Usher syndrome type 1C: Mechanisms, Animal Models and the hunt for a Cure

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Usher Coalition
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1. Lentz Lab Mission
2. Usher syndrome type 1C
3. Acadian Usher syndrome
4. *USH1C* and Harmonin
5. *Ush1c*.216A knock-in mouse
6. Treatment with Antisense Oligonucleotides (ASOs)
7. Future directions

Lentz Lab

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Mission

To develop a therapeutic approach to prevent or cure the deafness and blindness associated with Usher syndrome

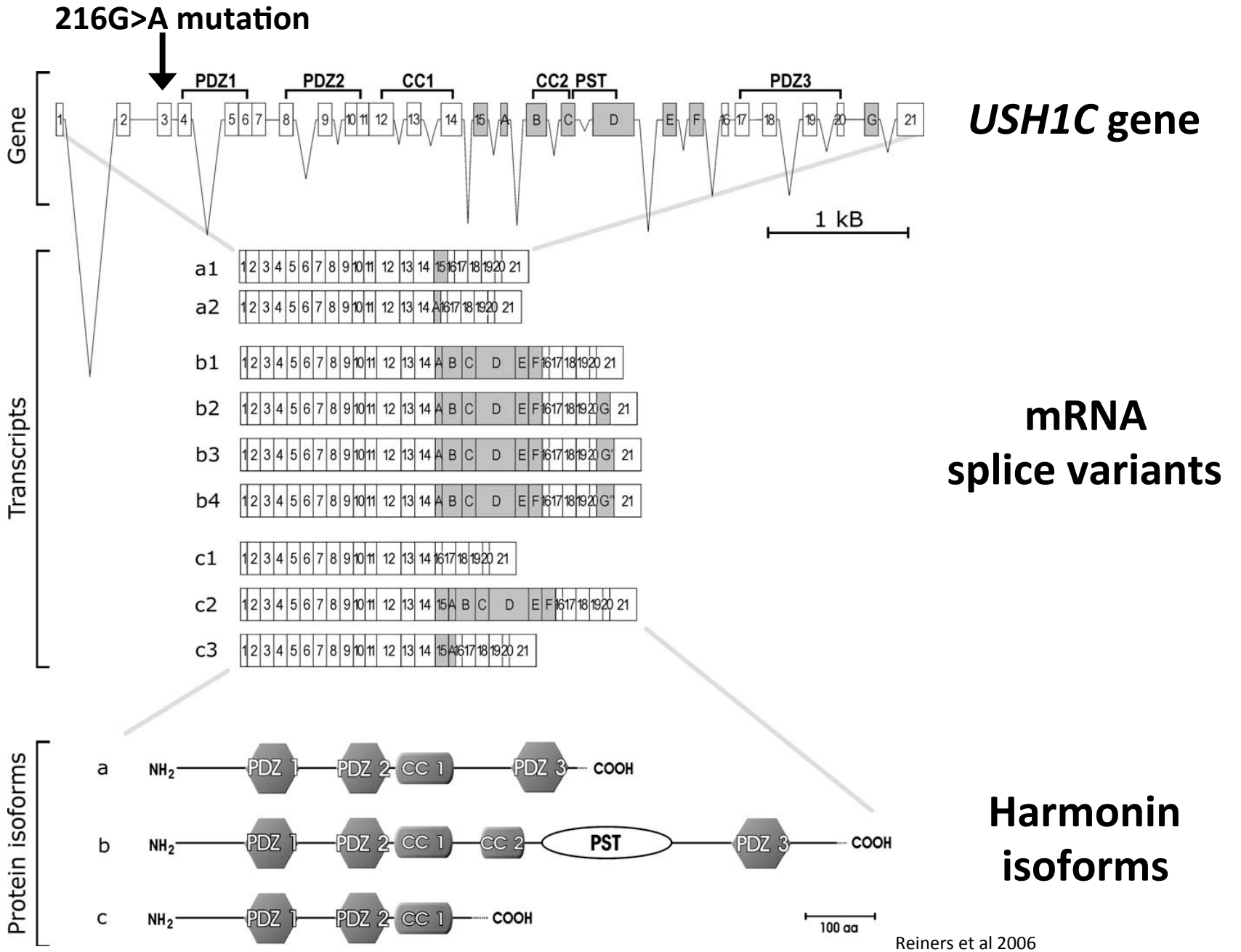
Focus: Usher syndrome type 1C

Congenital Deafness – born with severe to profound hearing impairment
Vestibular Areflexia – difficulty with balance
Retinitis Pigmentosa – begins in early adolescence with night-blindness

6-8% of Usher 1 cases are caused by mutations in the *USH1C* gene, which encodes the protein harmonin

All cases of Usher 1 in Acadian populations (South Louisiana and Canada) are caused by the USH1C.216G>A mutation (216A)

