Toward a Nonhuman Primate Model of USH1B

Martha Neuringer, Carol Hanna, Junghyun Ryu,
John Brigande, Lauren Renner, Jon Hennebold

Oregon National Primate Research Center
& Casey Eye Institute,
Oregon Health & Science University

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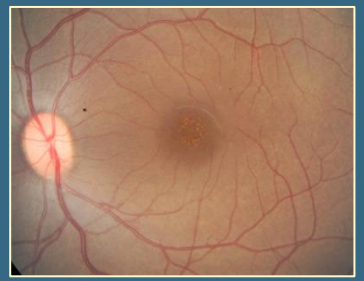
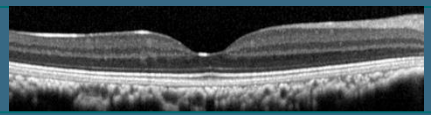
The need for primate models

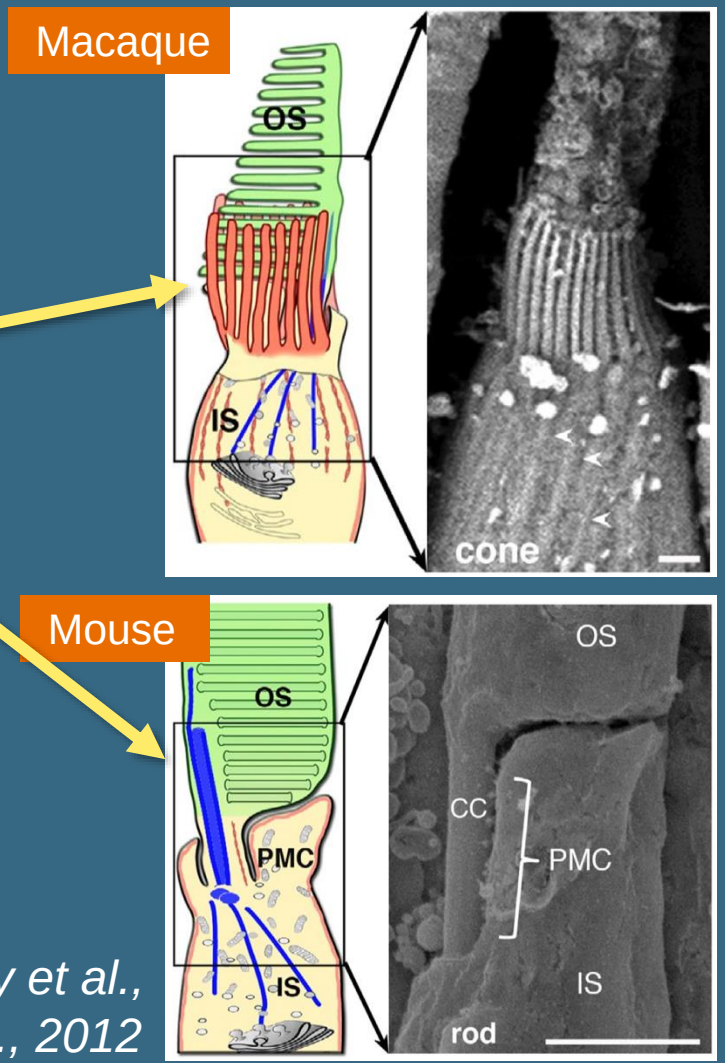


Snodderly, 1984



- *Lack of nonhuman primate models is seen as a key barrier to therapy development*
- Only higher primates have a retinal structure nearly identical to humans, rich in cones and having a fovea and macula that provide high acuity vision
- Key differences in photoreceptor structure exist between rodents and primates
- Accurate models are critical for understanding pathogenesis and for preclinical testing of therapies

- In primates, MYO7A and other USH1 proteins are localized to a special structure that encircles the base of the photoreceptor outer segments, *the calyceal processes*
- This structure is absent from rodent retinas
- This difference is thought to account for the lack of retinal phenotype in rodents with *MYO7A* and other Usher mutations



From Sahly et al., J. Cell Biol., 2012

Three paths to creating primate models

- Identification of naturally-occurring models
  BBS7 and CLN7 (Oregon), PDE6C (Davis)

