Toward a gene-edited nonhuman primate model of Usher syndrome

Martha Neuringer[1,2], Junghyun Ryu[1], Jon D. Hennebold[1], John P. Statz[1], William Chan[1], Cathy Ramsey[1], Fernanda C. Burch[1], J. Beth Kempton[3], Edward V. Porsov[3], Lauren Renner[1], Benjamin J. Burwitz[4], Carol B. Hanna[1], John V. Brigande[3]

[1]Oregon National Primate Research Ctr., [2]Casey Eye Inst., [3]Oregon Hearing Research Ctr. & [4]Vaccine & Gene Therapy Inst., Oregon Health & Science University

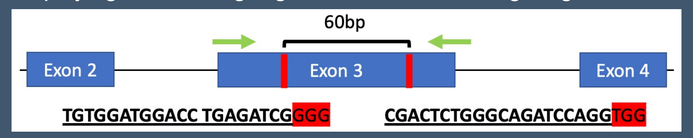
1944

Purpose

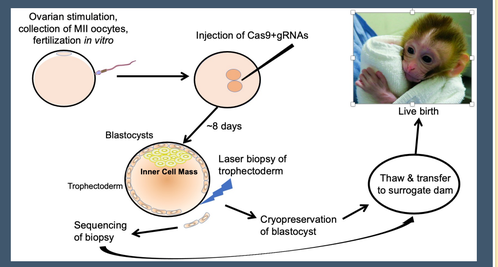
Better translational models are urgently needed for many retinal degenerative diseases to facilitate understanding pathogenetic processes and test potential therapies. **Usher syndrome** presents a particularly compelling need for such models, due to the devastating nature of the disease and the lack of rodent models showing retinal degeneration. **Usher 1B** is a primary target due to its rapid onset and prevalence among Usher subtypes. Nonhuman primates best mirror human retina anatomy and function by having a macula and fovea, as well as photoreceptor calyceal processes that are a major site of dysfunction in Usher syndrome but are absent in rodents.

Gene Editing

We used CRISPR-Cas9 editing to create rhesus monkey embryos with mutations in exon 3 of *MYO7A*. Two sgRNAs were designed for exon 3 (red bars). sgRNA sequences are underlined and PAM sequences are highlighted in red. Green arrows indicate primers for amplifying the flanking region of the MYO7A targeting locus.



Cas9 mRNA and two sgRNAs were injected into zygotes 16 hours after *in vitro* fertilization. Laser biopsies of the trophectoderm from day 8 blastocysts were genotyped by Sanger sequencing. Embryos with expected pathological mutations of the *MYO7A* gene were selected for transfer to surrogate dams, resulting in a live birth. Infant PBMCs, skin and cheek cells were sequenced to confirm genotype. Both trophectoderm biopsy and infant tissues showed a compound heterozygote pattern with 63bp and 1bp deletions.



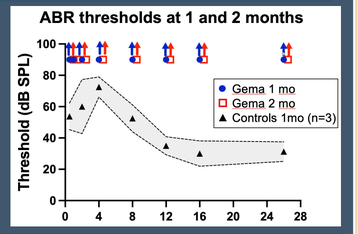
63bp deletion (in-frame mutation – 21 AA deletion)
AGGGGGACTATGTGTGGATGGACCTGAGA----------(63bp deletion)----------CAGGTGGTGGACGATGA

1bp deletion (Premature stop codon)
AGGGGGACTATGTGTGGATGGACCTGAGA-CGGGGCA---//---TGCGACTCTGGGCAGATCCAGGTGGTGGACGATGA

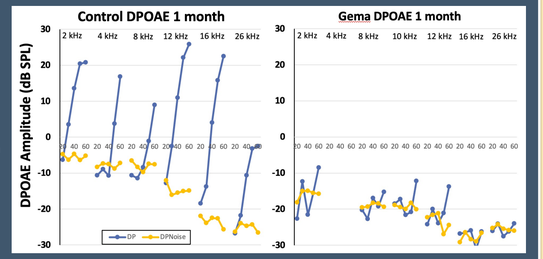
The live infant was named **Gema** (**G**ene-**E**dited ***MYO7A***).

Results: Absence of auditory function

- Auditory function, assessed by auditory brainstem response (ABR) at 1 and 2 months, showed absence of responses at all frequencies from 0.5 to 26 kHz, indicating profound hearing impairment.