USH1C and Harmonin Protein Isoforms



USH1C gene

mRNA splice variants

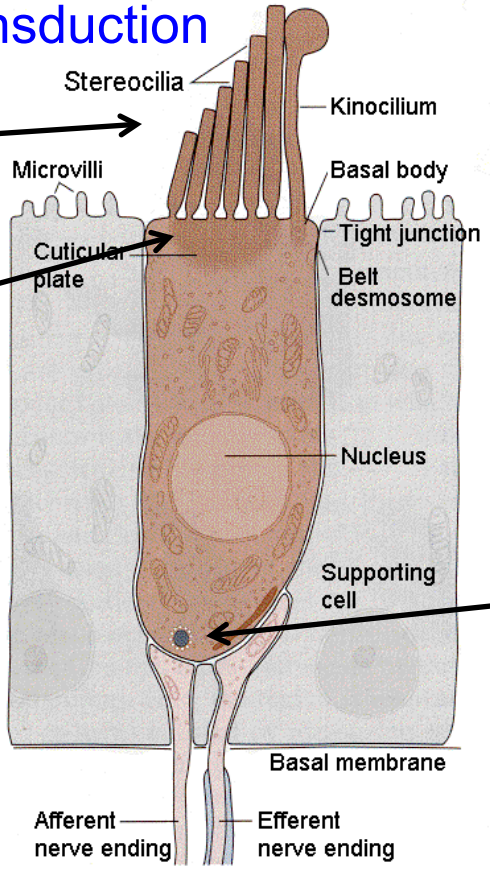
Harmonin isoforms

Reiners et al 2006

Harmonin

Cochlear Hair Cell

Bundle morphogenesis
Mechanotransduction



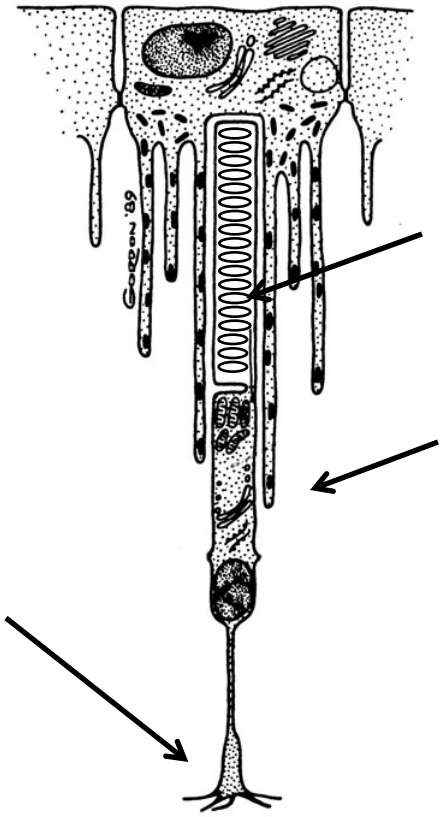
Bundle

Cuticular plate

Ribbon synapse

?

ROD photoreceptor



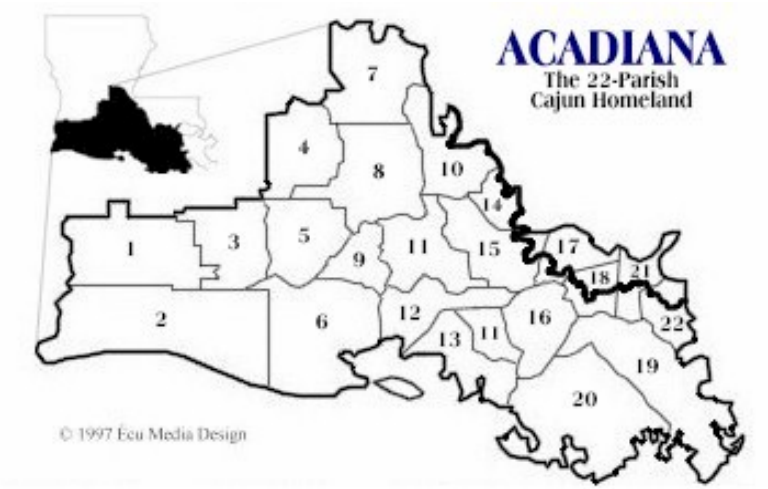
Outer Segments

?

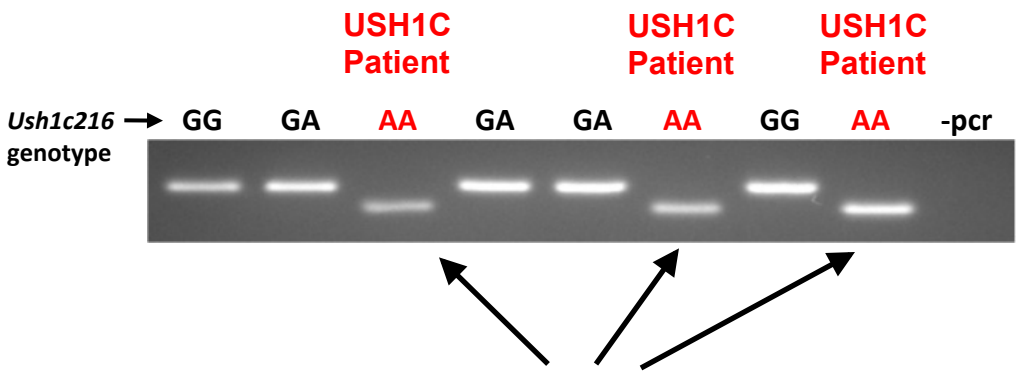
Inner Segments

?

