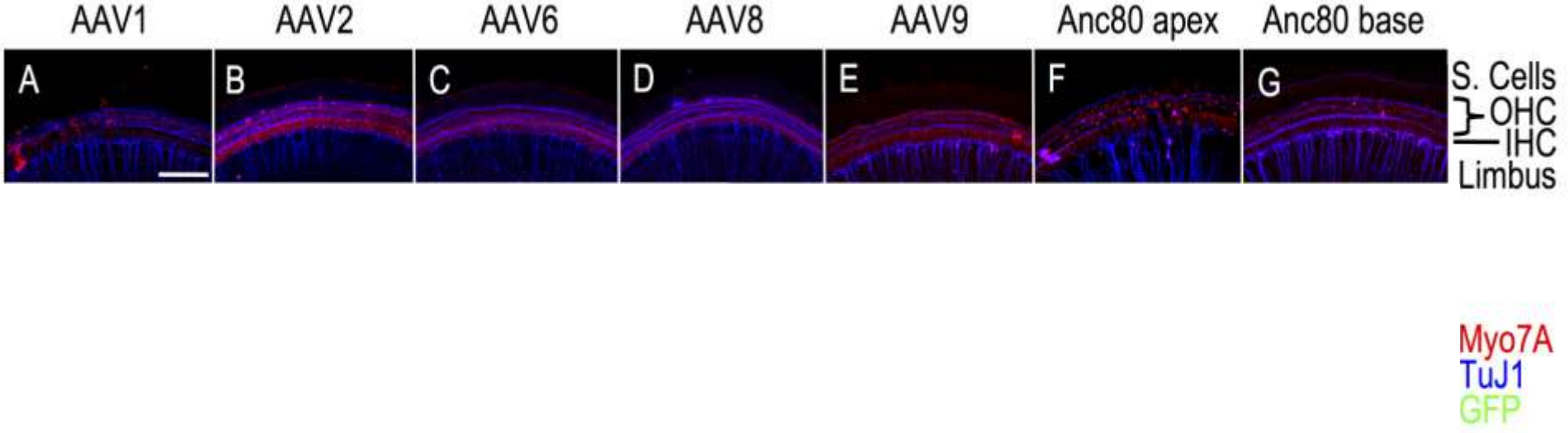
Predicted ancestor of AAV serotypes 1, 2, 8, and 9.
Hypothesis: Anc80 circumvents preexisting immunity.





Source: Zinn et al. In Silico Reconstruction of the Viral Evolutionary Lineage Yields a Potent Gene Therapy Vector. Cell Reports (open access) 2015.

First step: *in vitro* screening



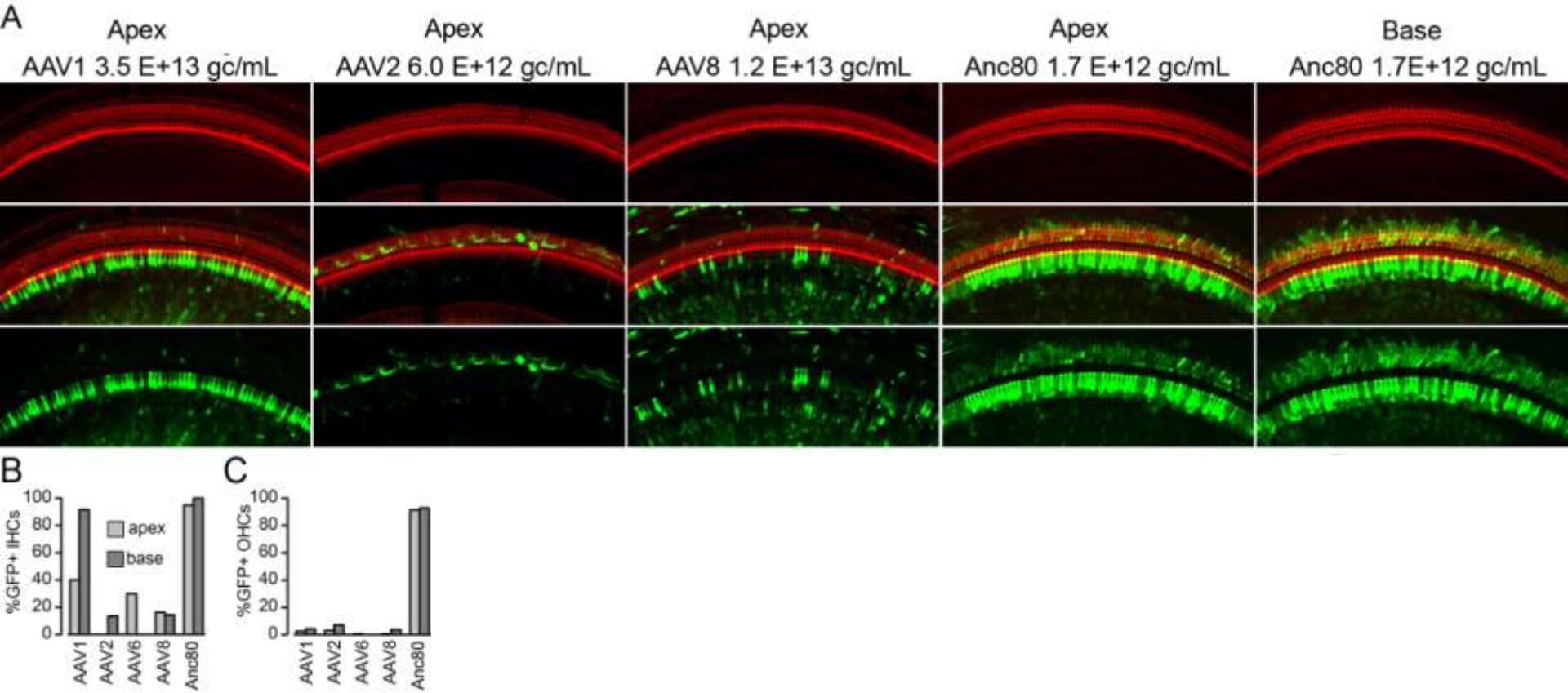
Error bars = SEM

Organotypic cultures ("cochlear explants"), p3-5 C57BL/6 pups, 10^{10} genome containing (GC) particles for 48h (±5d), CMV-driven eGFP expressing transgene cassette

Cochlear explants

Source: © Lukas Landegger

In vivo injections



Most promising serotypes chosen, p0-2 C57BL/6 pups, Round window membrane approach

