Gene Therapy for Usher Syndrome Type 1F: Engineering mini-PCDH15s for Viral Delivery

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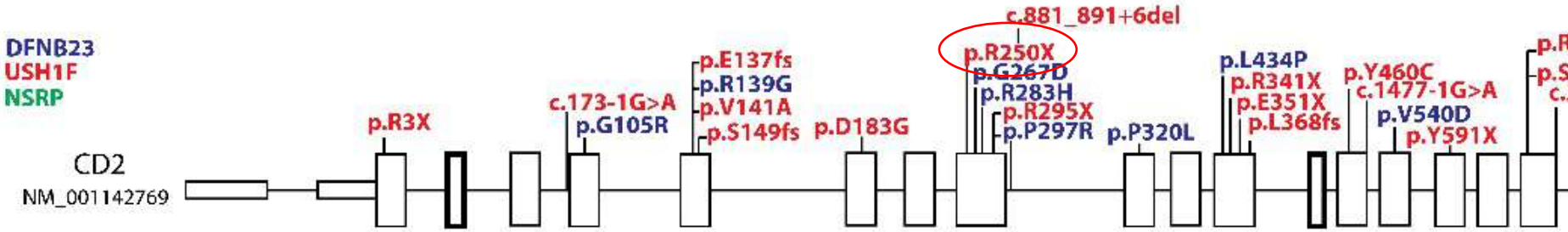
Usher syndrome type 1 (USH1) is the most severe form of inherited deafness and blindness in humans, with a prevalence from 1/6,000 to 1/10,000.

Gene Type	Clinical Type	Phenotype		
		HEARING	VISION	BALANCE
USH1B (myosin VIIa) USH1C (harmonin) USH1D (cadherin 23) USH1F (protocadherin 15) USH1G (Sans)	**Type 1**	Profound deafness from birth	Decreased night vision before age 10	Balance problems from birth
USH2A (Usherin) USH2C (VLGR1) USH2D (Whirlin)	**Type 2**	Moderate to severe hearing loss from birth	Decreased night vision begins in late childhood or teens	Normal
USH3A (Clarin-1)	**Type 3**	Normal at birth; progressive loss in childhood or early teens	Varies in severity; night vision problems often begin in teens	Normal to near-normal, chance of later problems

In the United States about 40-60 children are born each year with Usher 1F; 2,500-3,500 total patients.

Perhaps 10,000-12,000 Usher 1F patients worldwide.

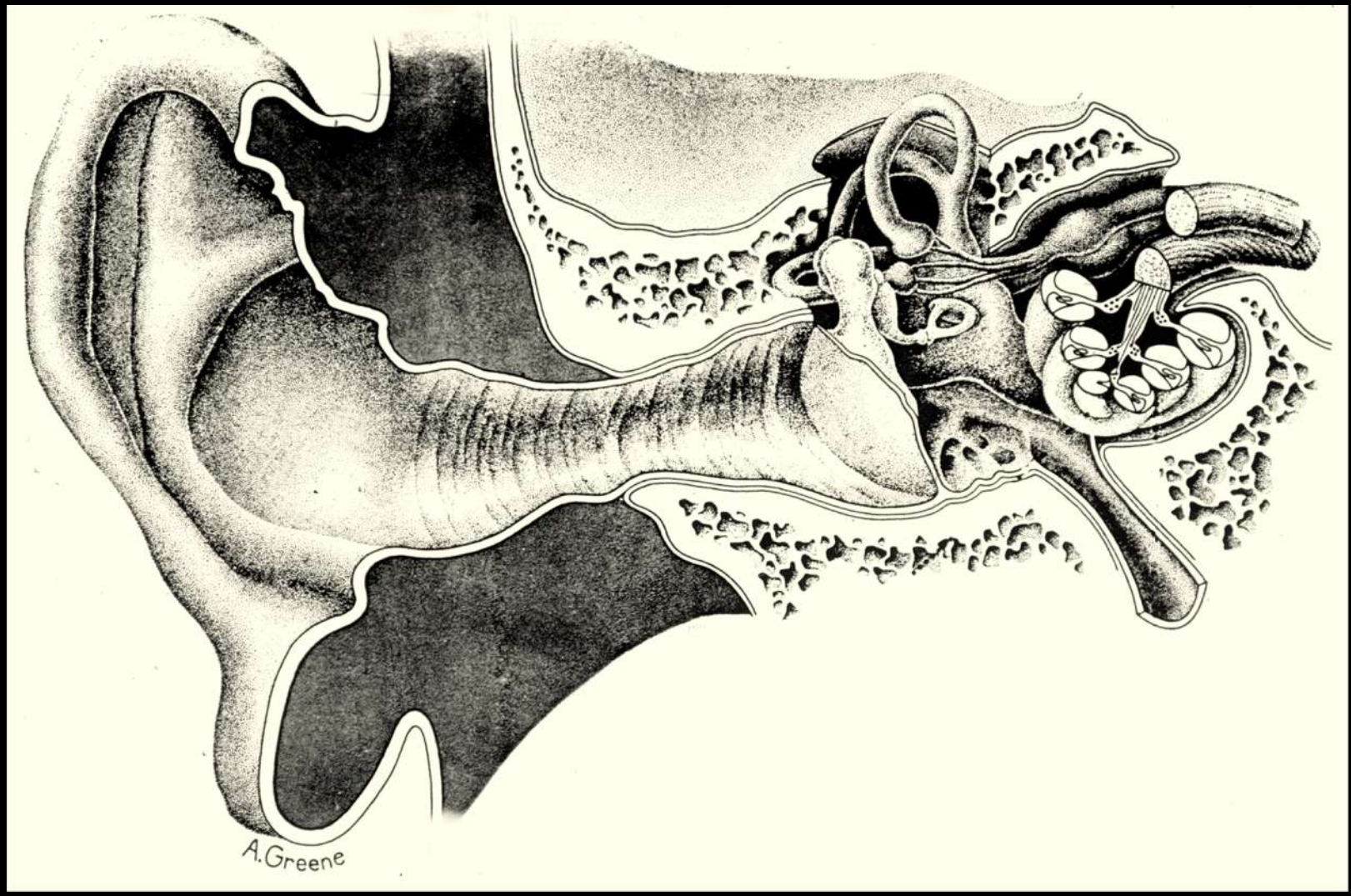
PCDH15 Mutations in Usher 1F



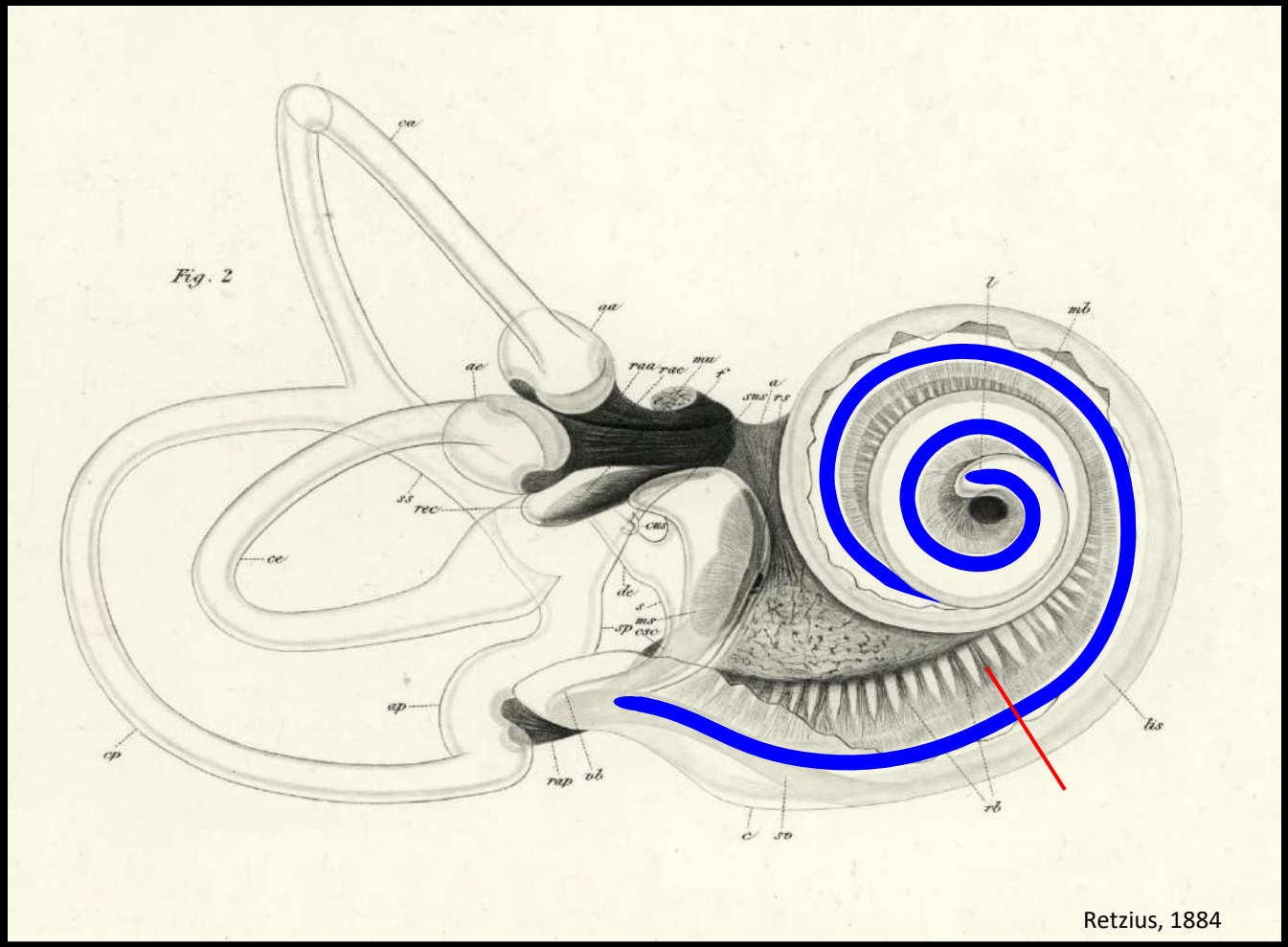
deafness and blindness
just deafness

There may be greater functional demands on PCDH15 in the ear than in the eye, so a therapy that restores function in the ear may be promising in the eye.

