Tropism of Novel AAV Capsids in the Inner Ear

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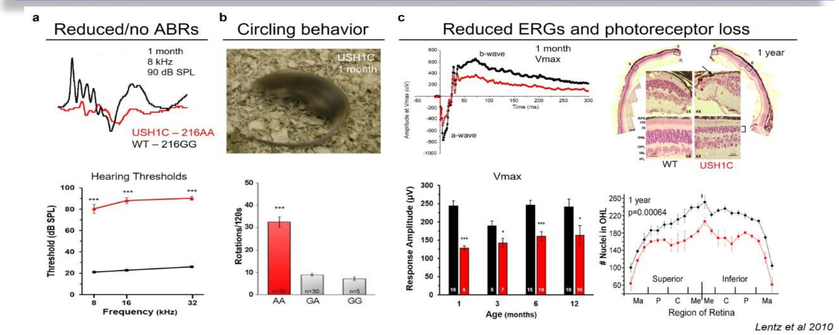
Abstract

- Usher syndrome (Usher) is the leading genetic cause of combined deaf-blindness.
- Current therapies are limited.
- Gene delivery therapies in the inner ear using adeno-associated viruses (AAV) provide a promising avenue for future treatment.
- Our knock-in murine model expresses the Type 1C Usher (USH1C*)* splice site mutation (c.216G>A) which results in a truncated protein, Harmonin (*Lentz et al 2005*).
- The mouse model displays the same dysfunctional visual, auditory, and vestibular phenotypes seen in USH1C patients *(Lentz et al 2010).*
- Gene therapy using AAV-Anc80L65-Harmonin shows short-term rescue of hearing and balance (*Pan et al 2017*).
- Optimizing AAV vectors will improve the specificity of *USH1C* gene delivery.
- **Our goal** is to find a gene therapy that can be used in both the inner ear and retina to rescue vision, hearing, and vestibular deficits in USH1C patients.

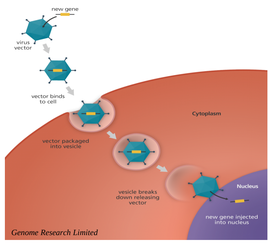
Aim: Examine the tropism of newly engineered AAV mutant capsids in the inner ear.

Background



Lentz et al 2010

Fig. Usher phenotype in USH1C^{216AA} mice. Knock-in mice with the *USH1C* c.216G>A splicing mutation (216AA) responsible for Acadian Type 1C Usher are profoundly deaf (**a**), have severe imbalance (**b**) and visual dysfunction (**c**).



Is there a single AAV capsid that can transduce cochlear hair cells, vestibular hair cells, and retinal photoreceptors?

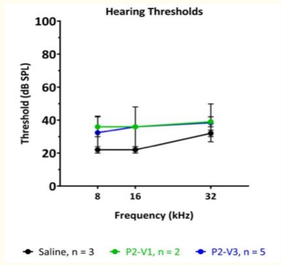
Methods

- **Mice:** Male and female CBA wild-type (WT) littermate mice.
- **AAV Injection:** WT mice are treated with 1.9x10E12 gc/ml of AAV2.P2-V1(Y-F+T-V)-CBA-GFP, AAV2.P2-V3-CBA-GFP, or saline at postnatal (P) day 1-2 by semicircular canal (SCC) injection.
- **Auditory Brainstem Response (ABR):** At P30, AAV-treated WT thresholds are analyzed at 8 kHz, 16 kHz, and 32 kHz.
- **Tissue Preparation:** Temporal bones are harvested at P30 and fixed in 4% paraformaldehyde (PFA). The cochlea were perfused using 0.5 mL of 4% PFA via round window (RW) and oval window (OW) injections. Post- fix, the tissues were stored on rocker at 4 degrees Celsius.
- **Microdissection:** The organ of corti (OC) and vestibular organs were micro-dissected in 1x PBS.
- **Immunohistochemistry (IHC):** Vestibular tissues are decalcified. Micro-dissected tissues are blocked using goat serum, stained with Rabbit anti-Myosin 7A primary antibody (1:250) and counterstained with DAPI and anti-rabbit 555 (1:500) secondary antibody. Stained tissues are mounted on cover slips using Prolong Diamond mounting media in the dark.
- **Confocal imaging**: Green fluorescence protein (GFP) and myosin 7A are used as a markers for transduced cells at 20x.