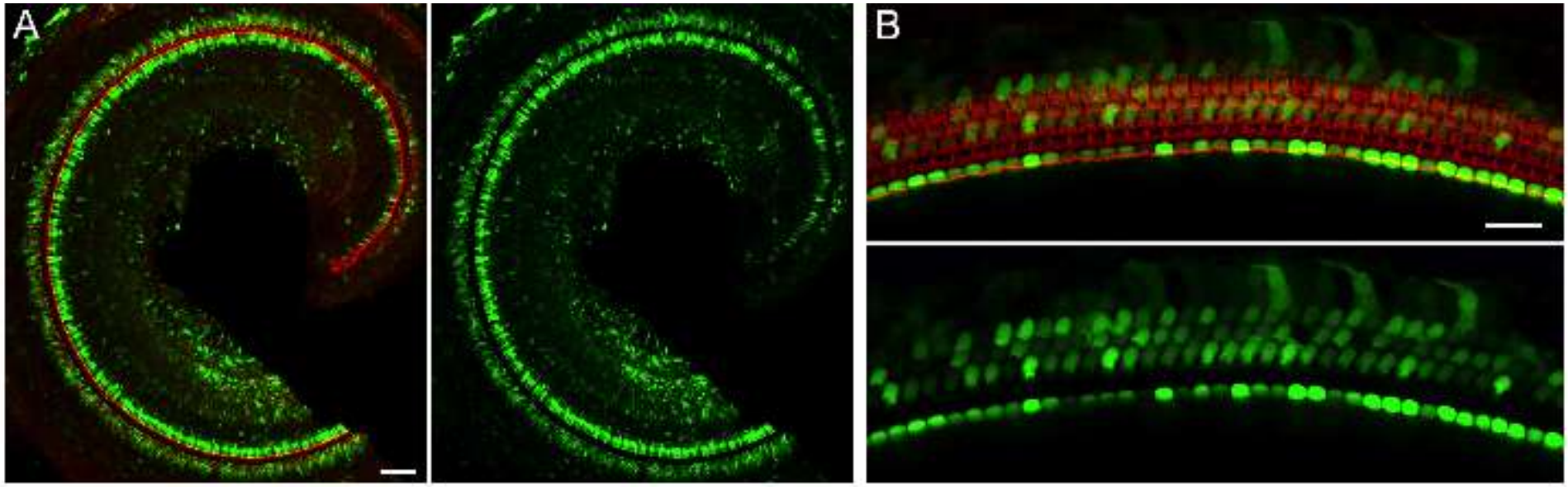
Source: Reprinted by permission from Springer Nature. Landegger et al. A synthetic AAV vector enables safe and efficient gene transfer to the mammalian inner ear. Nature Biotechnology © 2017.

Methods

- **Round window membrane (RWM) injections:** Mouse pups > glass micropipette through left RWM, application of 1 uL of the viral vector.

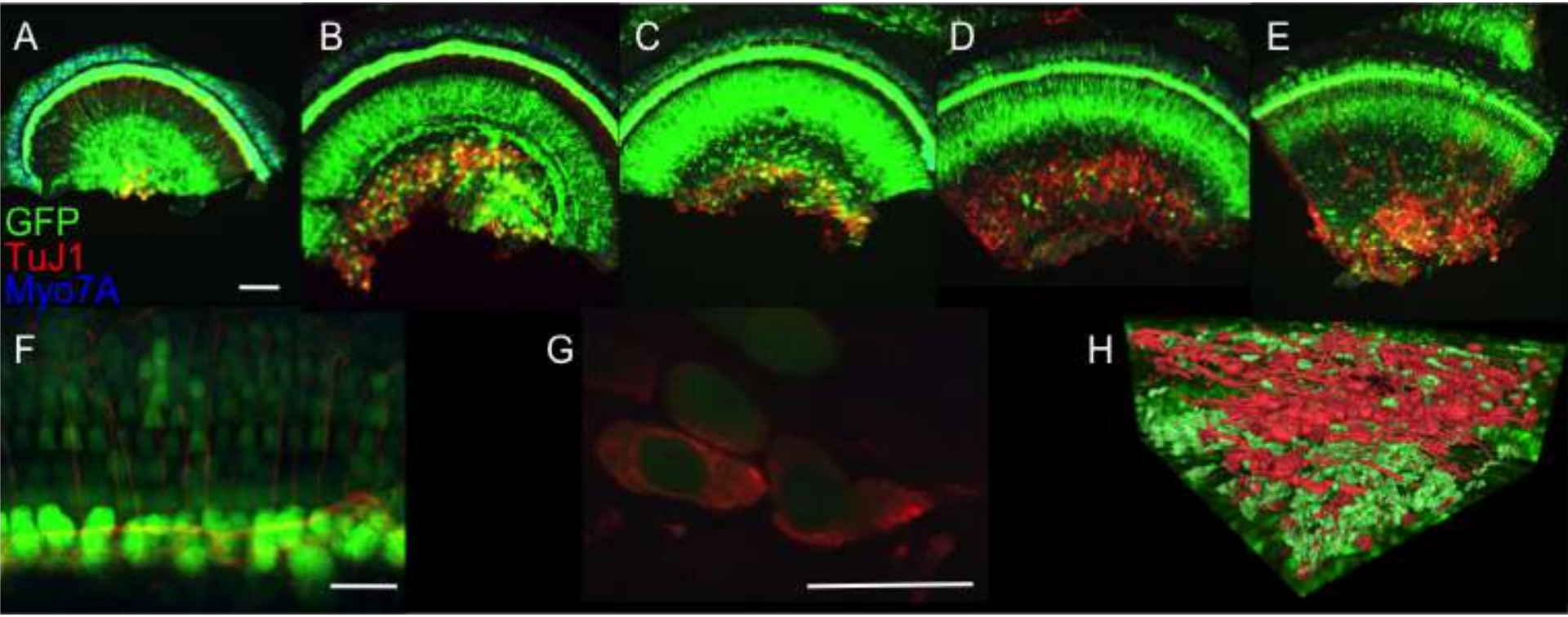
Source: © Maria Duarte

In vivo injections with Anc80



p0-2 C57BL/6 pups, RWM approach, nearly 100% of inner and outer hair cells
Follow-up of up to a month