- Sequencing to identify pathogenic variants
  ONPRC Primate Genetics Program is identifying variants in 2000 rhesus monkeys:

  The Macaque Genotype and Phenotype Resource: https://mGAP.ohsu.edu/

- ***Gene editing for high priority diseases***

Production of gene edited monkey models of inherited retinal degenerative diseases

- ONPRC Division of Reproductive & Developmental Sciences: implementing gene editing technologies in macaque monkeys

+

- ONPRC Assisted Reproductive Technologies (ART) Core: 40 years of experience with ART in macaque monkeys

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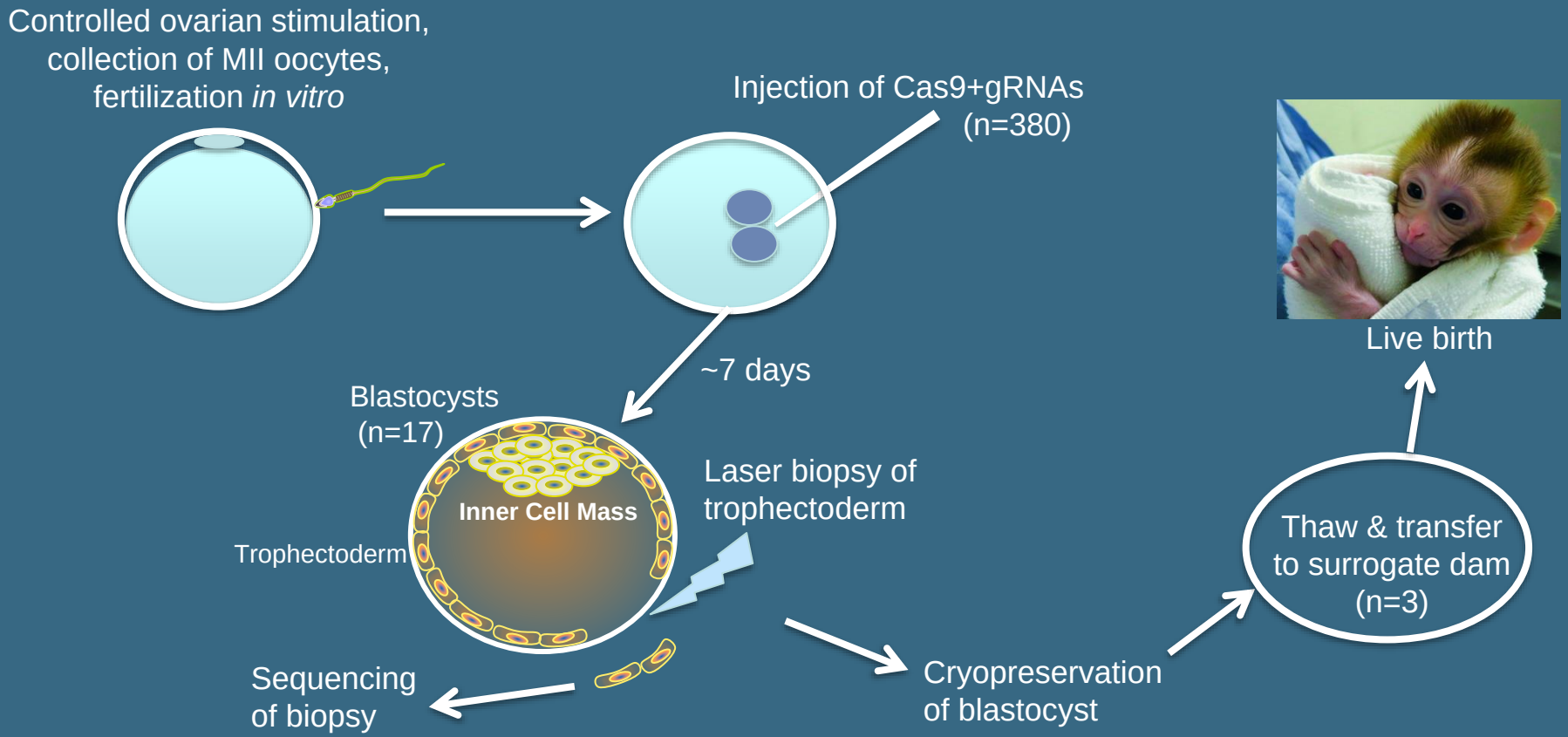
- Production of monkeys with mutations in *MYO7A*