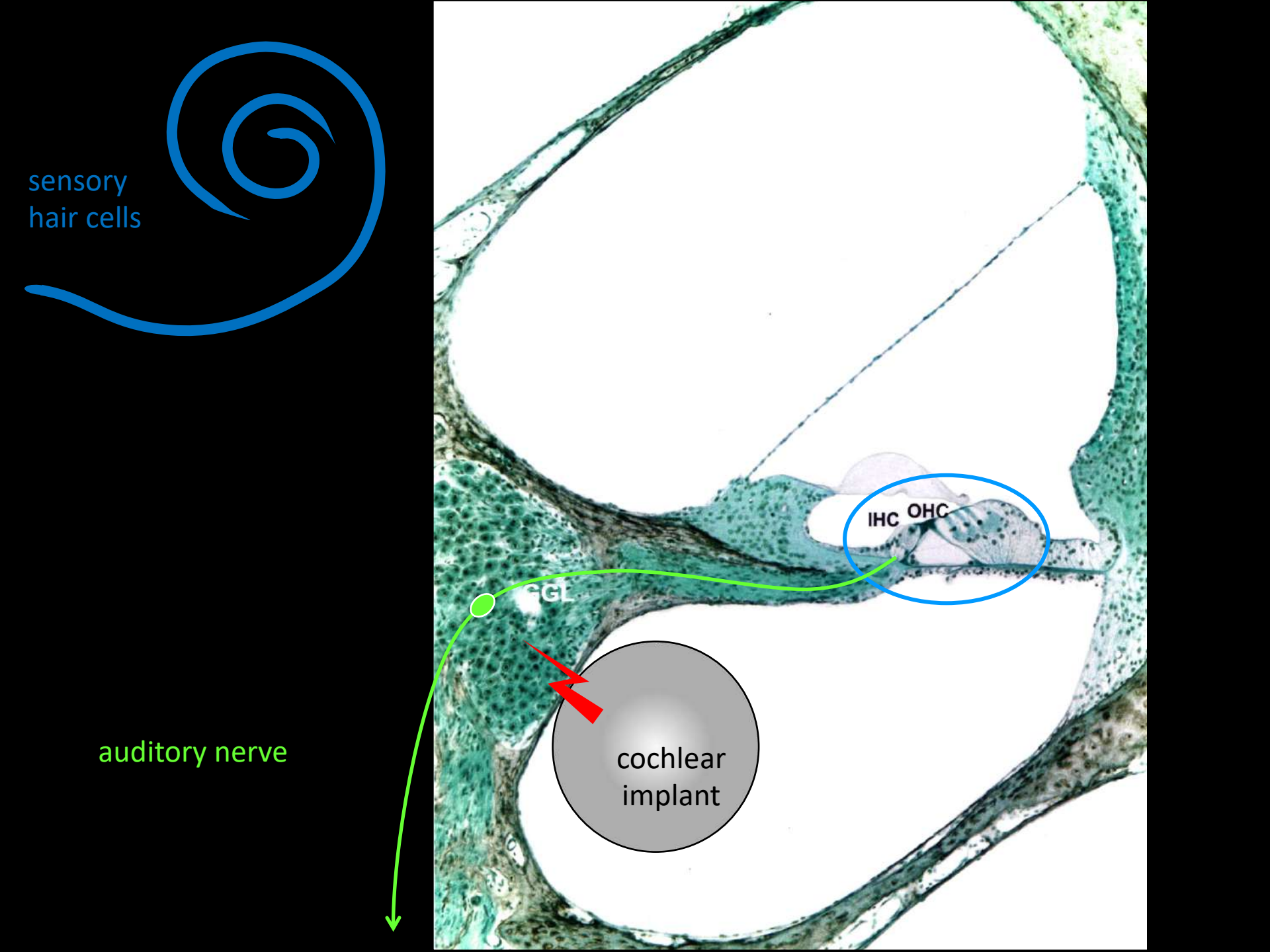
What is protocadherin-15?

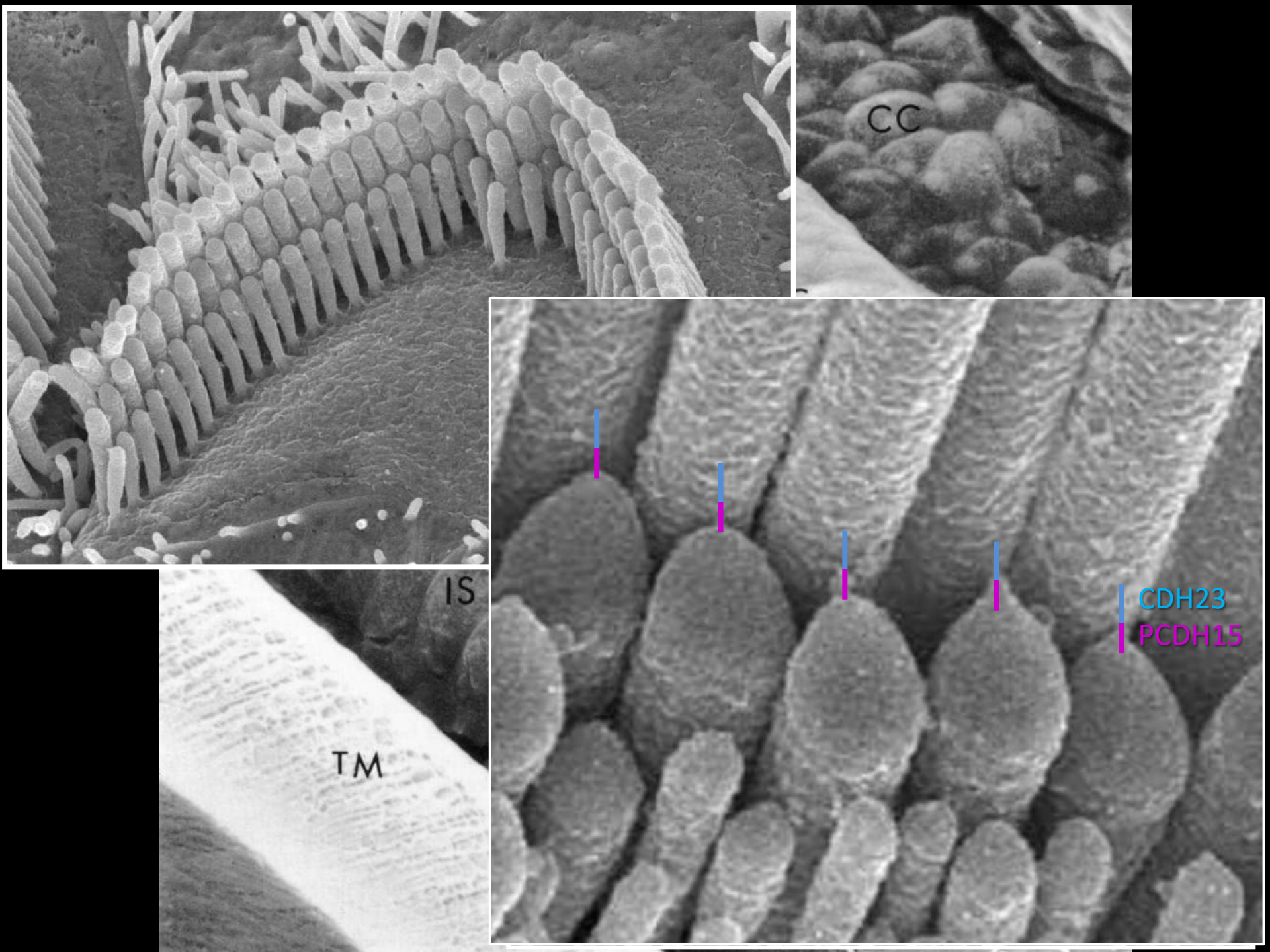


What is protocadherin-15?