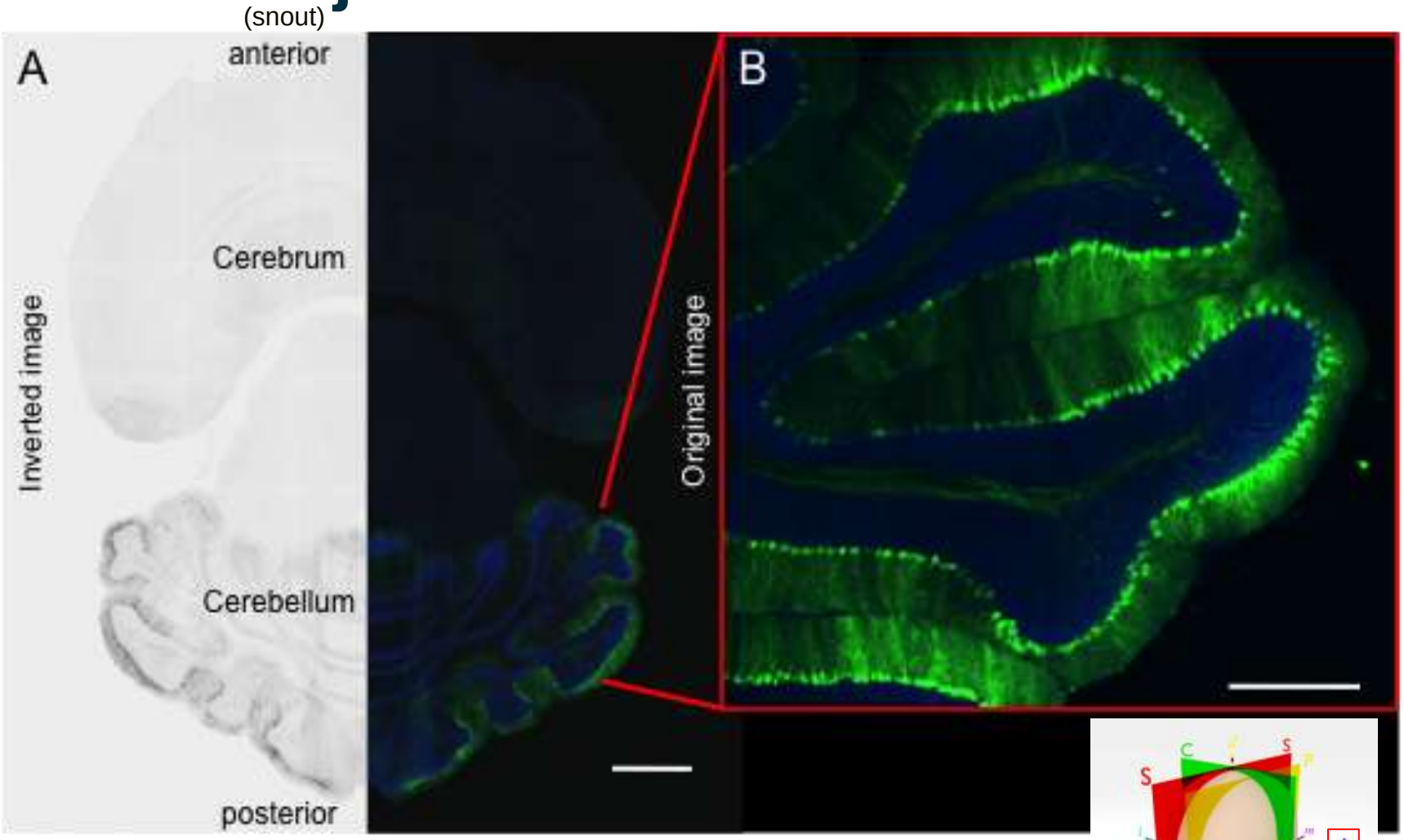
In vivo injections with Anc80



Close to 100% of GFP-positive IHCs and OHCs in the whole cochlea (A-E=from apex to base) of the injected (A/F) and also of the contralateral ear (B-E).

Source: Reprinted by permission from Springer Nature. Landegger et al. A synthetic AAV vector enables safe and efficient gene transfer to the mammalian inner ear. Nature Biotechnology © 2017.