Jon Hennebold
Division Chair

Carol Hanna
Director, ART Core

CGE
Somatic Cell Genome Editing

CRISPR/Cas9 editing of MYO7A in monkey embryos



First edited monkey infant ("Mya") born May 2019 .....but was mosaic

Sequencing of trophectoderm biopsy

gRNA-1 gRNA-2 gRNA-3

Wild type	TGTGGATGGACCTGAGATCGGGGCAGGAGTTTGATGTGCCCATCGGG//CGACTCTGGGCAGATCCAGGTGG	8%
Mutation	TGTGGATGGACCTGAGA**G**TCGGGGCAGGAGTTTGATGTGCCCATCGGG//CGACTCTGGGCAGATCCAGGTGG	92%

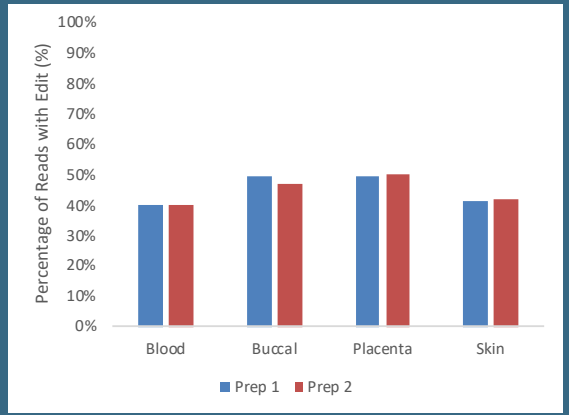
Resulting change in amino acid sequence:

GDYVWMDLRSGQEFDVPIGAVVKLCDSGQIQVVDDEGN → GDYVWMDLRVGAGV*

Wild type protein — Frameshift *stop codon

Sequencing of infant tissues:
blood, cheek cells, skin cells and placenta

40% of reads in blood and skin showed G insertion, but 50% in check cells and placenta, indicating a mosaic pattern of the mutation; this was confirmed by single-cell sequencing of blood lymphocytes.



Phenotyping of Mya:

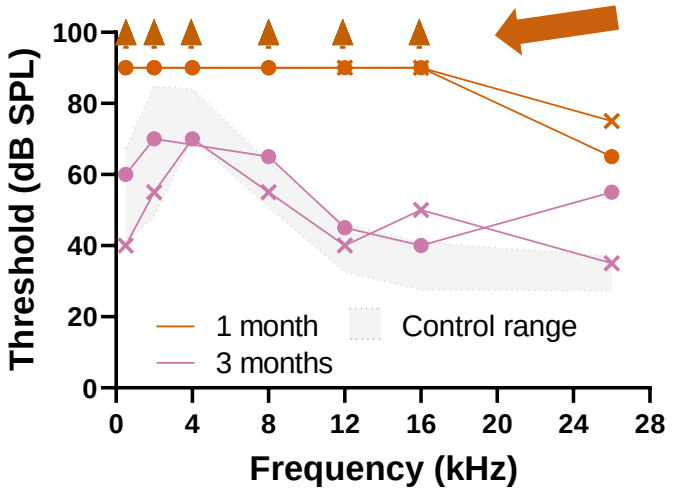
Auditory function

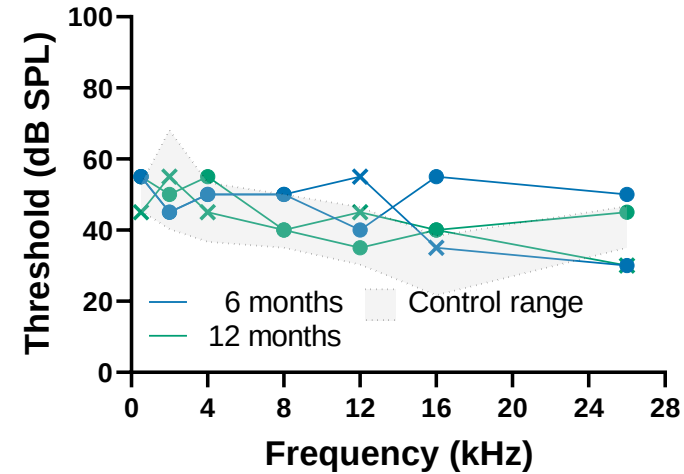
Brainstem auditory evoked responses (BAERs) and distortion product otoacoustic emissions (DPOAEs) were conducted by the Brigande lab at 1, 3, 6, 12 and 24 months of age.

At one month, responses were absent at lower frequencies by both measures and present but elevated at higher frequencies.

However, from 3 months onward, all thresholds were within or close to the normal range for age-matched infants, indicating functional inner and outer hair cells.

The early results could be due to the difficulty in stimulating through the very narrow infant ear canal.

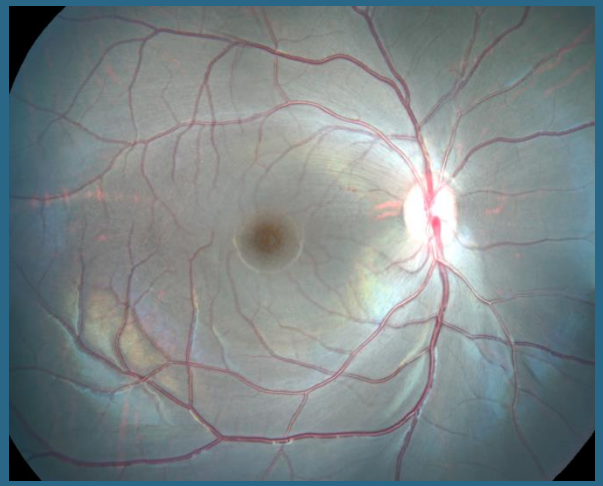


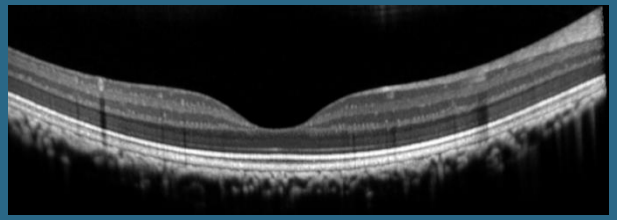


Phenotyping:

Multimodal retinal imaging

including colors, sdOCT, FAF, UWF, fluorescein and ICG angiography, adaptive optics