Acadian Usher Syndrome type 1C



Gene Expression in USH1C Patients



Truncated mRNA with 35 bp deletion at the end of exon 3

Wild-type Harmonin

MDRKVAREFRHKVDFLIENDAEKDYLYDVLRMYHQTMDVAVLVGDLKLVINEPSRLPLFDAIRPLIPL
KHQVEYDQLTPRRSRKLKEVRLDRLHPEGLGLSVRGGLEFGCGLFISHLIKGGQADSVGLQVGDEIVR
INGYSISSCTHEEVINLIRTKKTVSIKVRHIGLIPVKSSPDEPLTWQYVDQFVSESGGVRGSLGSPGN
RENKEKKVFISLVGSRGLGCSISSGPIQKPGIFISHVKPGSLSAEVGLEIGDQIVEVNGVDFSNLDHK
EAVNVLKNSRSLTISIVAAAGRELFMTDRERLAEARQRELQRQELLMQKRLAMESNKILQEQQEMERQ
RRKEIAQKAAEENERYRKEMEQIVEEEEKFKKQWEEDWGSKEQLLLPKTITAEVHPVPLRKPKYDQGV
EPELEPADDLDGGTEEQGEQDFRKYEEGFDPYSMFTPEQIMGKDVRLLRIKKEGSLDLALEGGVDSPI
GKVVVSAVYERGAAERHGGIVKGDEIMAINGKLVTDYTLAEADAALQKAWNQGGDWIDLVVAVCPPKE
YDDELTFLLKSKRGNQIHALGNSELRPHLVNTKPRTSLERGHMTHTRWHPWDLNLSPRNLKLPLALNQ
GQIRNSSGHFFEGQCGGKGAASRLGEDLKDPDSHSFPLAQ

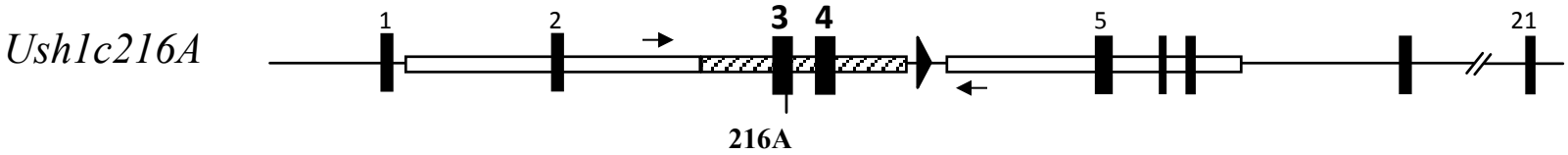
Truncated 216A mRNA Translation

MDRKVAREFRHKVDFLIENDAEKDYLYDVLRMYHQTMDVAVLVGDLKLVINEPSRLPLFDAIRPLIPL
KHQ***EAEGGASGPSAPRRPRPECAWWPGVWLWALHLPPHQRRSGRQRRAPGRGRDRPDQWIFHLLLYP***

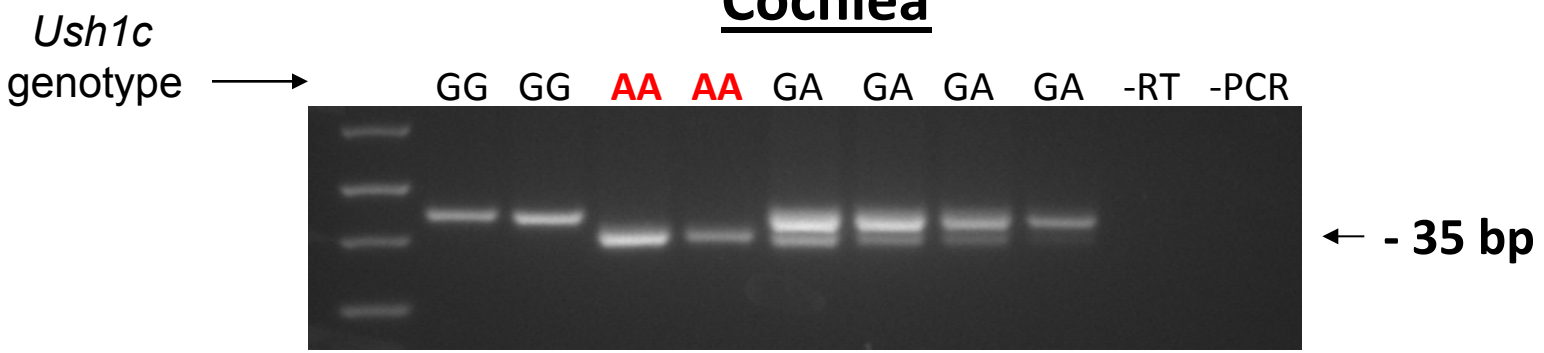
Predicted to encode a truncated harmonin

Lentz et al 2005

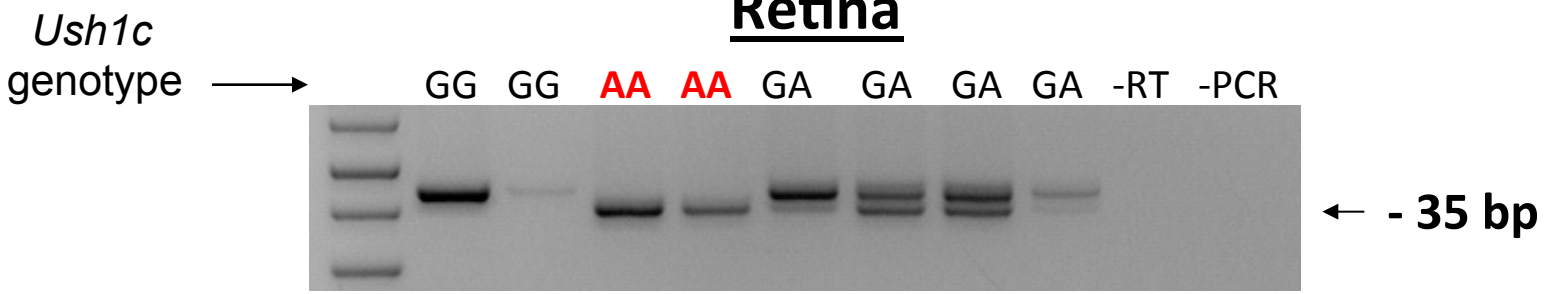
Put the Human 216A mutation into the Mouse *Ush1c* gene (knock-in)