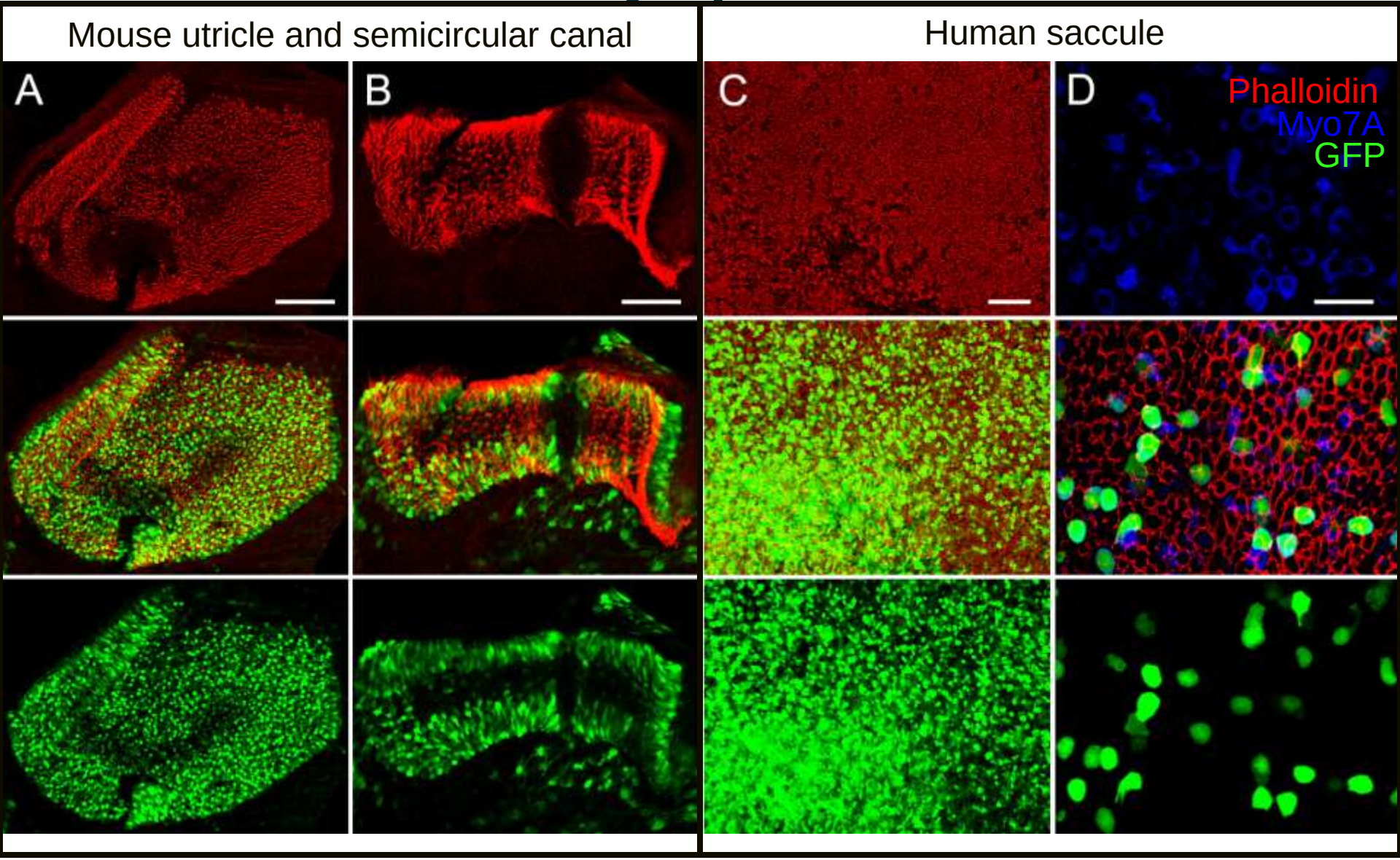
In vivo injections with Anc80



Contralateral transduction presumably via cochlear aqueduct > CSF

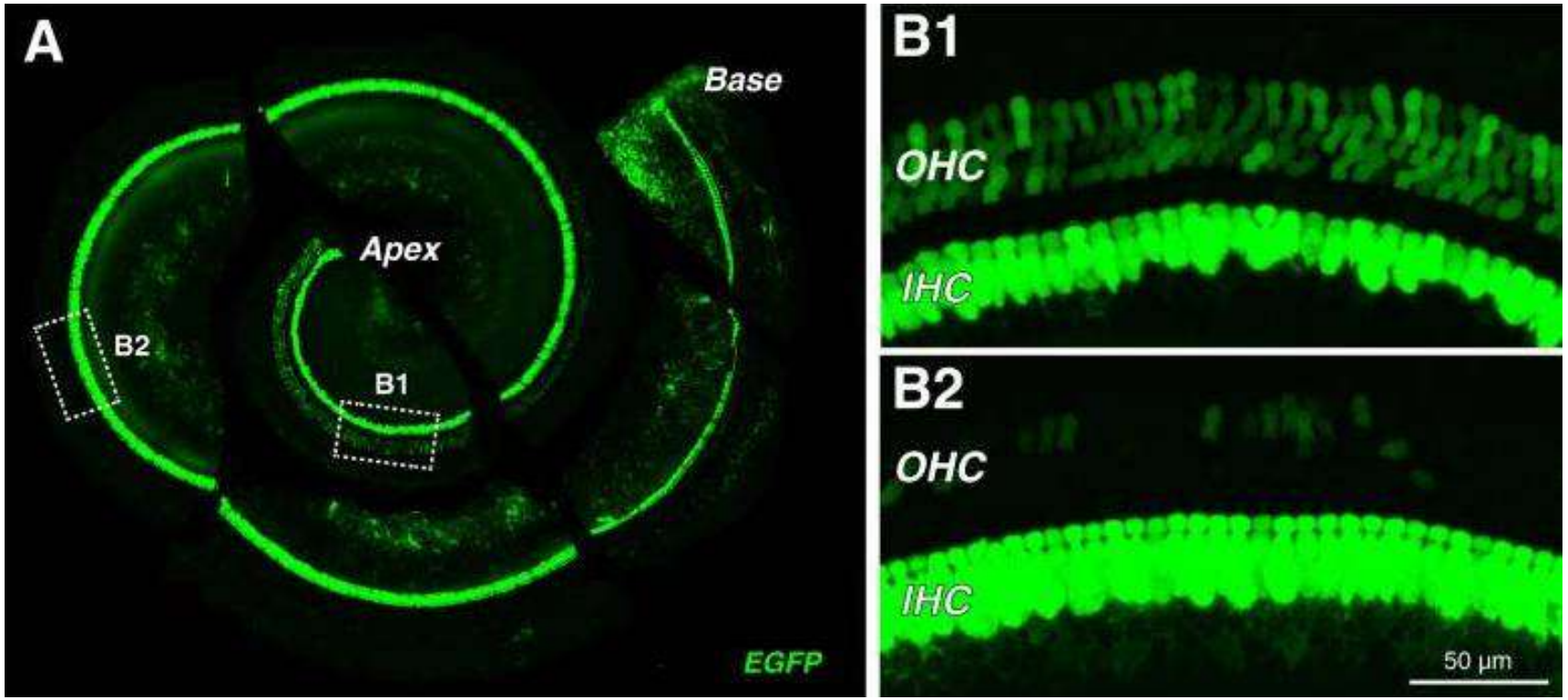
Source:
- Reprinted by permission from Springer Nature. Landegger et al. A synthetic AAV vector enables safe and efficient gene transfer to the mammalian inner ear. Nature Biotechnology © 2017.
- https://commons.wikimedia.org/wiki/File:Human_head_with_labeled_anatomic_planes.jpg

Vestibular sensory epithelia



Excellent expression also in human tissue > promising candidate for clinical studies