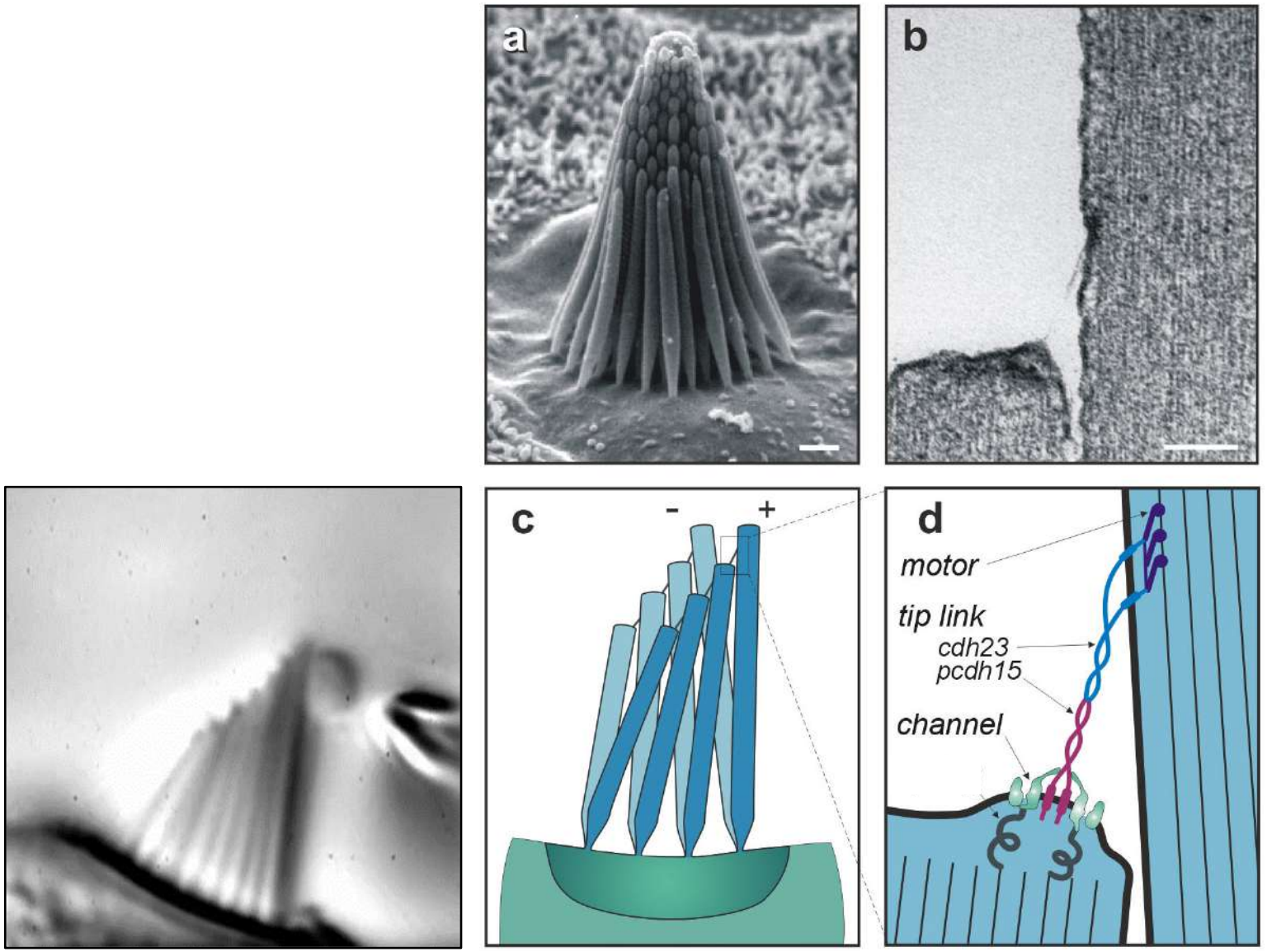


Retzius, 1884

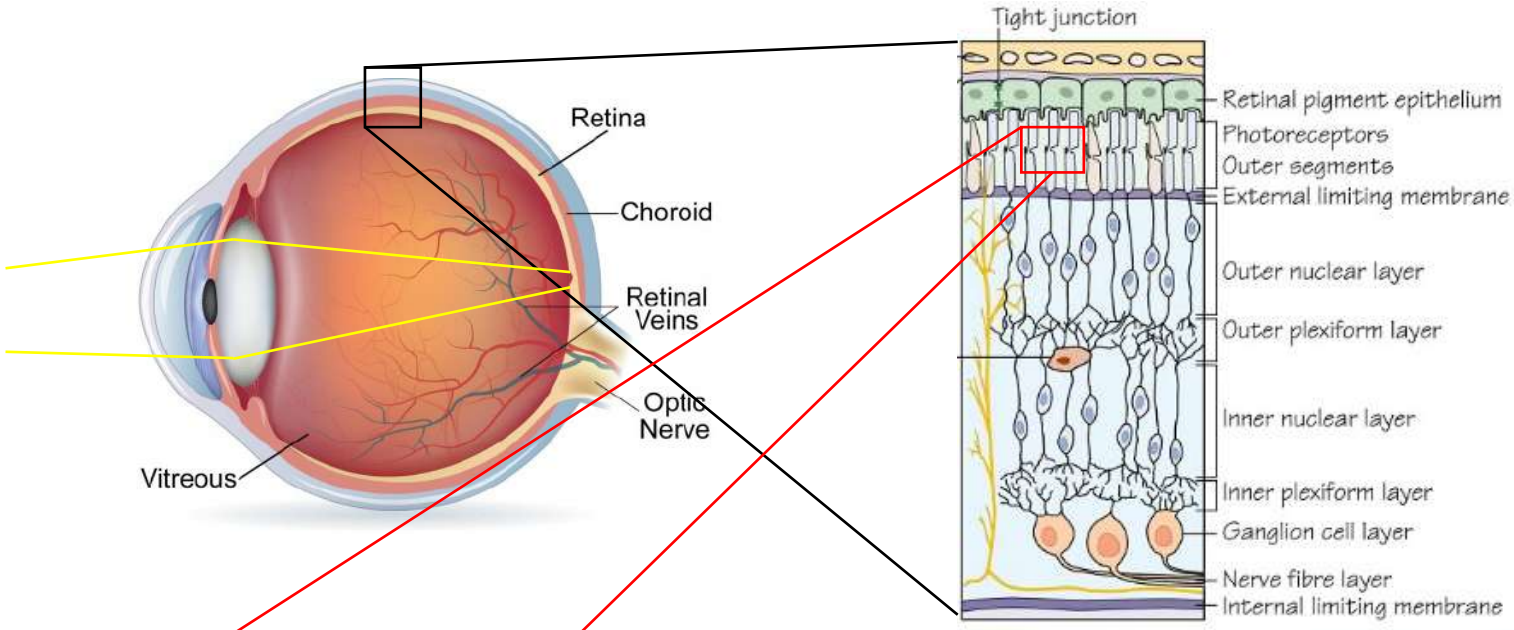

sensory
hair cells
IHC
OHC
GGL
cochlear
implant
auditory nerve

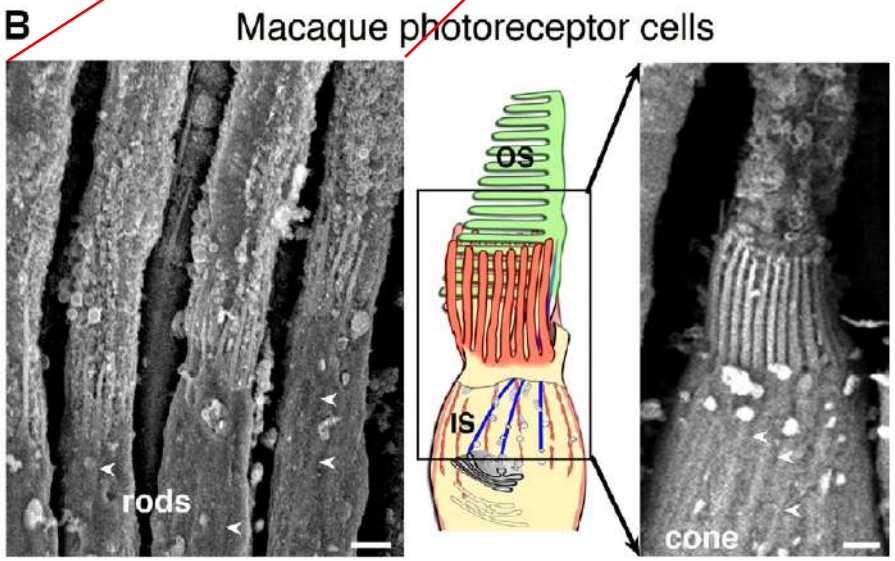

CC
IS
TM
CDH23
PCDH15

The hair-cell transduction mechanotransduction complex



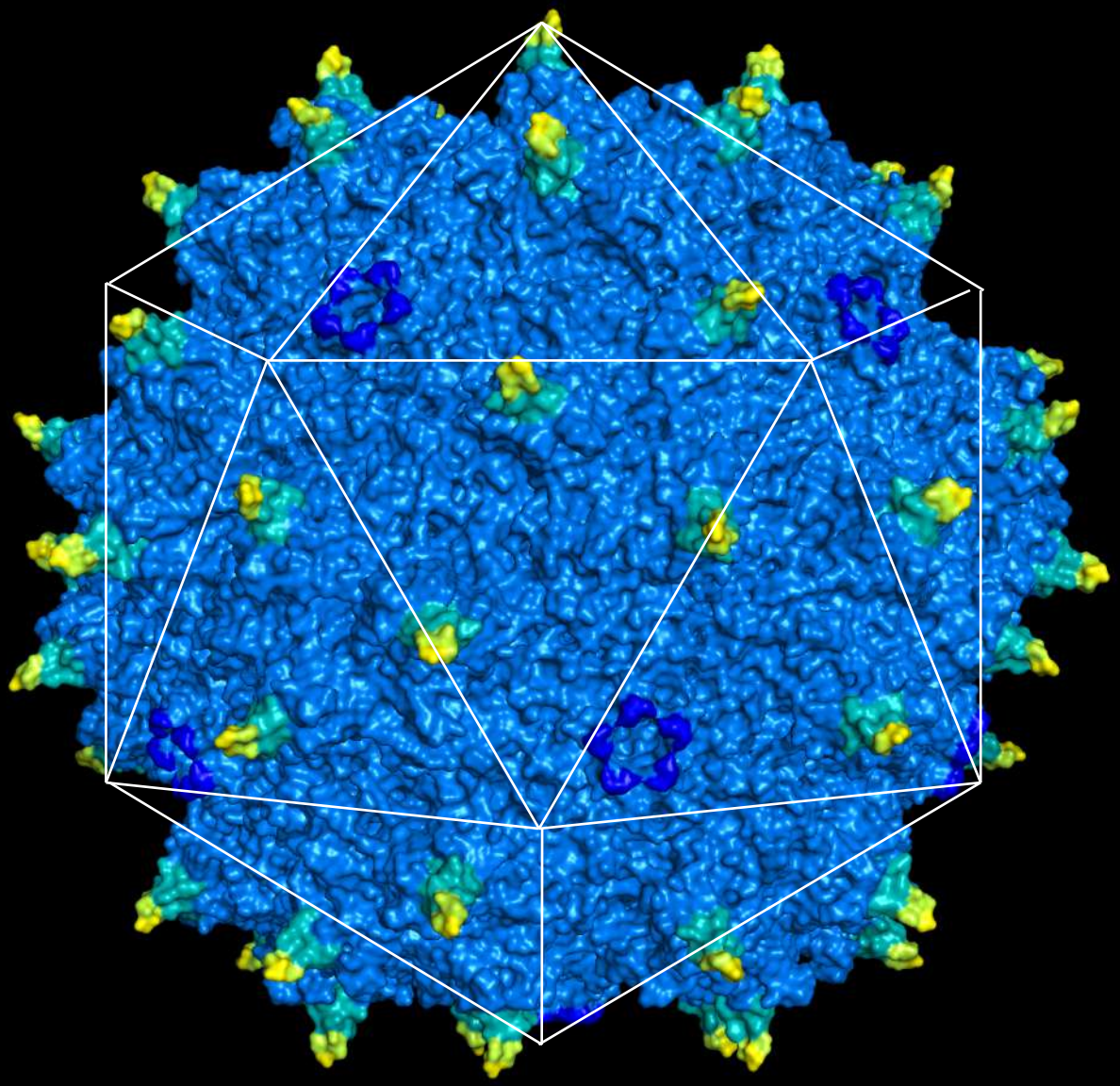
PCDH15 is also in retinal photoreceptors





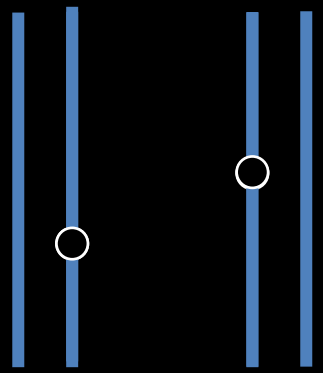
Sahly et al., 2012

Adeno-associated virus (AAV) can deliver therapeutic genes



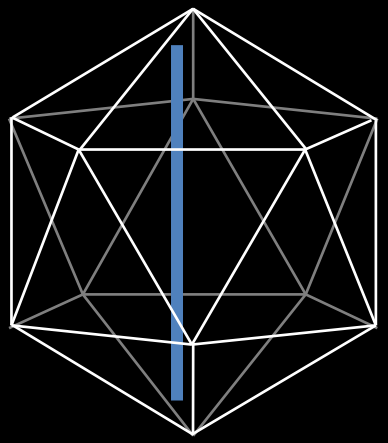
Gene Addition

recessive, small (<4.7 kb)



PCDH15 coding sequence is ~ 6 kb

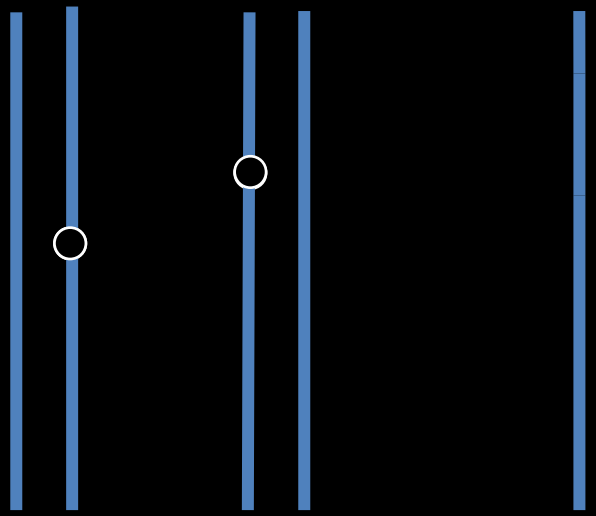
Too big for AAV!