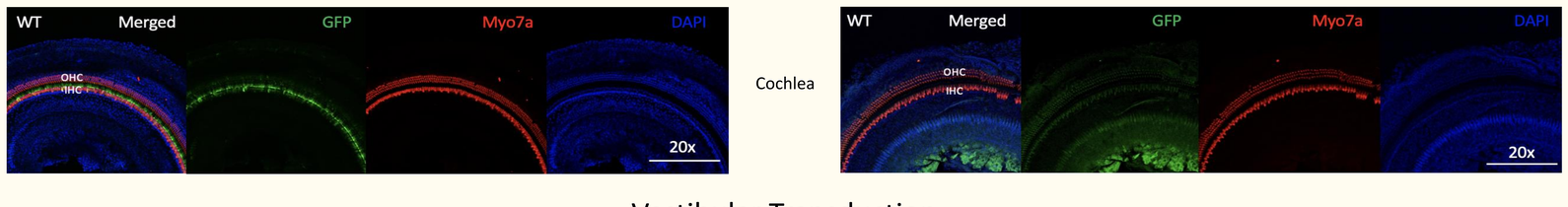
Results

- Mice treated by SCC injection with AAVs had ABR thresholds similar to saline treated WT control mice
- P2-V1 capsid transduced supporting cells in the cochlea and vestibular end organs.
- Little-no transduction was observed in cochlear or vestibular cells with P2-V3 capsid.

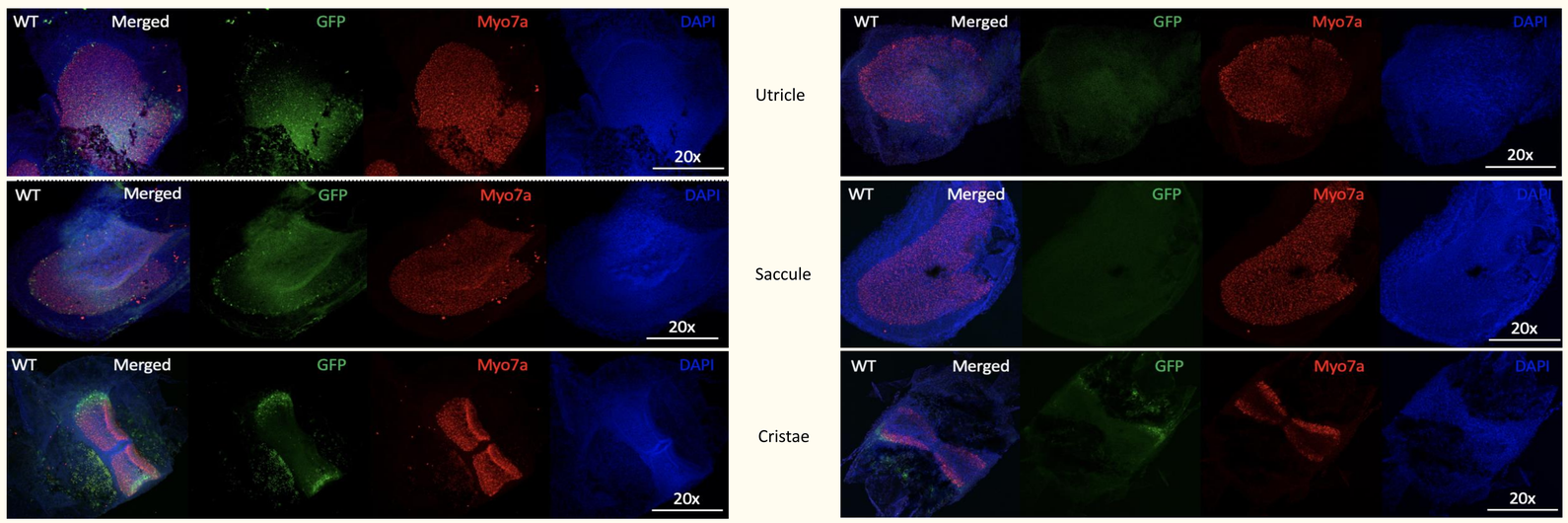
Auditory Brainstem Response (ABRs)



Cochlear Transduction



Vestibular Transduction



Conclusion

- Delivery of novel AAV vectors via SCC injection leads to safe and effective transduction of cochlear and vestibular cells in mice.
- AAV2.P2-V1-CBA-GFP transduces inner ear support cells that may be a target for some forms of genetic deafness.
- Studies are ongoing to evaluate additional novel capsids to further expand the toolbox for effectively treating diseases of the inner ear.

References

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