Anc80 injections in adult animals

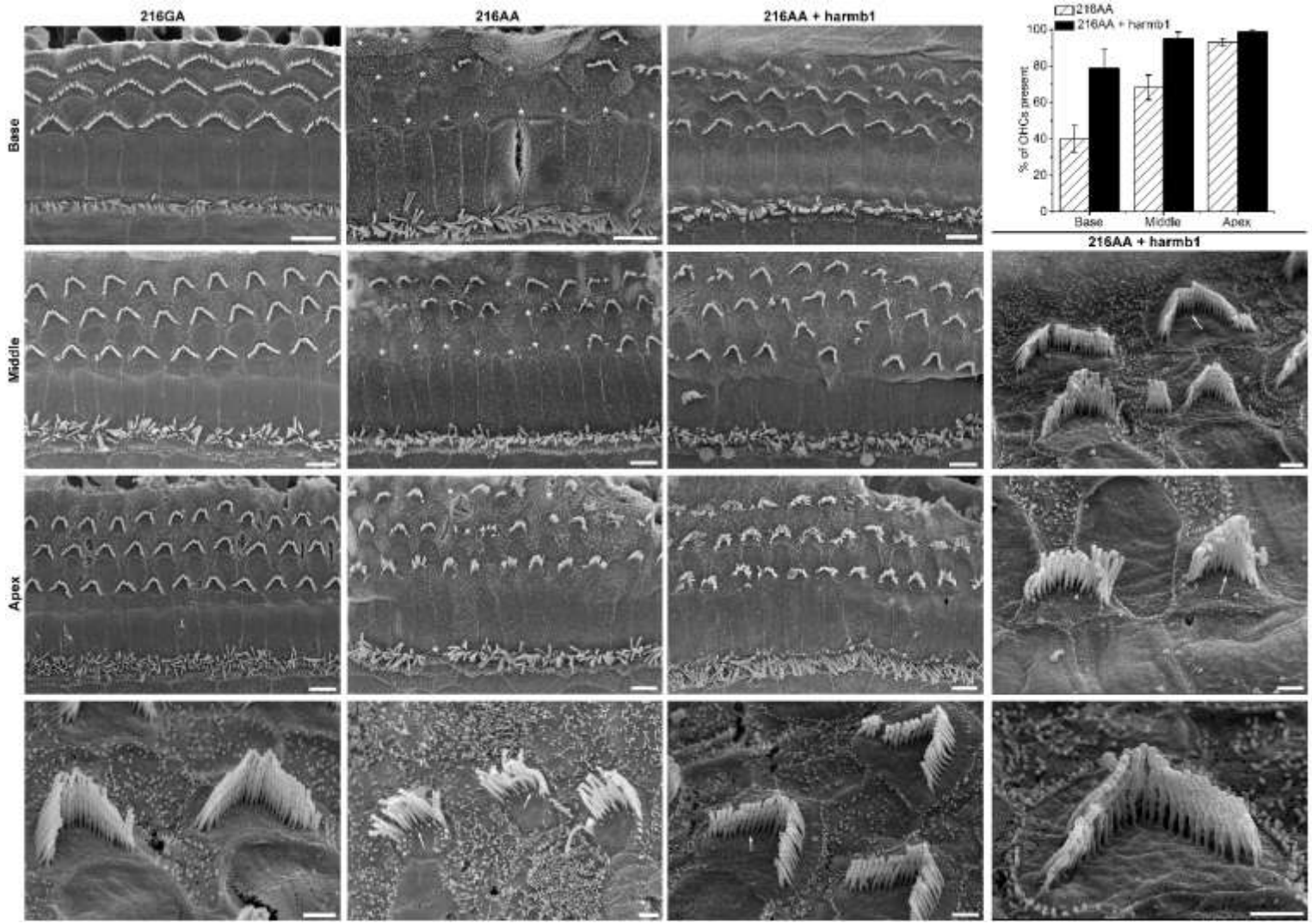


7-week-old animals injected through posterior semicircular canal
Transduction also possible in adult animals

Source: Suzuki et al. Cochlear gene therapy with ancestral AAV in adult mice: complete transduction of inner hair cells without cochlear dysfunction. Scientific Reports (open access) 2017.

Anc80 in a mouse model of Usher syndrome

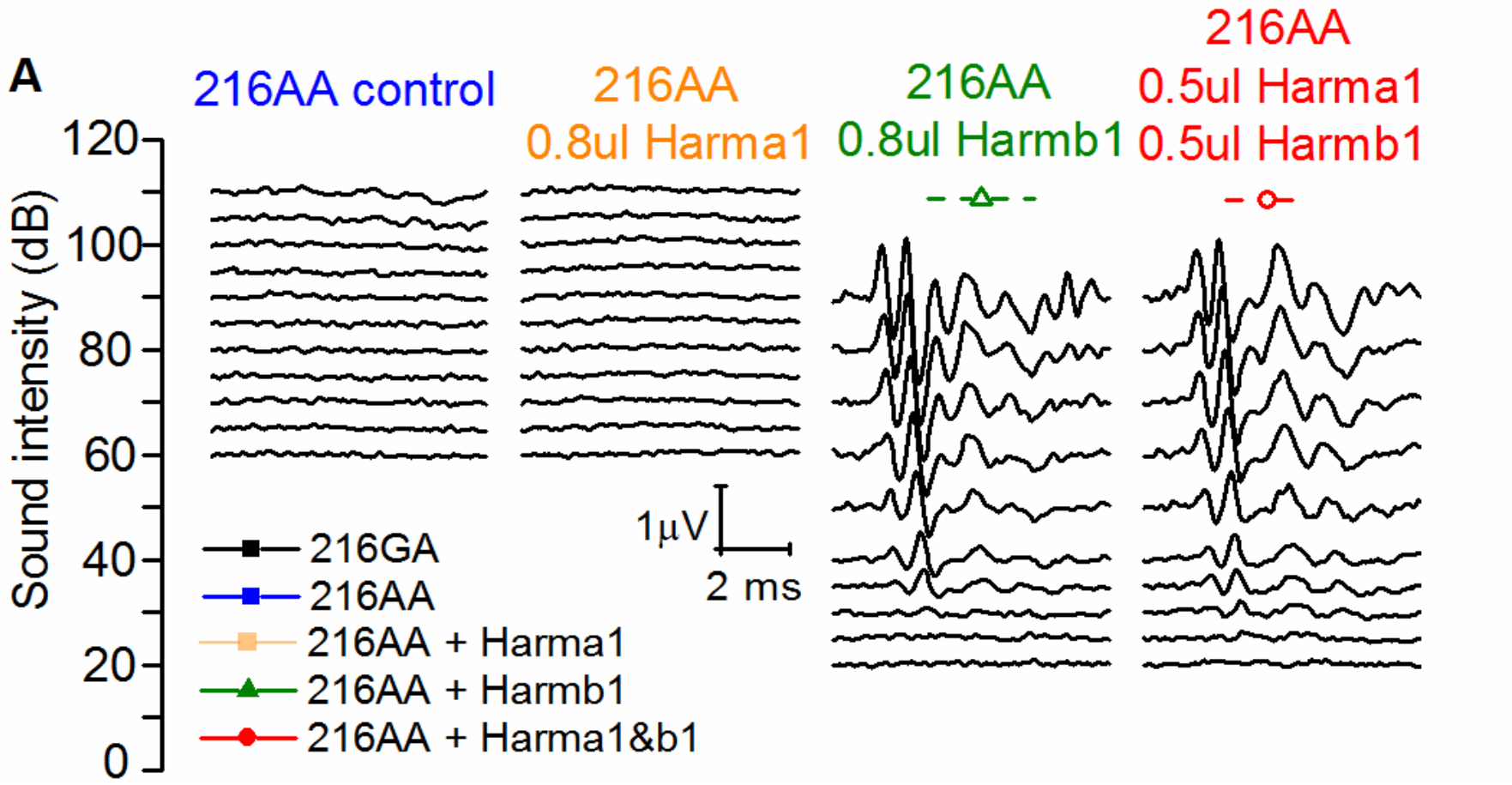
Different types, leading cause of deafblindness, recessive inheritance. Scanning electron microscopy of hair cells in mice after treatment with AAV2/Anc80.CMV.harmonin-b1: restitution/stabilization of hair cells & hair bundle morphology 6 weeks after therapy



Source: Reprinted by permission from Springer Nature. Pan et al. Gene therapy restores auditory and vestibular function in a mouse model of Usher syndrome type 1c. Nature Biotechnology © 2017.

Anc80 in a mouse model of Usher syndrome

Many additional experiments to confirm functionality of cells.
ABR measurements ("objective hearing tests"): positive in mice that were injected with the viral construct. Stable for > 6 months.