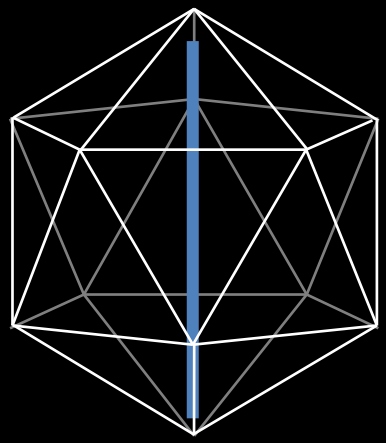
AAV

Gene Addition for Large Genes

recessive, large (>4.7 kb)



PCDH15 coding sequence is ~ 6 kb

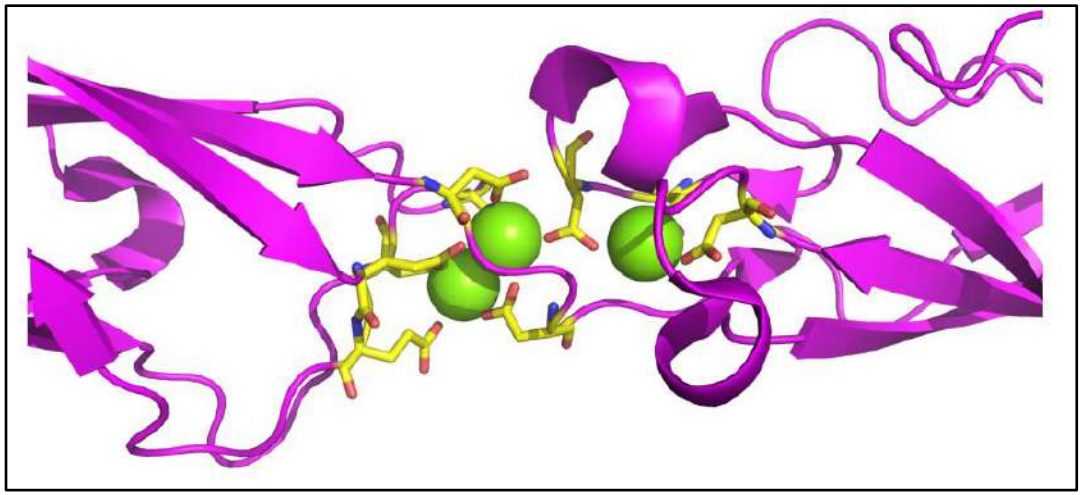


Gene Therapy for Usher 1F: Gene Addition

Problem: The coding sequence for PCDH15 is too large to fit in AAVs.

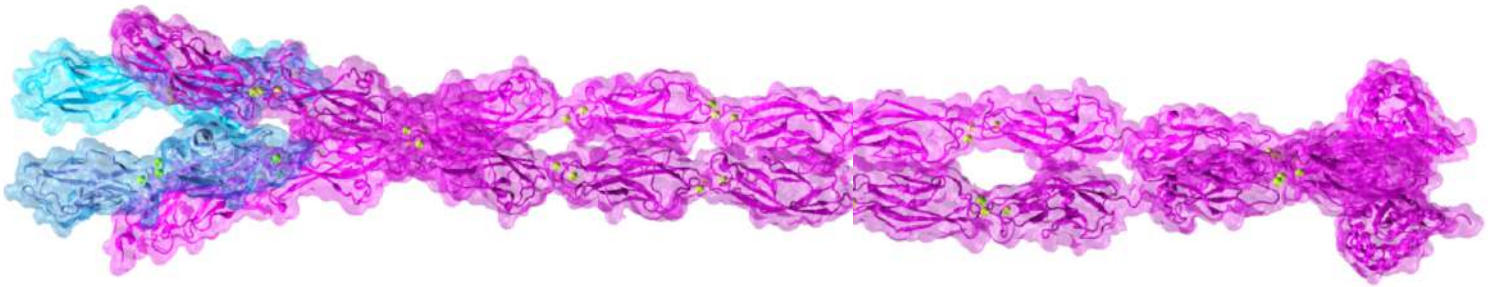
Strategy: Remove unnecessary segments to make “mini-PCDH15.”

Marcos Sotomayor



The position of every one of about 20,000 atoms in PCDH15 has been solved.

Delivery of mini-PCDH15s to cochlear hair cells and photoreceptors



√ Do mini-PCDH15 proteins behave properly in a test tube?

Is there a good animal model?

How can mini-PCDH15 coding sequences be delivered to the inner ear?

How do we assay successful rescue of function?

Delivery of mini-PCDH15s to cochlear hair cells and photoreceptors

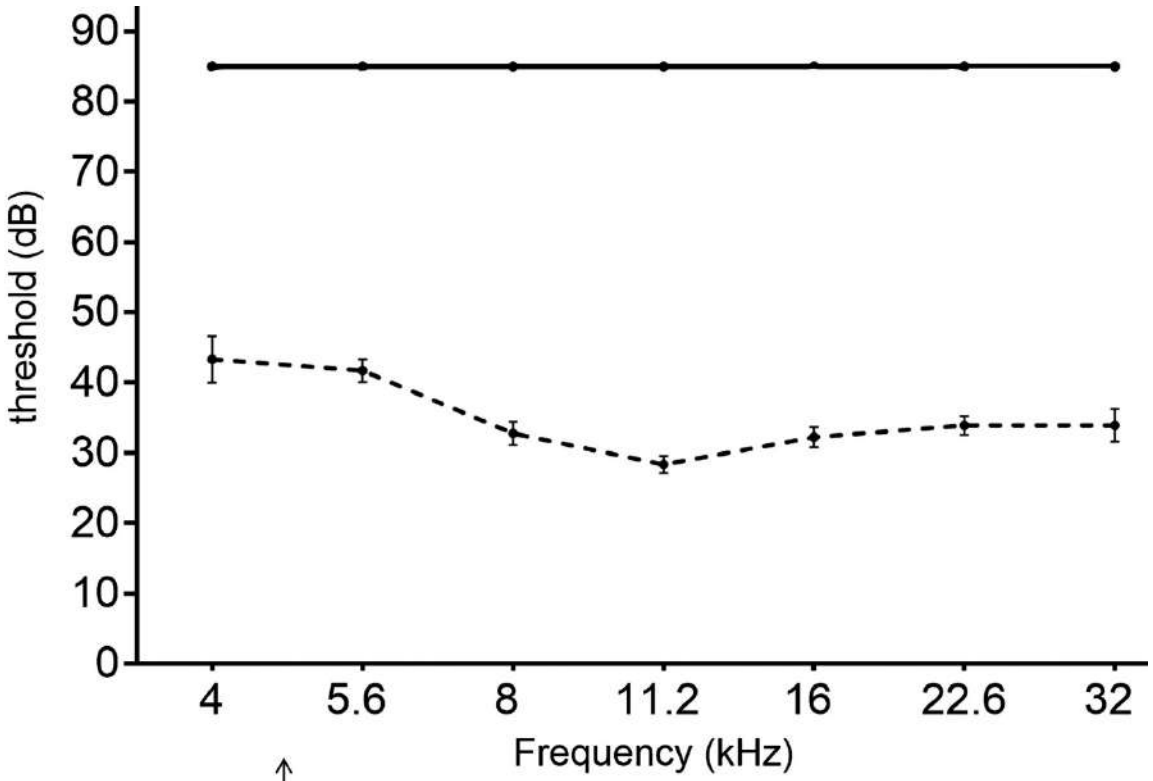
Do mini-PCDH15 constructs behave properly in vitro?

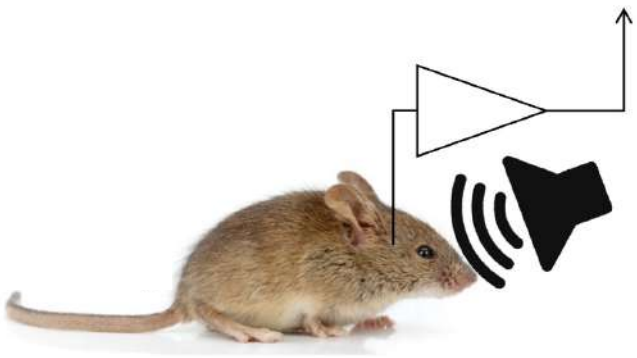
Is there a good animal model? *We made a mouse line that lacks PCDH15 in the inner ear*

How can mini-PCDH15 coding sequences be delivered to the inner ear?

How do we assay successful rescue of function?

Pcdh15 knockout mice are completely deaf





adult (P35)

Delivery of mini-PCDH15s to cochlear hair cells and photoreceptors

√ Do mini-PCDH15 constructs behave properly in vitro?

√ Is there a good animal model? Mouse line that lacks PCDH15 in the inner ear

How can mini-PCDH15 coding sequences be delivered to the inner ear?

How do we assay successful rescue of function?

Delivery of mini-PCDH15s to cochlear hair cells and photoreceptors

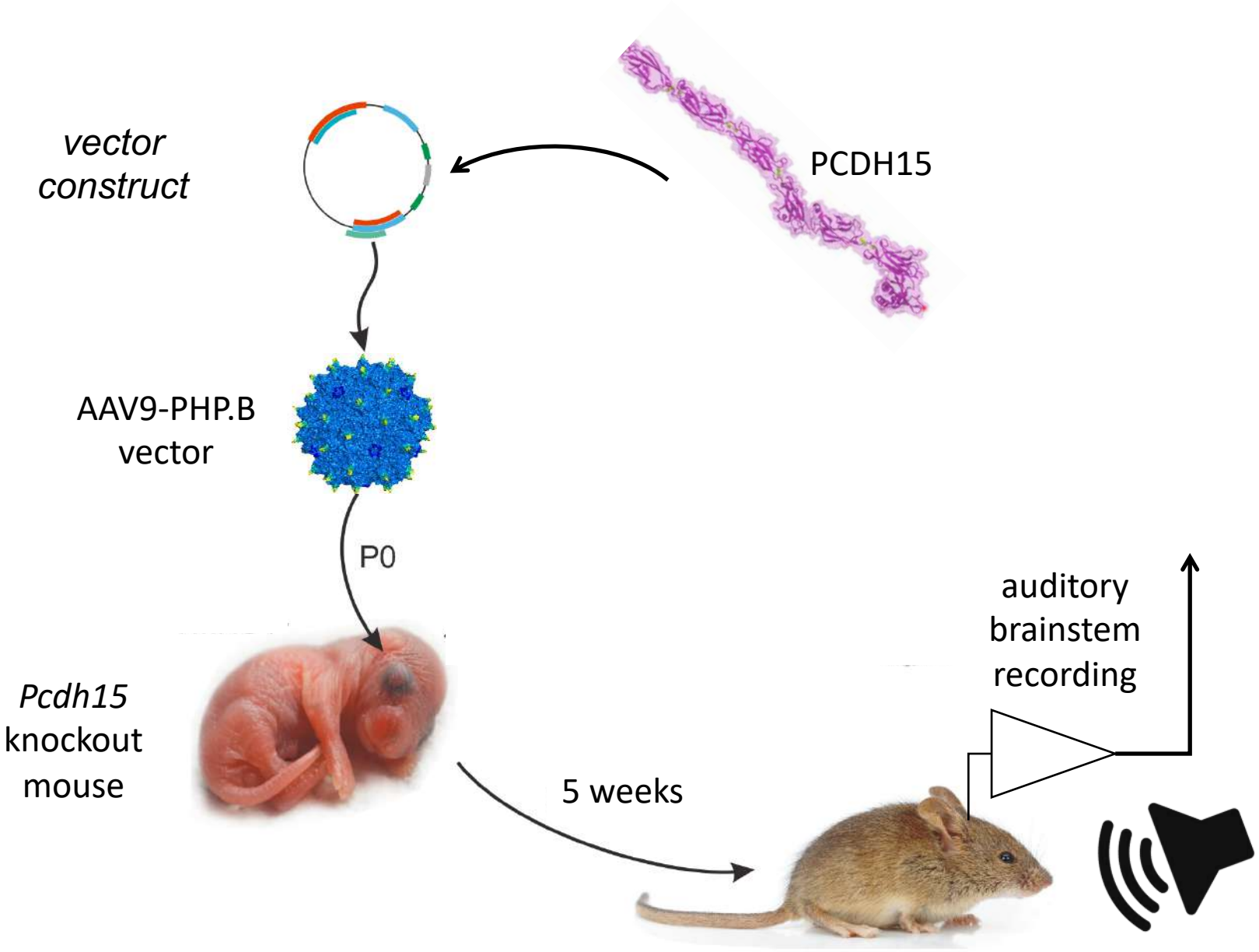
√ Do mini-PCDH15 constructs behave properly in vitro?