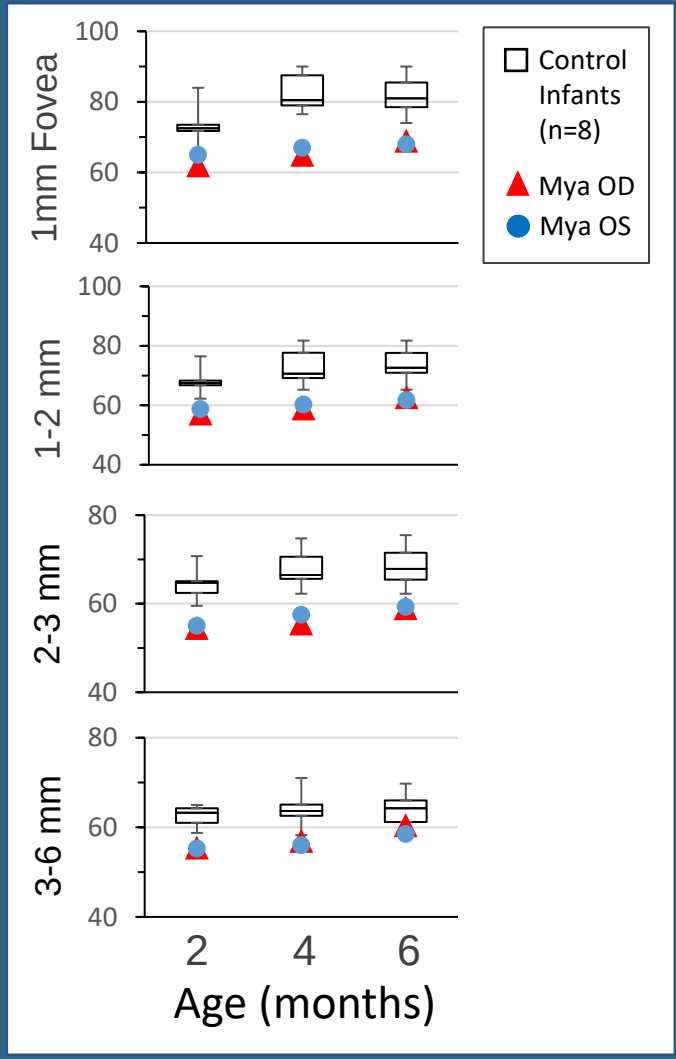




No abnormalities were detected at ages 2, 4, 6, 9, 12, 18 or 24 months of age --

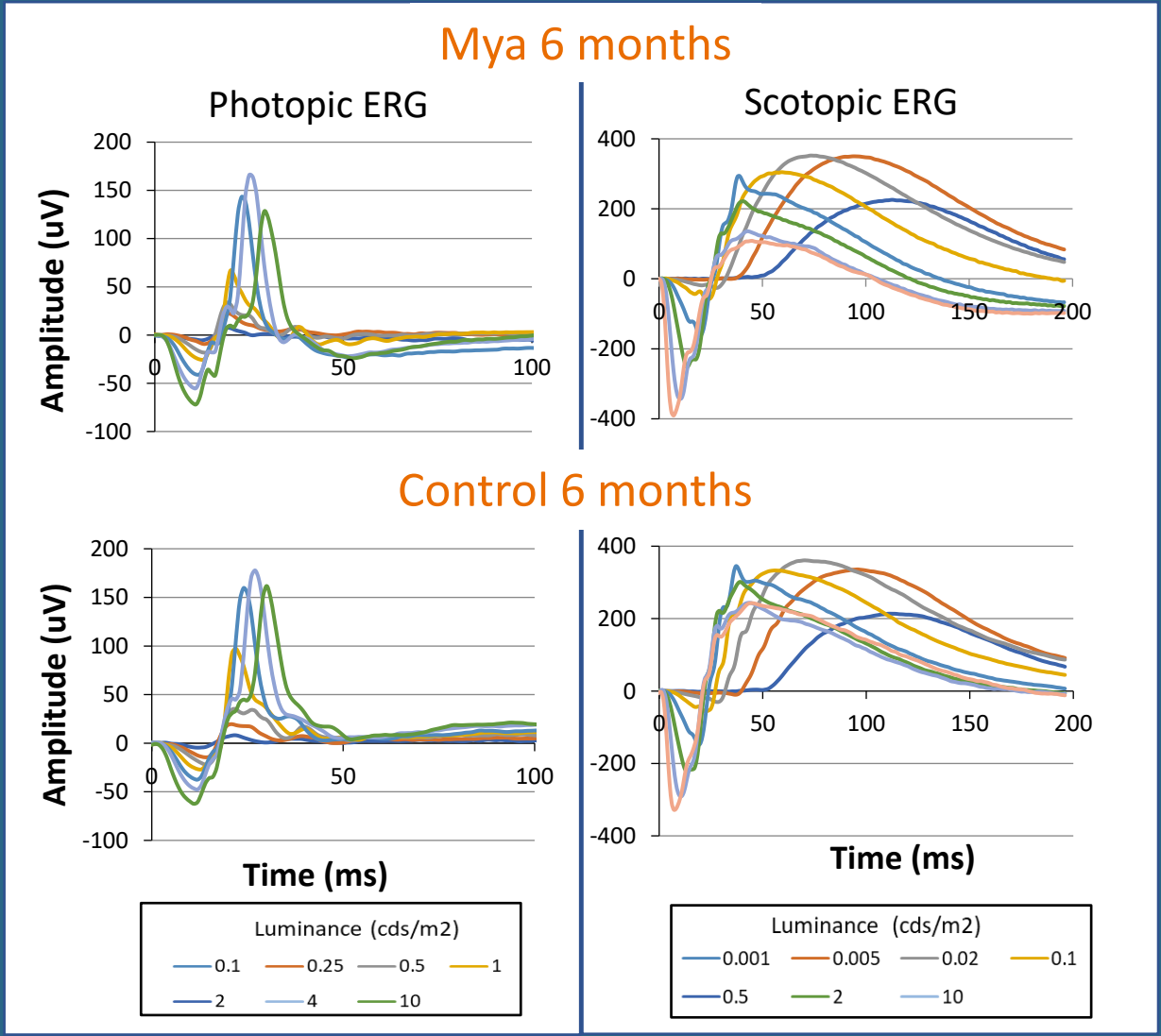
with the exception of a small decrease in the thickness of the photoreceptor inner/outer segment layers within the central 6 mm.