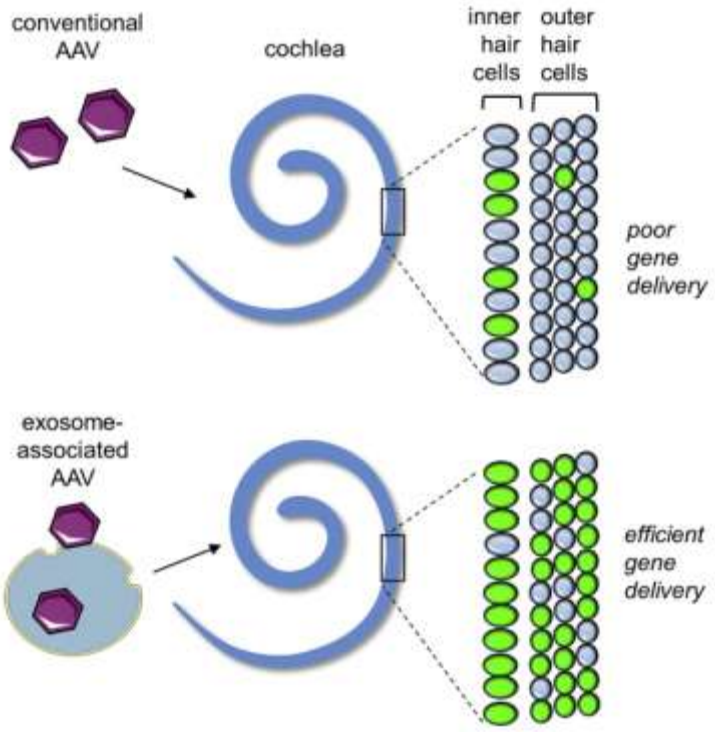
Successful therapy of USH1G in Emptoz et al. PNAS 2017 and WHRN mutation (USH2D) in Chien et al. Mol. Ther. 2016 and Isgrig et al. Mol. Ther. 2017.

Other changes to make viruses target more cells

Exosomes are small vesicles (“bubbles filled with information”) secreted from cells > communication between cells

Other cells take these exosomes up to process content

Viruses in nature have “hijacked” this approach

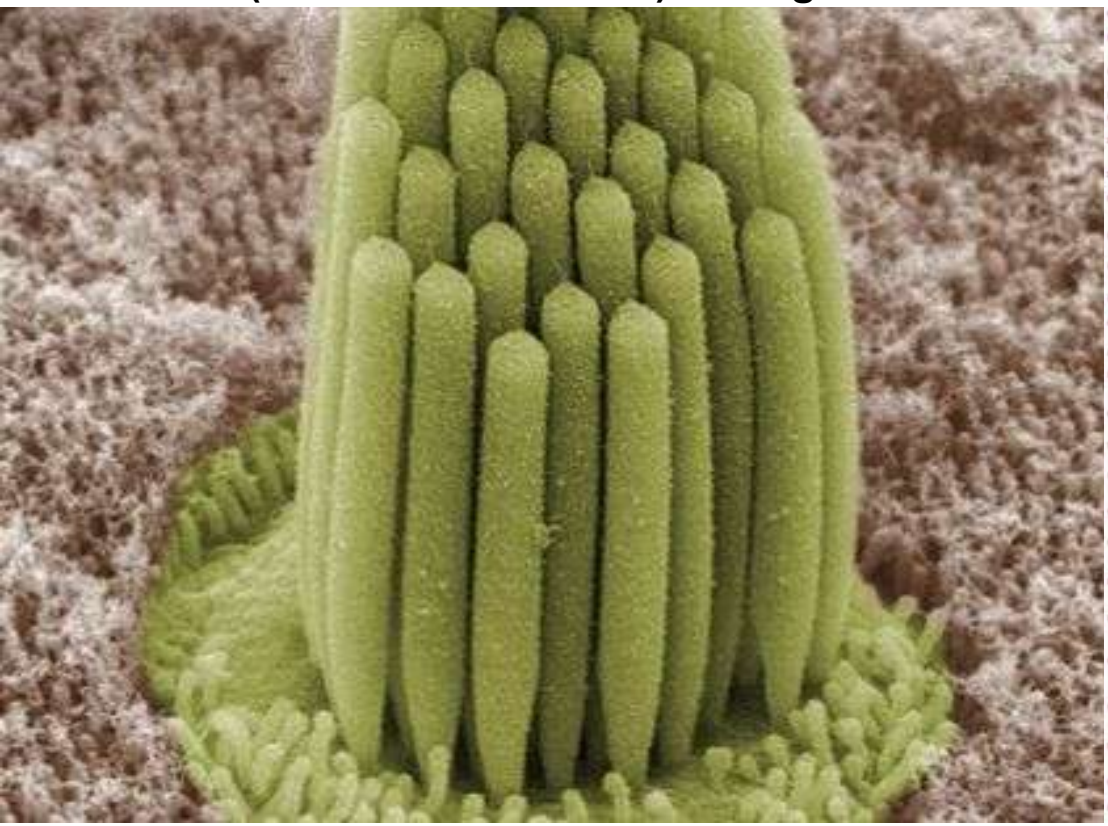


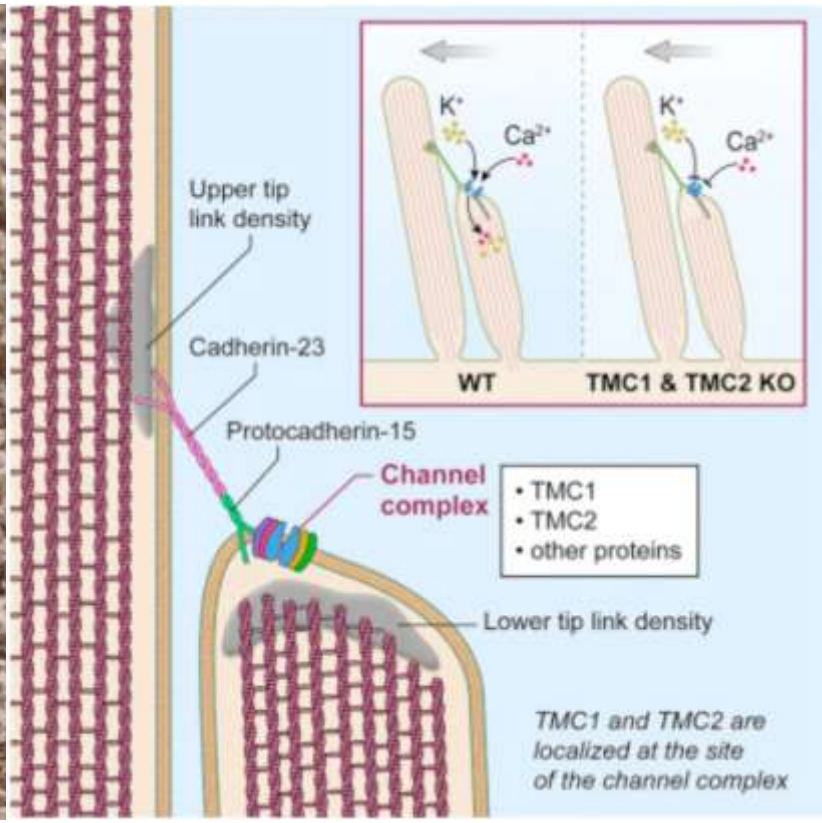
Common AAVs packaged in exosomes > more cells targeted

Source: György et al. Rescue of Hearing by Gene Delivery to Inner-Ear Hair Cells Using Exosome-Associated AAV. Molecular Therapy (open access) 2017.