Cochlea



Retina



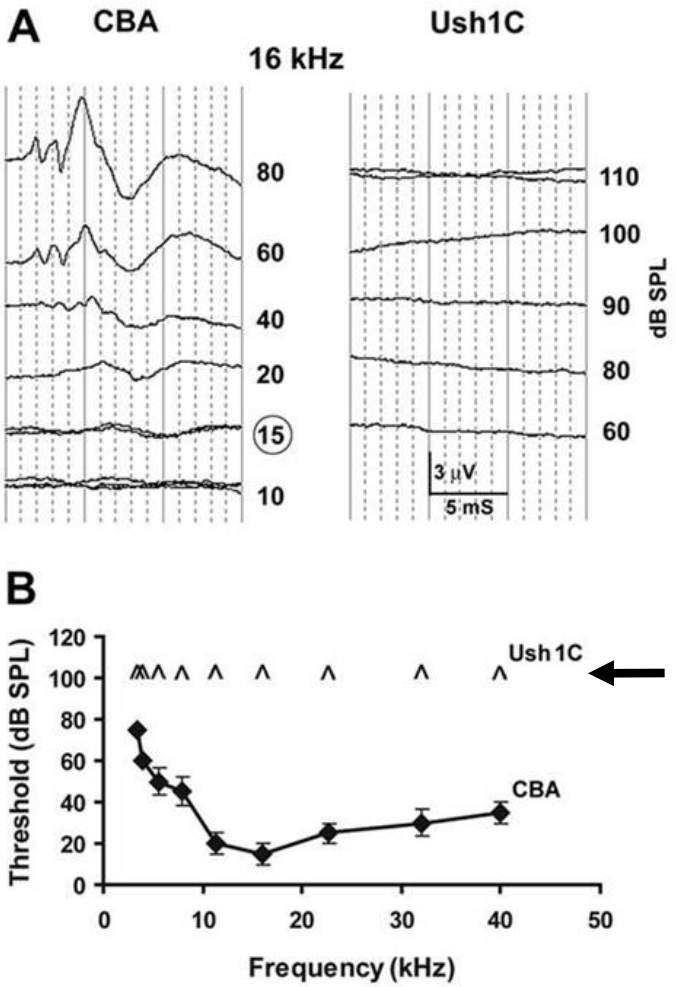
216AA mice express truncated mRNA in cochlea and retina

Lentz et al 2007

216AA mice have vestibular dysfunction and are deaf

Circling and head tossing behavior

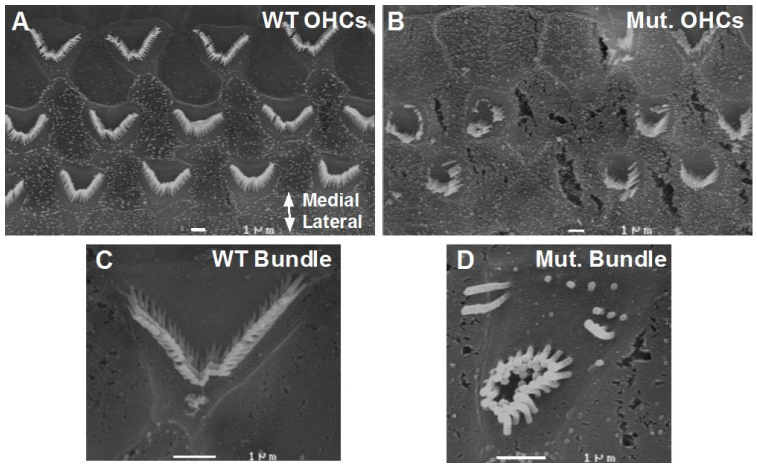
No Auditory-evoked Brainstem Response (ABR)