Inner/Outer Segment Layer Thickness (µm)



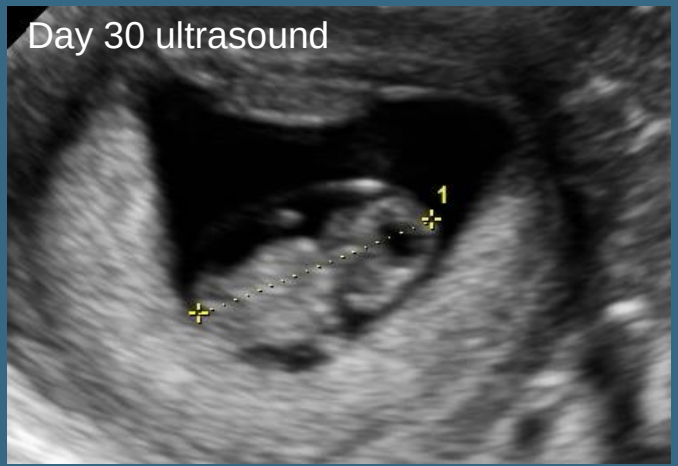
Phenotyping: Electroretinogram

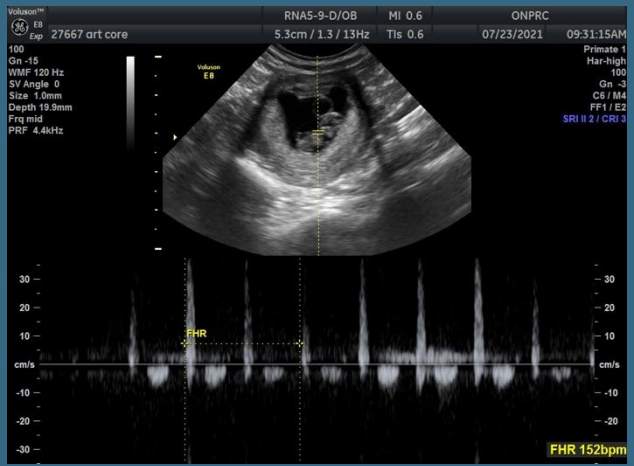
Photopic, scotopic and flicker amplitudes and latencies all were similar to age-matched normal infants across the intensity-response range at ages 2.5, 6, 9, 12 and 24 months



Production of fully edited infants: progress in 2020-2021

- Controlled hormonal stimulations: 11
- Oocytes collected: 307
- Oocytes fertilized and edited: **243**
- Surviving blastocysts (day 7-8): 64
- Blastocysts biopsied & sequenced **58**
- Biopsies with 100% editing: 18
- Embryos transferred: 15 biopsied + 4 fresh = **19**
- *Pregnancies:* *2* *due in December*





Future Directions

- Produce enough affected animals to test multiple therapeutic approaches
  - Gene therapy
  - Gene editing
  - Photoceptor cell replacement

- Provide a model to test treatments for multiple forms of Usher syndrome
- Work toward prenatal gene therapy for hearing loss (John Brigande)
- With the experience gained from this project, use gene editing to develop other valuable models of inherited retinal disease

Acknowledgements

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