Gene editing with CRISPR-Cas9

Stereocilia (“hairs” of hair cells) of frog inner ear





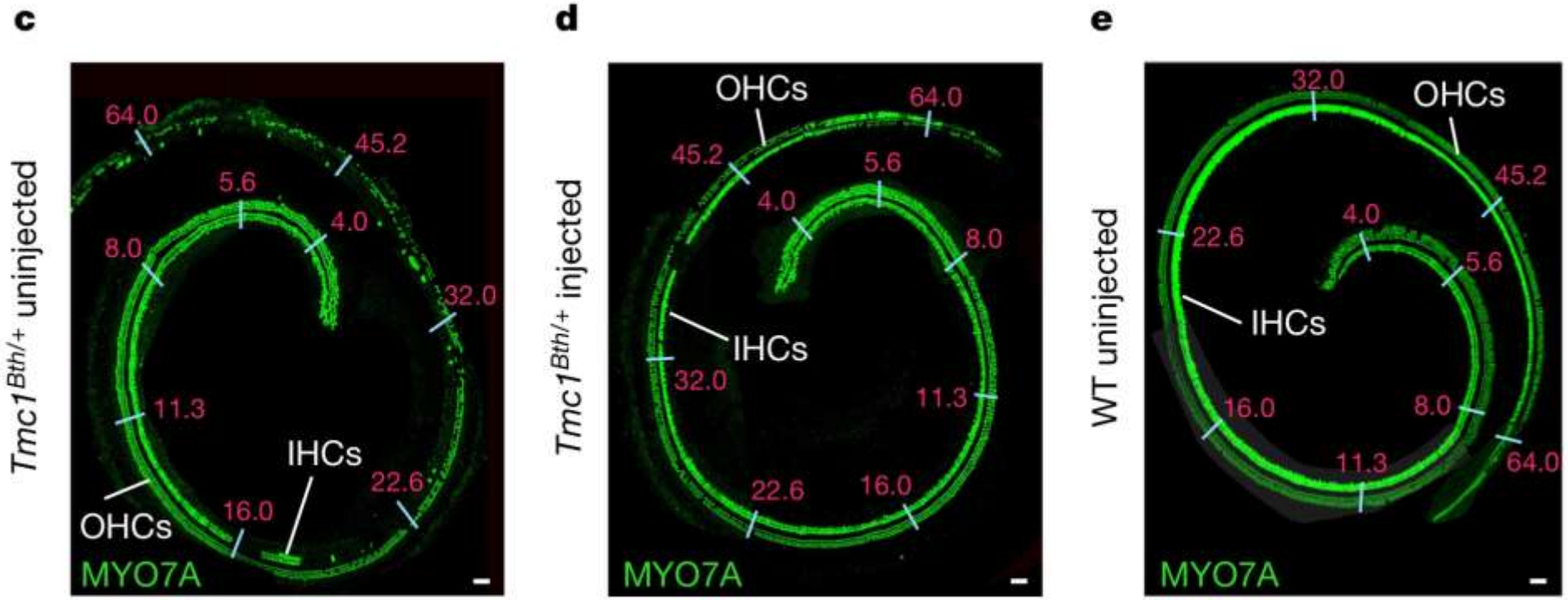
Tmc1 = transmembrane channel-like gene family 1

“Beethoven” mouse has Tmc1 mutation, leads to slow degeneration of hair cells
Also relevant in humans (described in a Chinese family)

Source:
- https://en.wikipedia.org/wiki/Stereocilia_(inner_ear)#/media/File:Stereocilia_of_frog_inner_ear.01.jpg
- Kurima et al. TMC1 and TMC2 Localize at the Site of Mechanotransduction in Mammalian Inner Ear Hair Cell Stereocilia. Cell Reports (open access) 2015.
- Vreugde et al. Beethoven, a mouse model for dominant, progressive hearing loss DFNA36. Nature Genetics © 2002.
- Zhao et al. A novel DFNA36 mutation in TMC1 orthologous to the Beethoven (Bth) mouse associated with autosomal dominant hearing loss in a Chinese family. PLoS One (open access) 2014.

Gene editing with CRISPR-Cas9

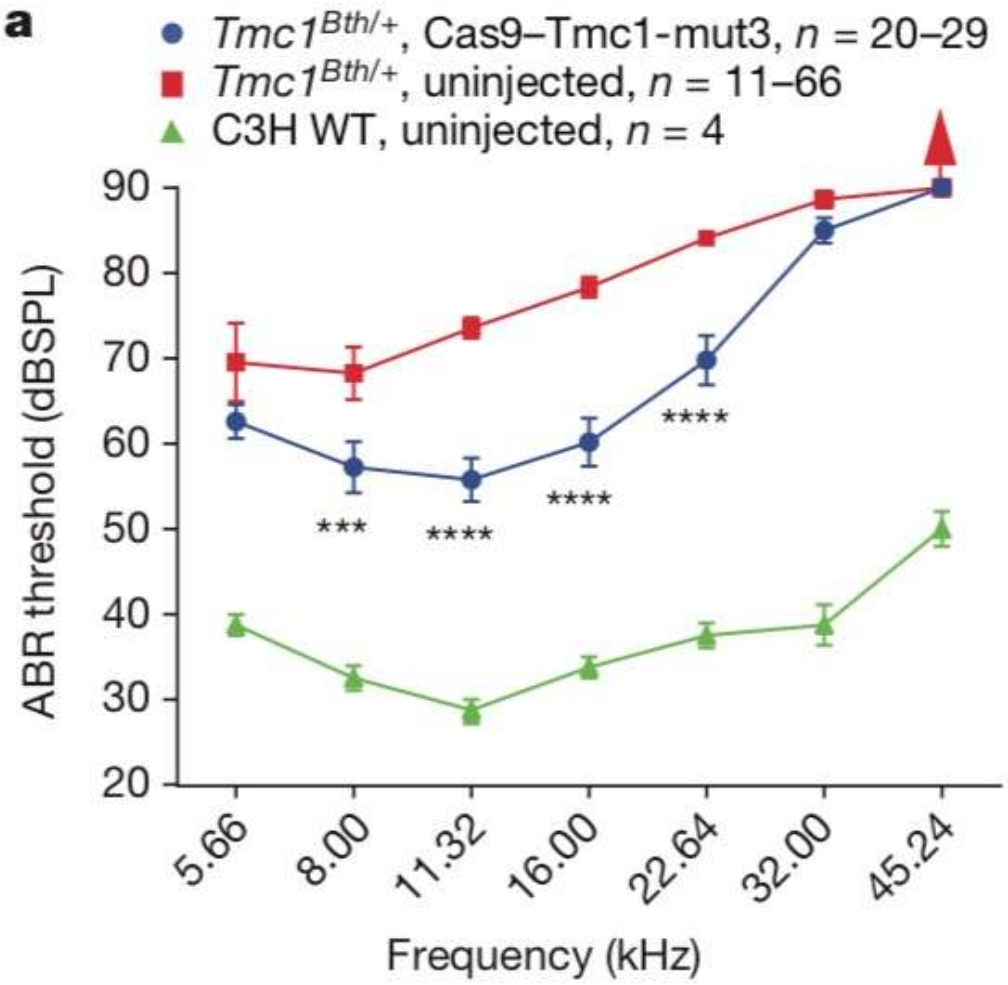
“Beethoven” mouse pups injected (or controls)
Protein-RNA complex delivery (no virus) targets affected copy of gene without influencing the other gene