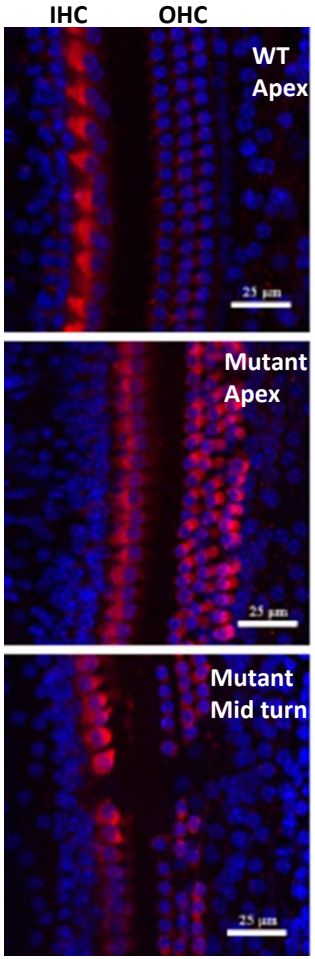
No ABR at high, mid or low frequencies

Lentz et al 2010

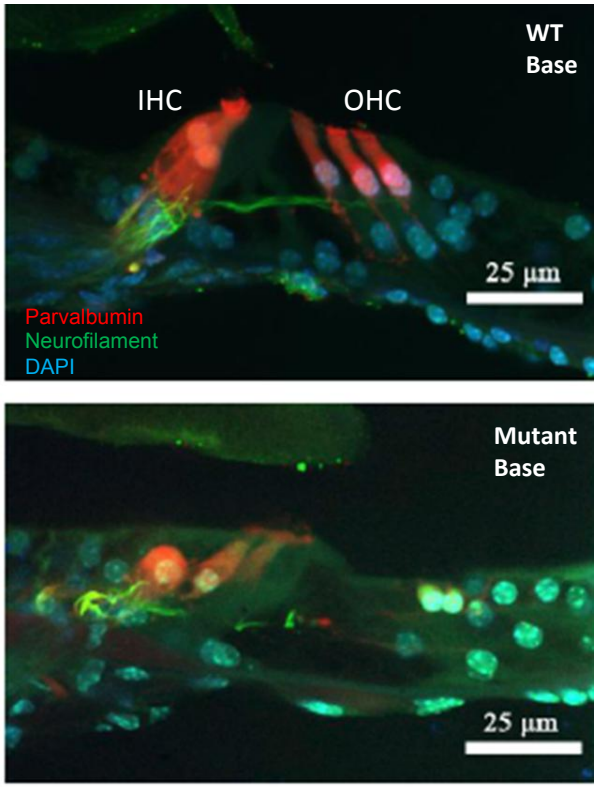
216AA mice have abnormal bundles and missing hair cells