√ Is there a good animal model?

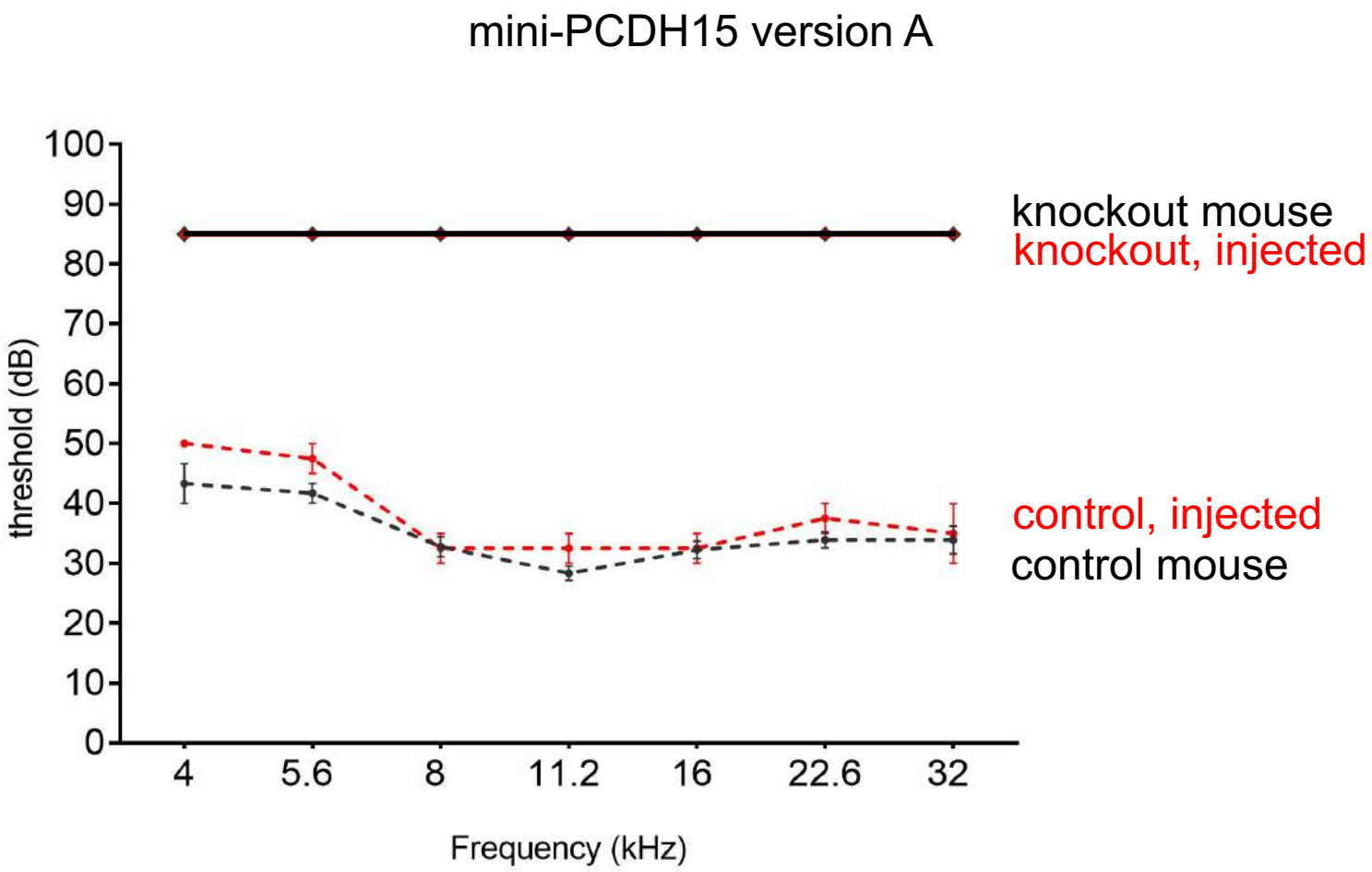
How can mini-PCDH15 coding sequences be delivered to the inner ear?

How do we assay successful rescue of function?

Delivery of mini-PCDH15 to Hair Cells using AAV Vectors

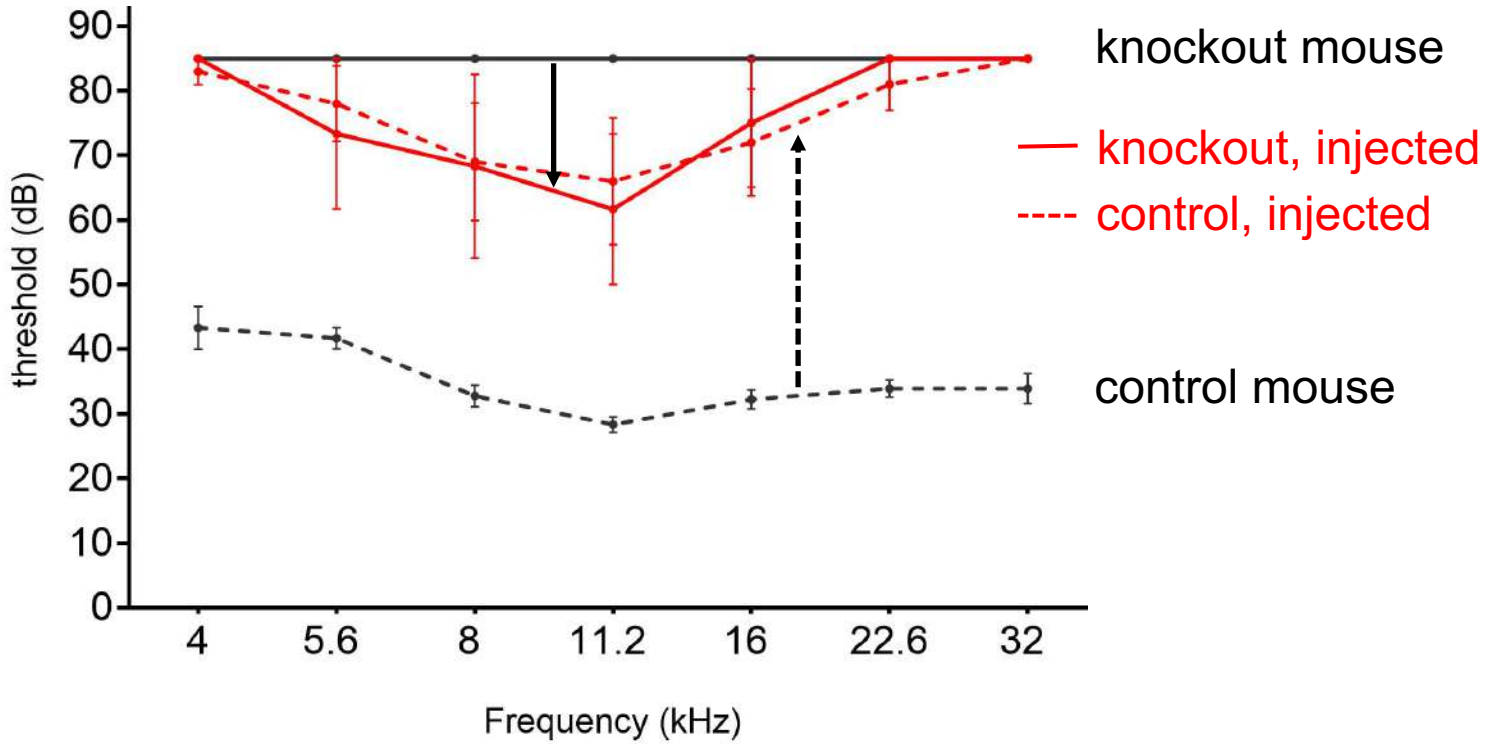


Auditory Brainstem Recording after 35 Days



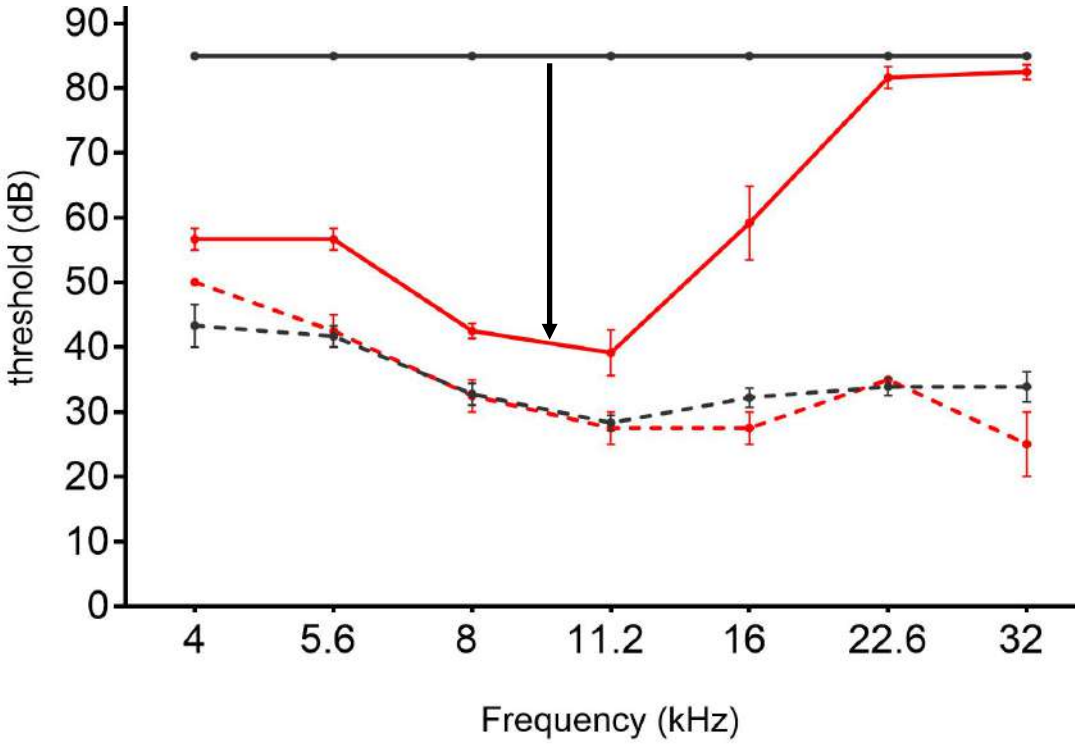
Auditory Brainstem Recording after 35 Days