Source: Reprinted by permission from Springer Nature. Gao et al. Treatment of autosomal dominant hearing loss by in vivo delivery of genome editing agents. Nature © 2017.

Gene editing with CRISPR-Cas9

“Objective hearing test” results



Suggests that gene disruption might be a potential strategy for the treatment of some forms of dominant hearing loss

Source: Reprinted by permission from Springer Nature. Gao et al. Treatment of autosomal dominant hearing loss by in vivo delivery of genome editing agents. Nature © 2017.

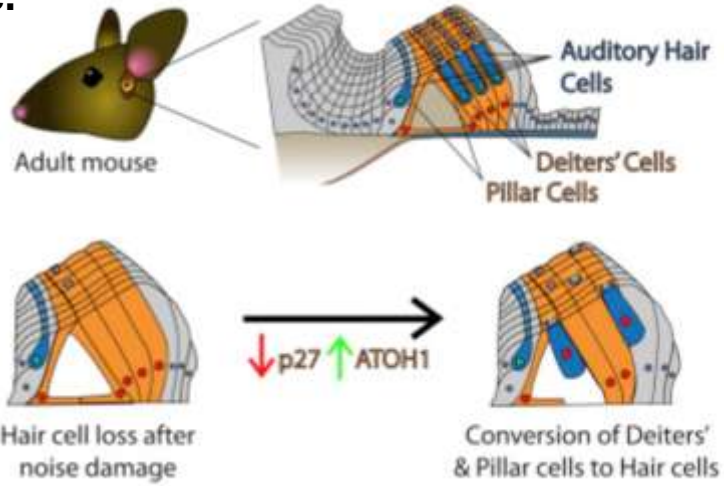
Primarily stabilization, what about restoration?

Genetic hearing loss vs. age-related hearing loss

Gene therapy, molecular therapy, and stem-cell therapy (overlap between all three fields)

Important targets to convert supporting cells into hair cells established in mouse pups: Wnt/Notch signaling, $p27^{Kip1}$, GATA3, ATOH1, POU4F3, etc.

"Co-activation of GATA3 or POU4F3 and ATOH1 promoted conversion of supporting cells to hair cells in adult mice. Activation of POU4F3 alone also converted mature supporting cells to hair cells in vivo."



Only clinical study at the moment targets ATOH1 (with adenovirus that transiently gets into hair cells)

Source:
- Chai et al. Wnt signaling induces proliferation of sensory precursors in the postnatal mouse cochlea. Proceedings of the National Academy of Sciences of the United States of America (open access) 2012.
- Bramhall et al. *Lgr5*-Positive Supporting Cells Generate New Hair Cells in the Postnatal Cochlea. Stem Cell Reports (open access) 2014.
- Walters et al. In Vivo Interplay between $p27^{Kip1}$, GATA3, ATOH1, and POU4F3 Converts Non-sensory Cells to Hair Cells in Adult Mice. Cell Reports (open access) 2017.
- clinicaltrials.gov: Safety, Tolerability and Efficacy for CGF166 in Patients With Unilateral or Bilateral Severe-to-profound Hearing Loss. NCT02132130.

Summary

Gene therapy potential solution to restore “perfect hearing” in millions of people affected by (hereditary) hearing loss

Anc80 potent viral vector for cochlear gene delivery

Several animal mouse model(s) could be rescued
(best results for major deafness genes at the moment with Anc80)

Gene editing with CRISPR-Cas9 feasible

Outlook – hurdles on the way to the clinic

Studies in large animal models (dosing, safety, etc.) > last step prior to starting multiple independent human experiments > injection of sufficient volumes into inner ears of rhesus monkeys without worsening of objective hearing (Botond Roska: vector result correlation <30% between mice and humans vs. >75% between monkeys and humans)

Specific targeting of cells with different promoters (cerebellum?)

Size limitation for AAVs (ca. 4.7 kilobases – dual vectors, trans-splicing etc.)

Time window to treat diseases (degeneration of cells)? Therapy in the womb? Treatment of age-related hearing loss?

Most (gene therapy) hearing research labs have now ordered Anc80 > hope to accelerate translational research

For antisense applications, optogenetics etc. definitely more research necessary

Source: Dai et al. Rhesus Cochlear and Vestibular Functions Are Preserved After Inner Ear Injection of Saline Volume Sufficient for Gene Therapy Delivery. Journal of the Association for Research in Otolaryngology © 2017.

Collaborations

Stankovic Lab (Mass. Eye & Ear/Harvard Medical School, Boston)

Brown/Lee Lab (Mass. Eye & Ear/Harvard Medical School, Boston)

Sewell Lab (Mass. Eye & Ear/Harvard Medical School, Boston)

Vandenberghe Lab (Schepens/Harvard Medical School, Boston)

Holt Lab (Children's hospital/Harvard Medical School, Boston)

Géléoc Lab (Children's hospital/Harvard Medical School, Boston)

Forge Lab (University College London, London)

Funding

National Institutes of Health

5DP1EY023177

NIDCD
National Institute on Deafness and Other Communication Disorders

T32DC00038
R01DC015824

DEPARTMENT OF DEFENSE
UNITED STATES OF AMERICA

W81XWH-15-1-0472

Nancy Sayles Day Foundation

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Lauer Tinnitus Research Center

FOUNDATION FIGHTING BLINDNESS

GROUSBECK FAMILY FOUNDATION

Jeff and Kimberly Barber Gene Therapy Research Fund

Ush2A Consortium

Support our research

http://teameyeandear.org/lukaslandegger

(donations accepted until April 30, 2018)