Abnormal bundles



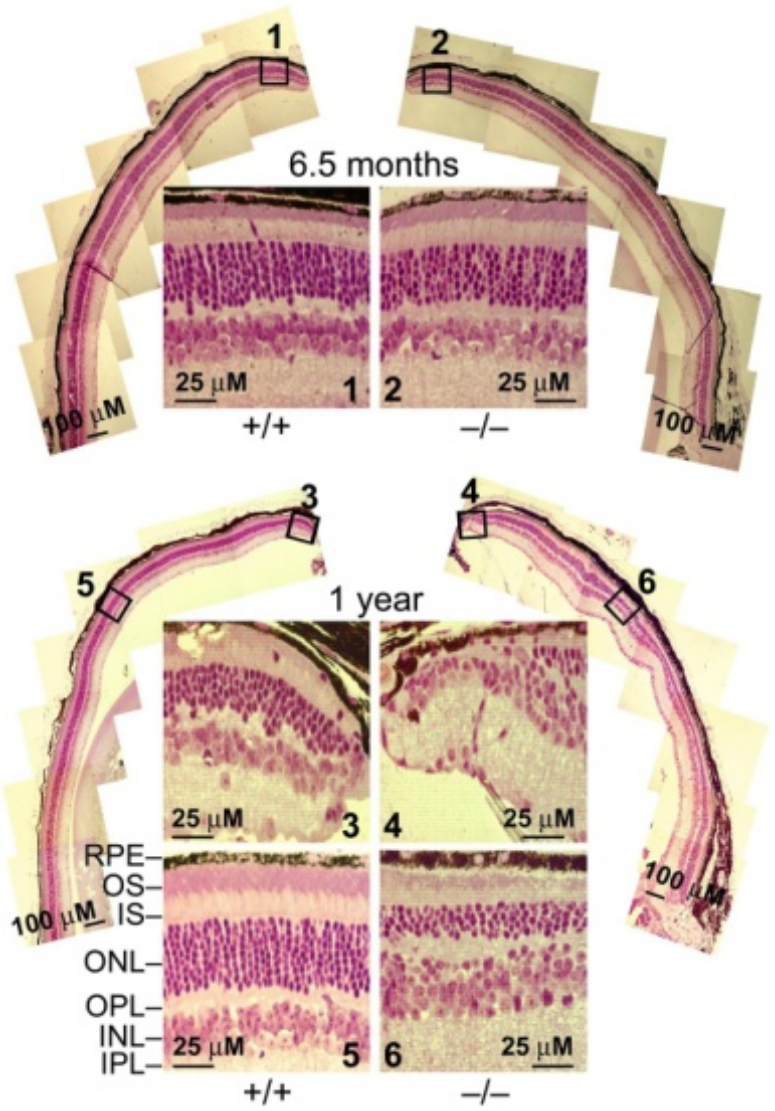
Disorganized
OHC rows

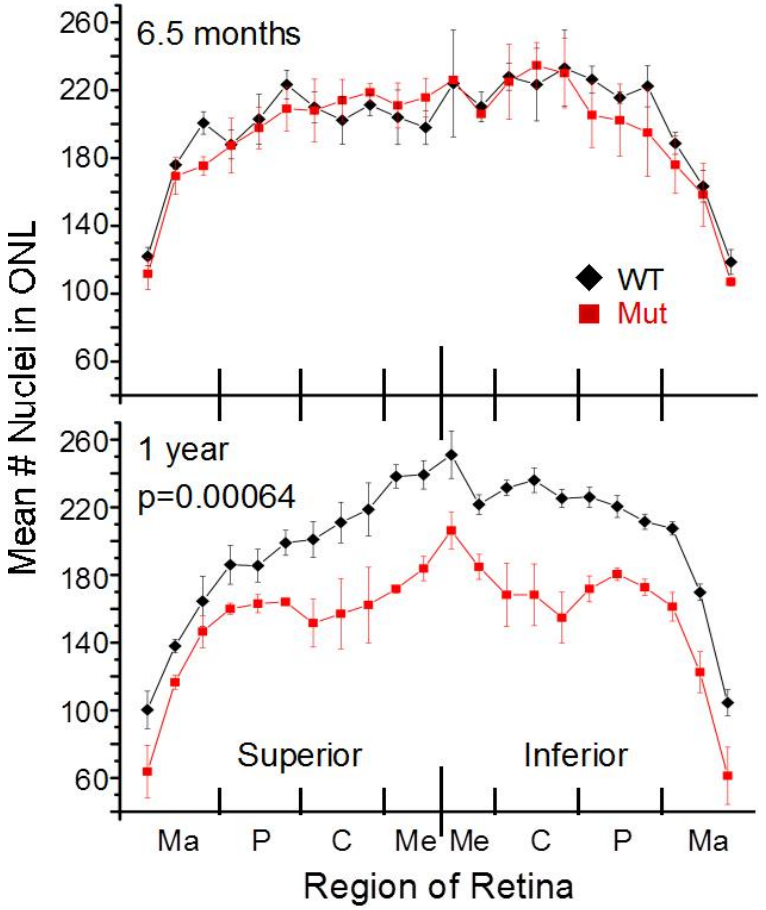


Missing IHC & OHCs

Lentz et al 2010

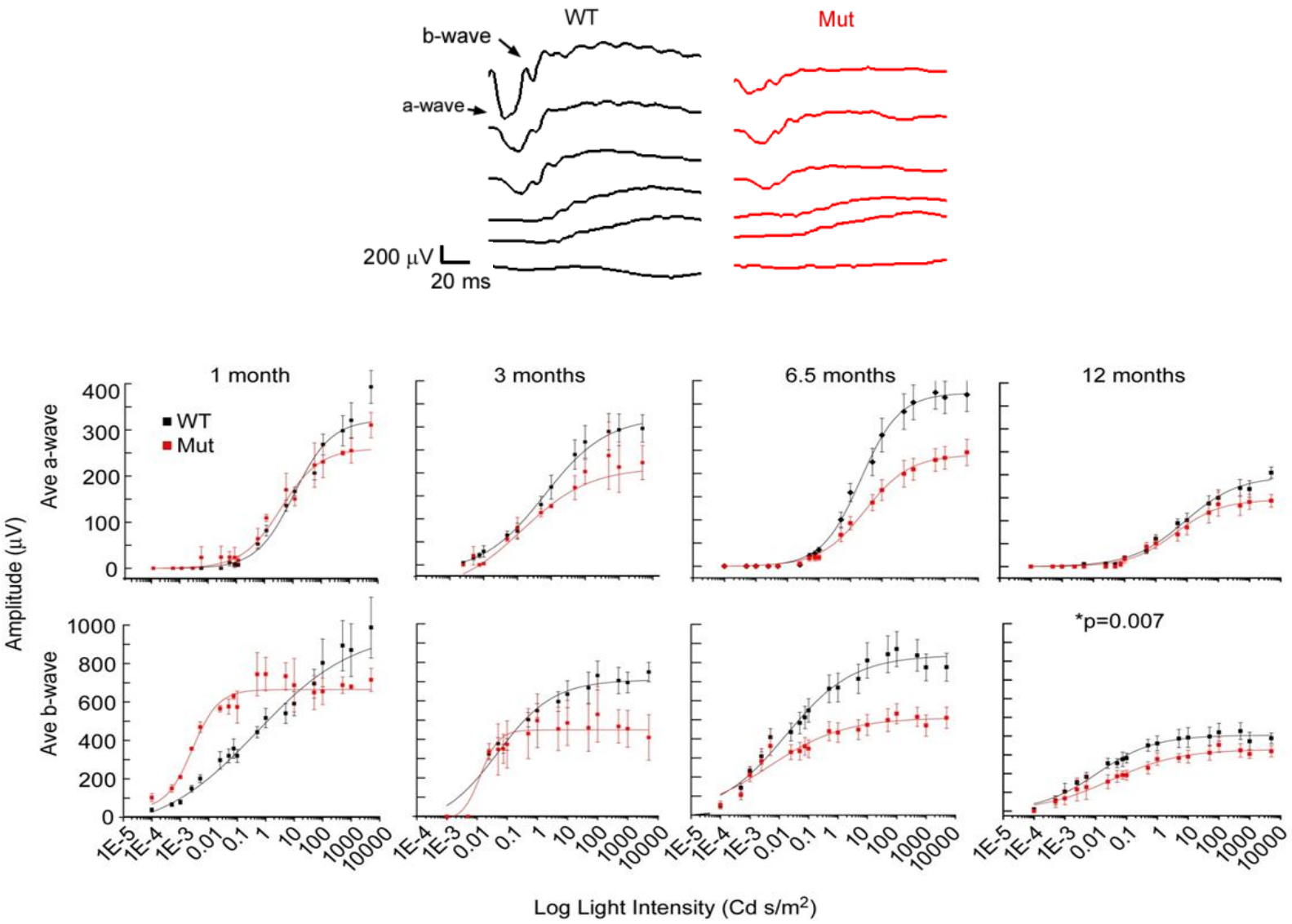
216AA mice have progressive photoreceptor degeneration