mini-PCDH15 version D



Auditory Brainstem Recording after 35 Days

mini-PCDH15 version E



knockout mouse

knockout, injected

control mouse

control, injected

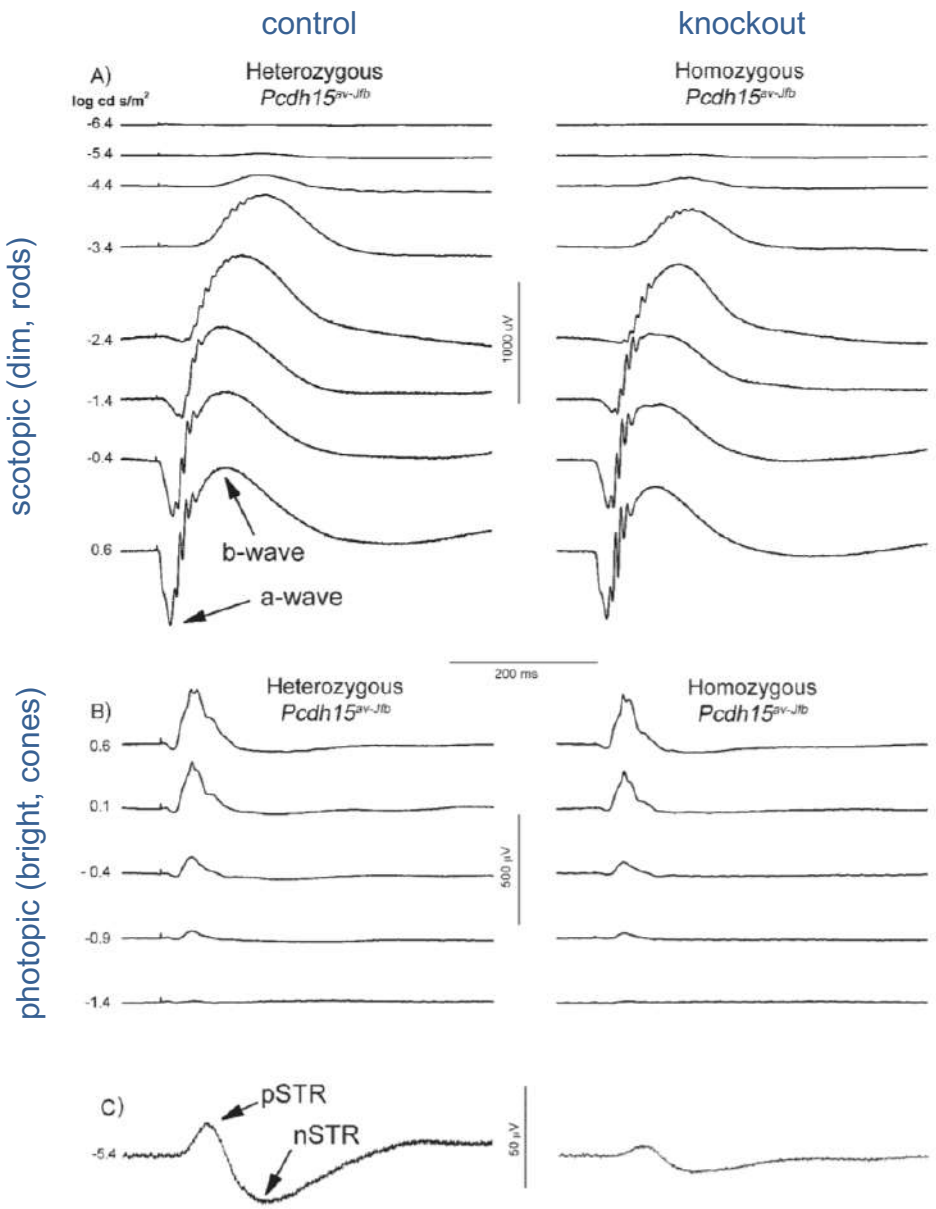
Delivery of mini-PCDH15s to cochlear hair cells and photoreceptors

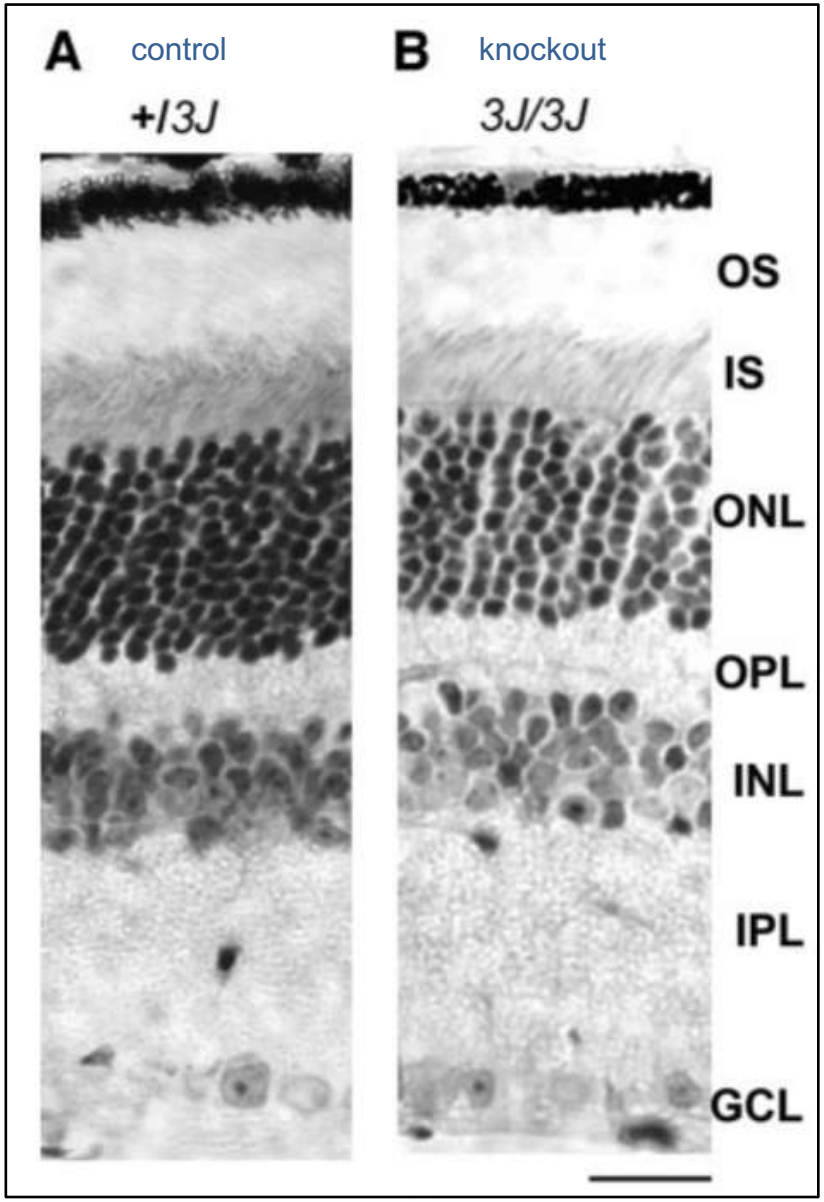
Do mini-PCDH15 constructs behave properly in vitro?

Is there a good animal model for the *loss of vision*?

How can mini-PCDH15 coding sequences be delivered to the inner ear?

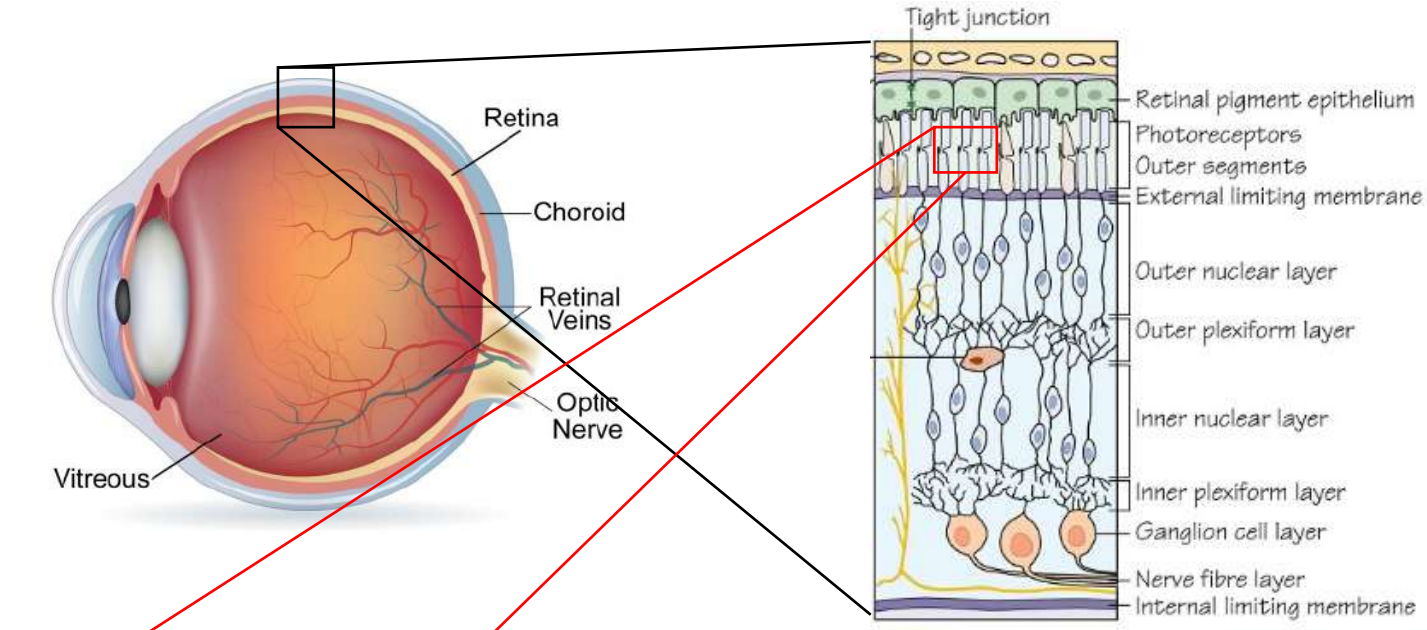
Pcdh15 mutant mice have nearly normal vision