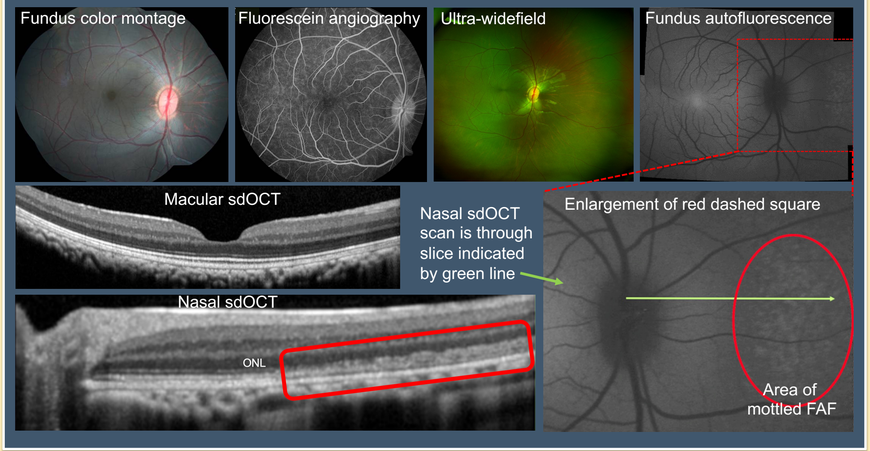
- Distortion product otoacoustic emissions (DPOAE) showed no responses above the noise floor at either age, confirming the absence of cochlear outer hair cell function.



- Tympanometry showed normal function of eardrum/middle ear.

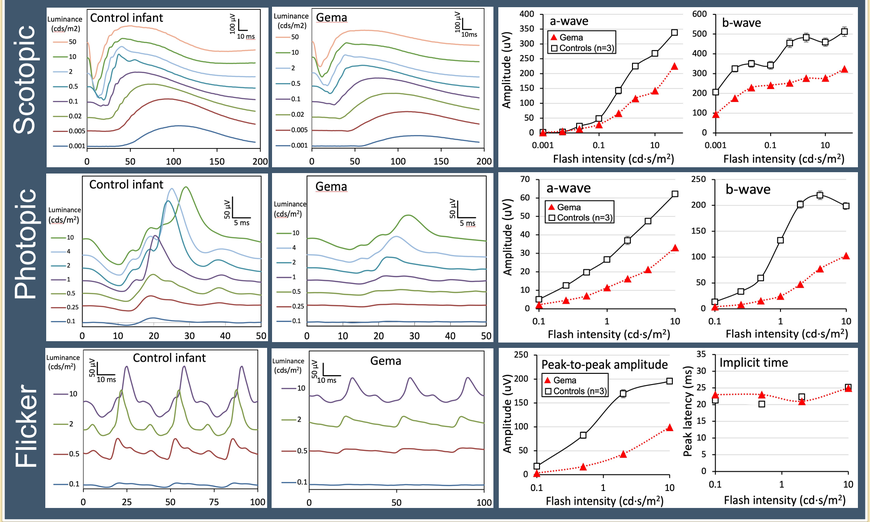
Incipient photoreceptor degeneration at 4 mo

- Multimodal retinal imaging at 1, 2 and 4 months included sdOCT; macular and ultra-widefield color, fundus autofluorescence (FAF) and fluorescein angiography; OCTA and adaptive optics.
- No abnormalities were detected at 1 or 2 months.
- At 4 months, the nasal periphery showed mottled FAF (red oval). OCT of the same area showed thinning of the ONL and marked disruption of outer retinal layers (red rectangle in nasal OCT).



Results: Subnormal ERG

Full-field scotopic, photopic and flicker ERG amplitudes were reduced by ~50%. Little or no change was seen in implicit times.



Abnormal vestibular function

Skilled observers and neurological ratings confirmed abnormal balance, hind limb bradykinesia, and wide, asymmetric gait.

Conclusions

- This study is the first to create a gene-edited monkey model of retinal disease.
- We showed the ability to induce pathogenic mutations in the *MYO7A* gene in rhesus macaques, resulting in auditory, vestibular and retinal disease phenotypes mirroring those in human USH1B patients.
- Continued confirmation of the USH1B phenotype, and production of additional infants with *MYO7A* mutations, will set the stage for studies of dual-AAV gene therapy and other therapeutic approaches in this first gene-edited nonhuman primate model of Usher syndrome.

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