Lentz et al 2010

216AA mice have reduced visual function and retinal degeneration

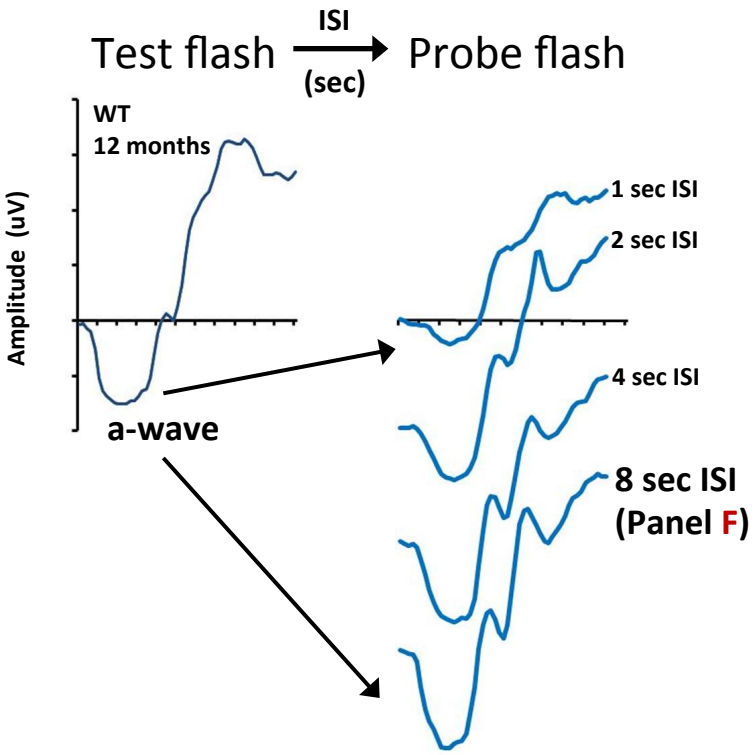


Lentz et al 2010

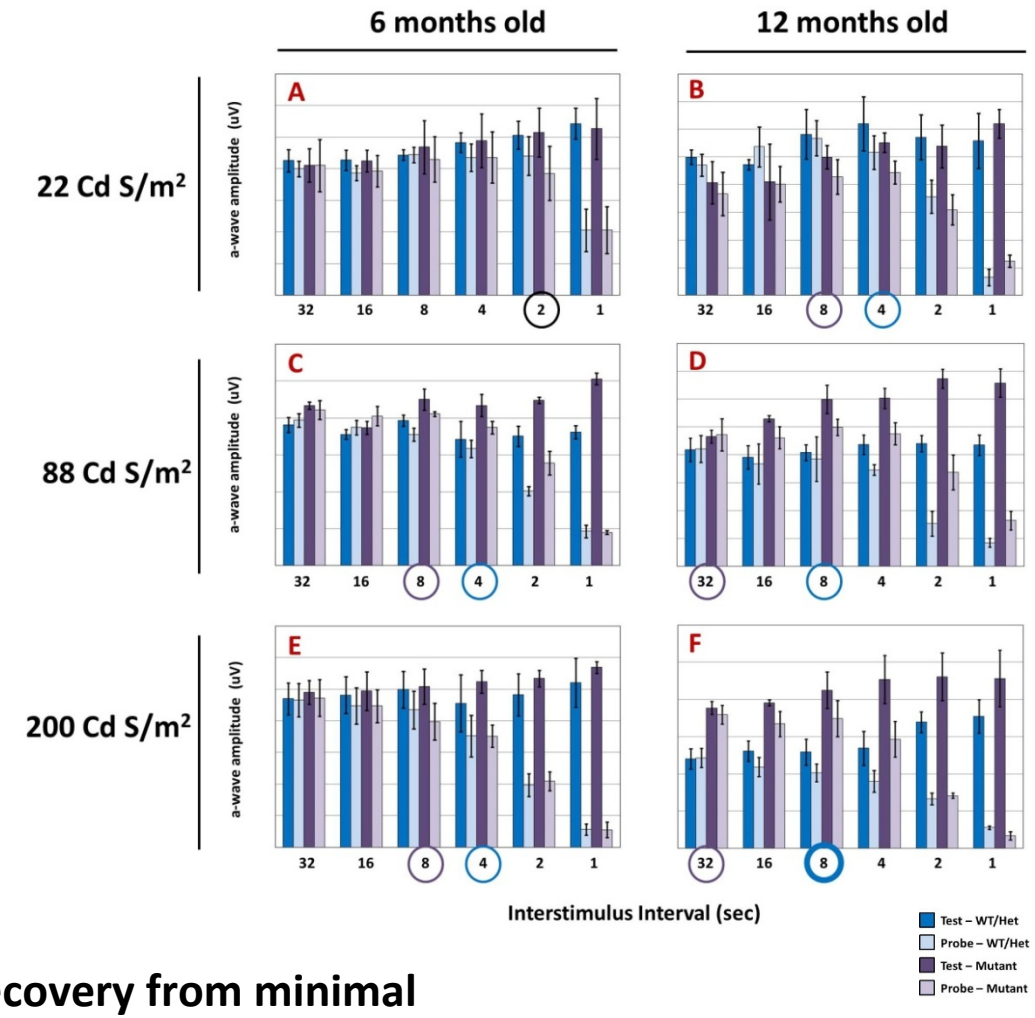
216AA mice have slow rod adaptation

Twin flash protocol

Minimal Bleach (> 1% rhodopson)