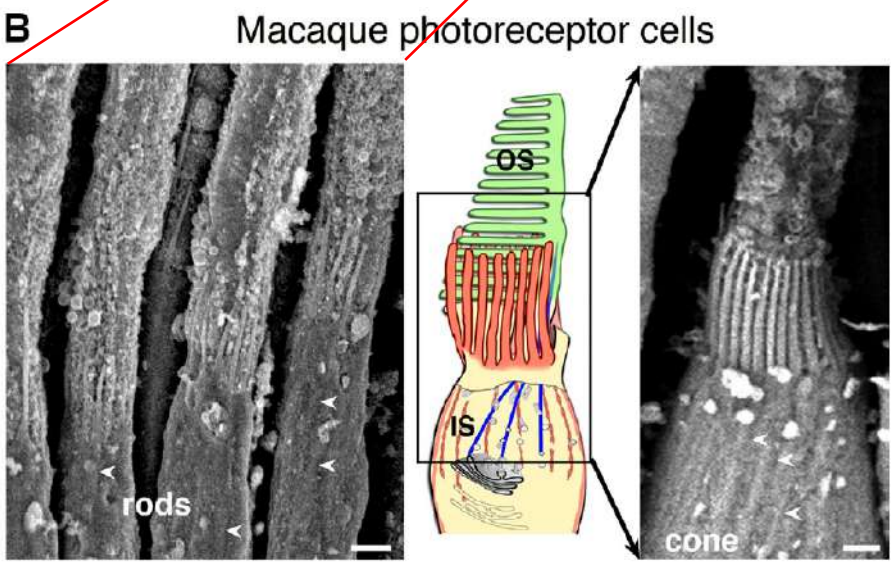


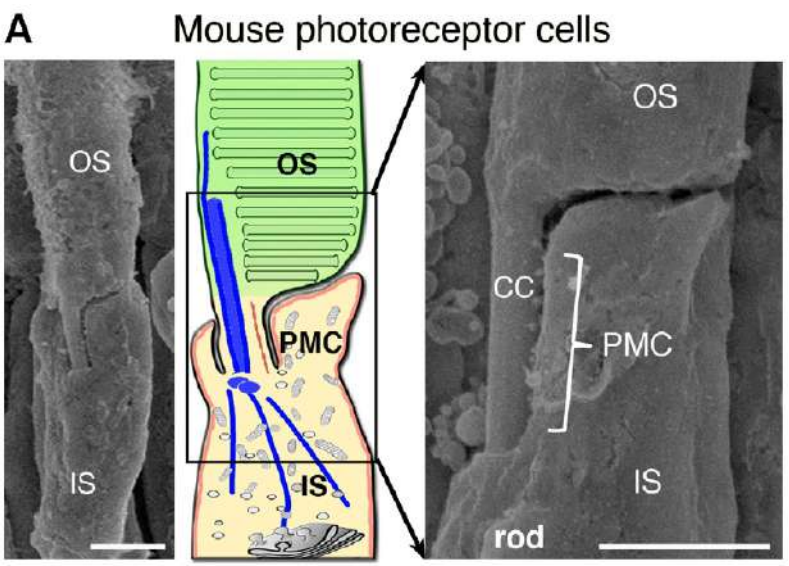


Haywood-Watson et al., 2006

PCDH15 is also in retinal photoreceptors



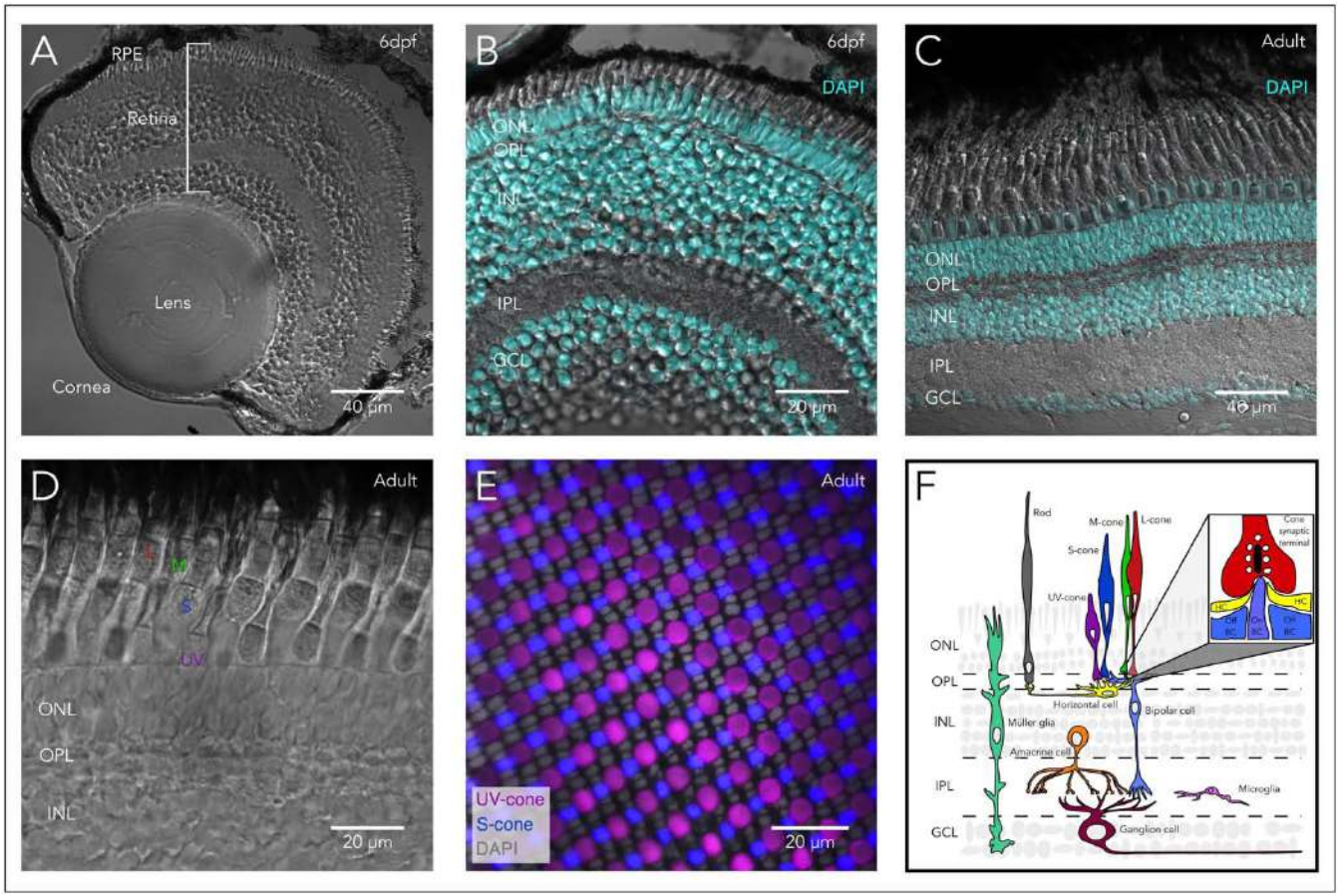




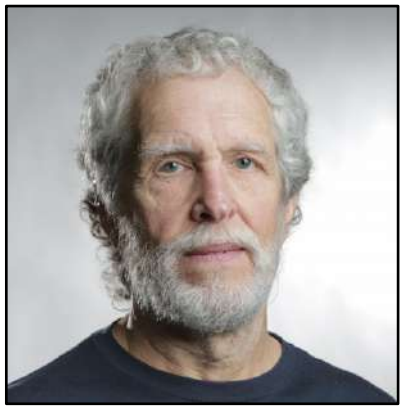
Sahly et al., 2012

Zebrafish are an excellent model for studying Usher syndrome in retina

- retina is similar to humans (cone-dominated retina; 40% rods and 60% cones)
- have the same USH genes
- USH gene mutations produce retinal cell death



Phillips and Westerfield used CRISPR/Cas9 gene editing to delete Pcdh15b in zebrafish



Monte Westerfield

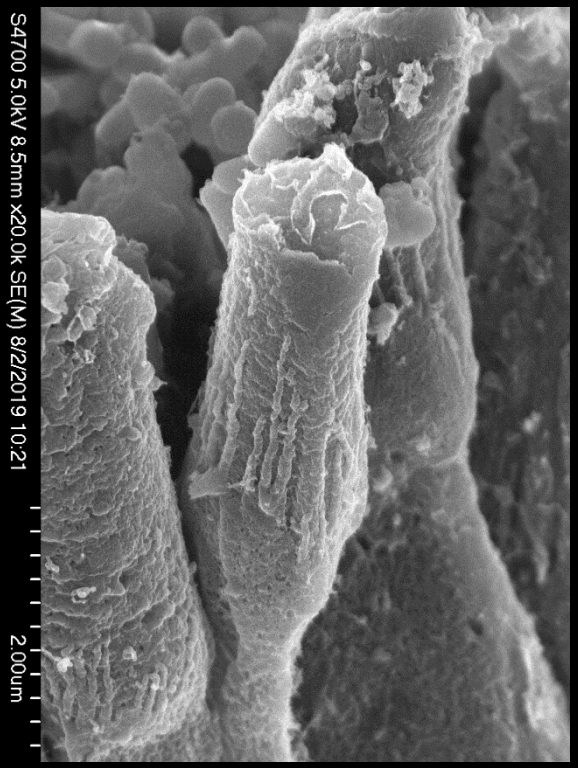
Jen Phillips

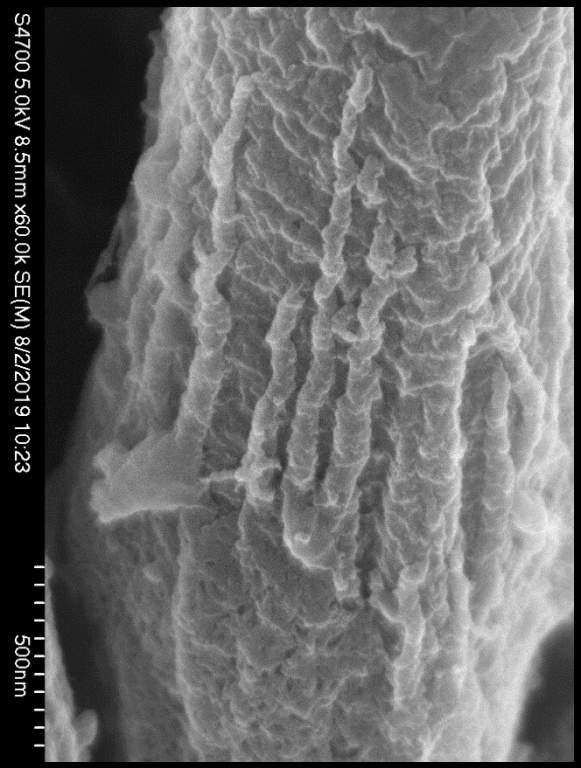
University of Oregon

Gene	Allele	Consequence
pcdh15a	b1255 b1256	Exon 8 frameshift
	b1340	In-frame Exon 8 skip
	b1388	R245X knock-in*
pcdh15b	b1257 b1266	Exon 8 frameshift
	b1341	In-frame Exon 8 skip
	b1389	R245X knock-in*

Electron Microscopy of Photoreceptors in Zebrafish Larva

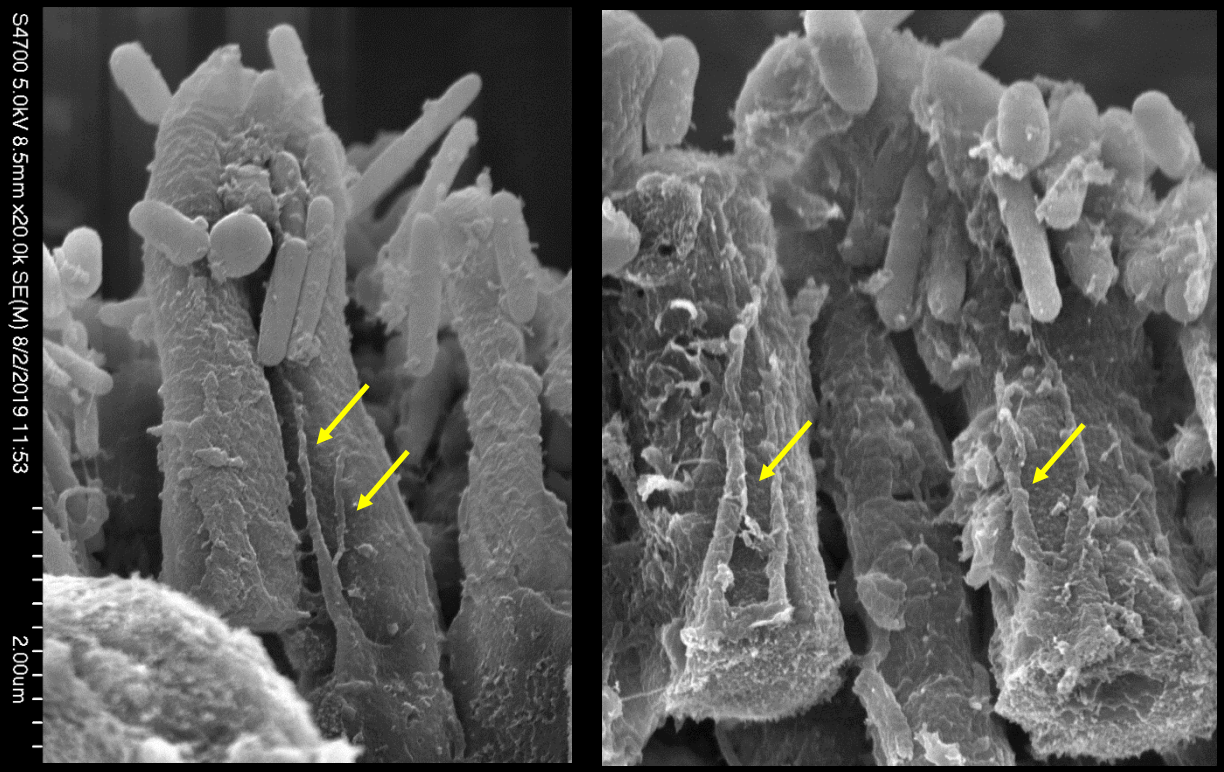
normal larva, 7 days old



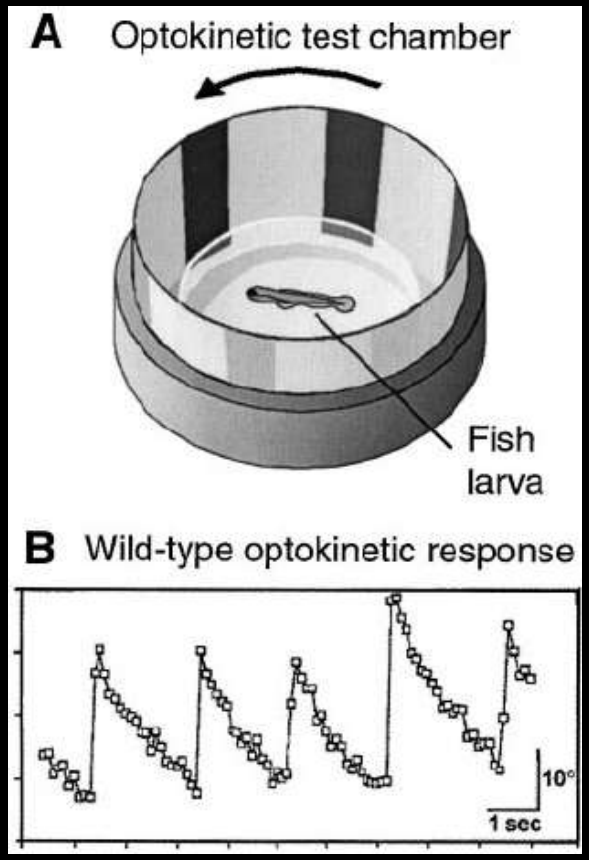


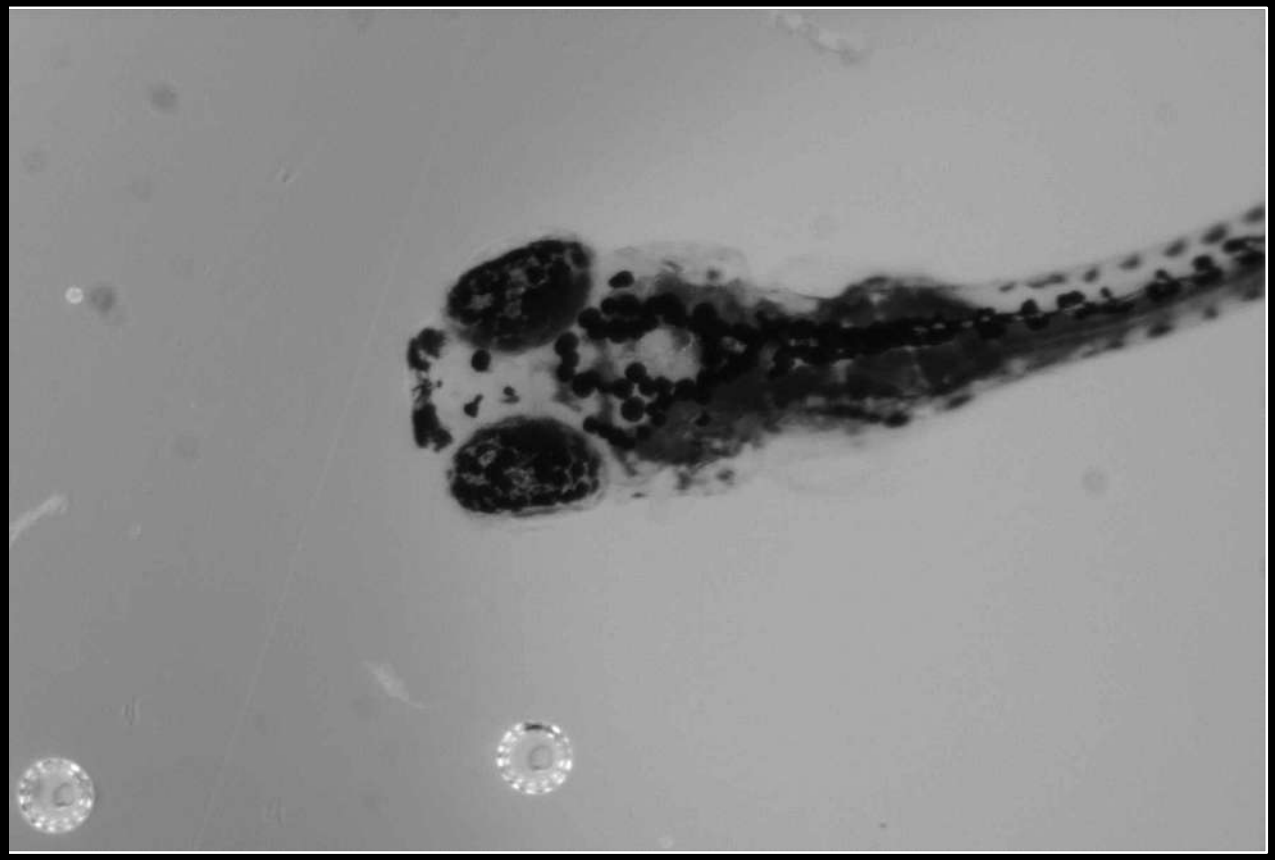
Electron Microscopy of Photoreceptors in Zebrafish Larva

knockout larva, 7 days old



Optokinetic Reflex





6 day old zebrafish larva

Current Status of mini-PCDH15 Approach

Hearing

- Produced AAV vectors that encode the first three mini-PCDH15s
- Injected vectors into the inner ears of *Pcdh15* knockout mice
- mini-PCDH15 version E preserves hearing without toxicity

Vision

- Obtained zebrafish animal model for Usher 1F
- Produced zebrafish DNA for the first three mini-PCDH15s
- Wiil inject mini-PCDH15 DNA into fertilized eggs of knockout zebrafish
- Will test rescue of retina morphology and function at 7 days

The Usher 1F Team

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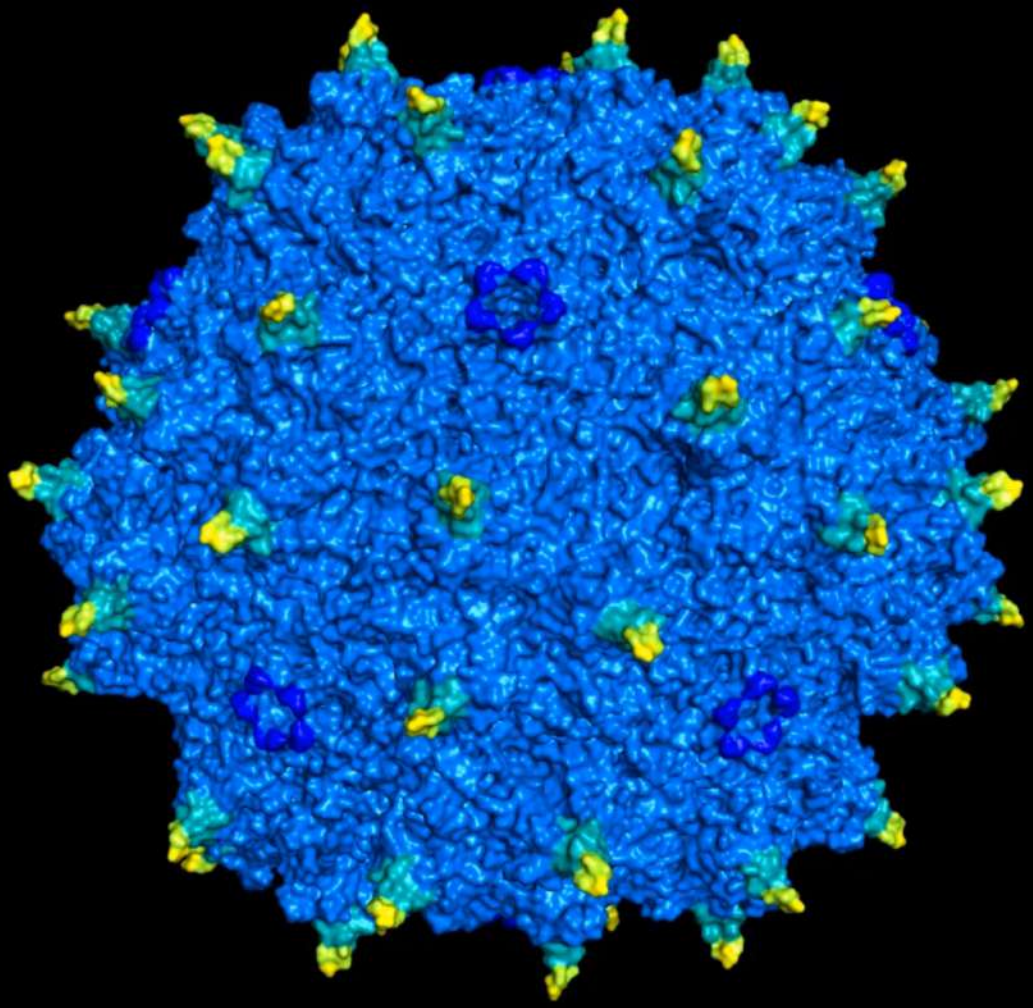

In that day the deaf will hear the words of the scroll, and out of gloom and darkness the eyes of the blind will see.

Isaiah 29:18