Deactivation of Rhodopsin Kinetics



a-wave recovery from minimal bleach takes 2 – 4 times as long

Lentz unpublished data

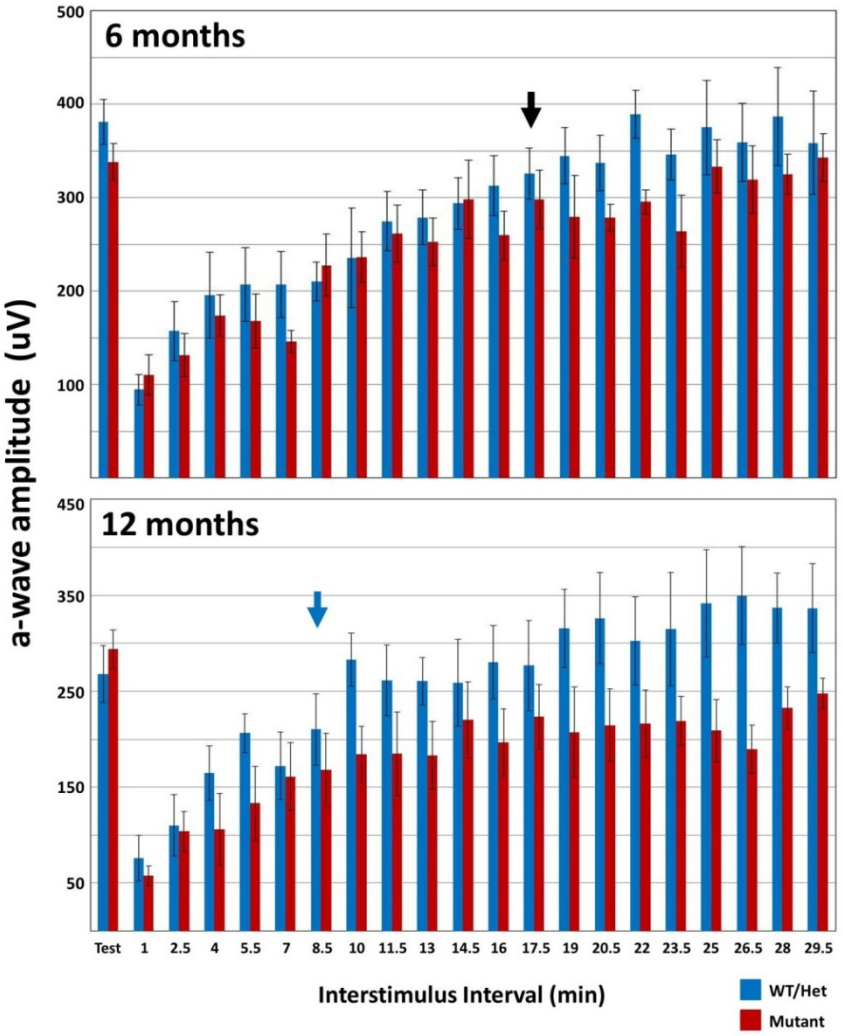
Visual Cycle Kinetics

Paired flash protocol

Bleach 30-40 % Rhodopson

Test flash —ISI (min)→ Probe flash

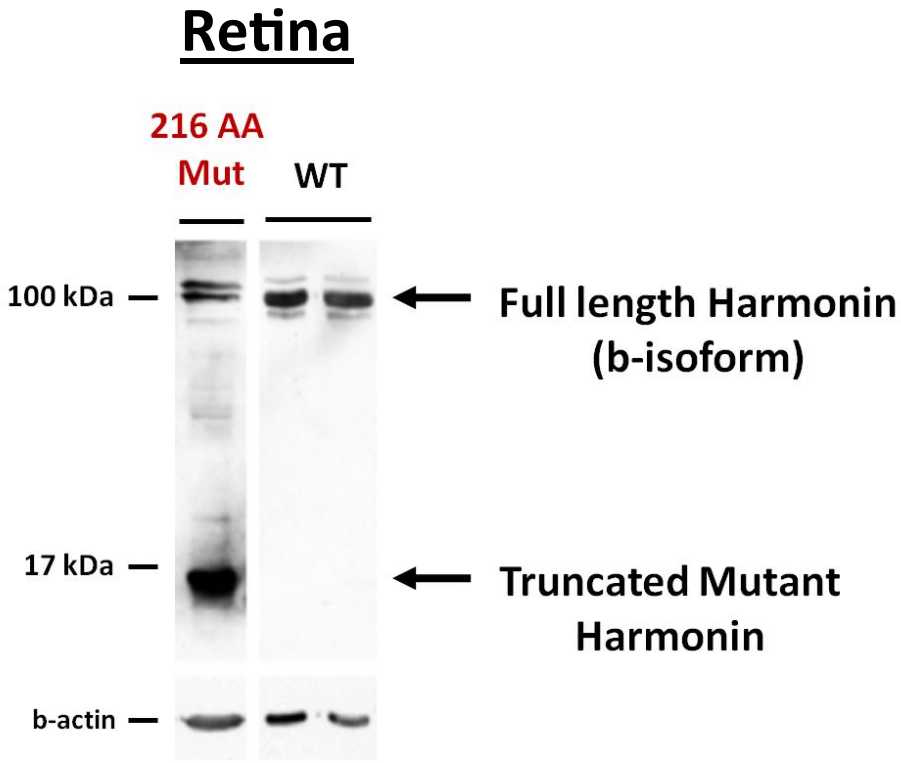
3000 lux
2 min



Mutant a-wave not recovered after 30 min

Lentz unpublished data

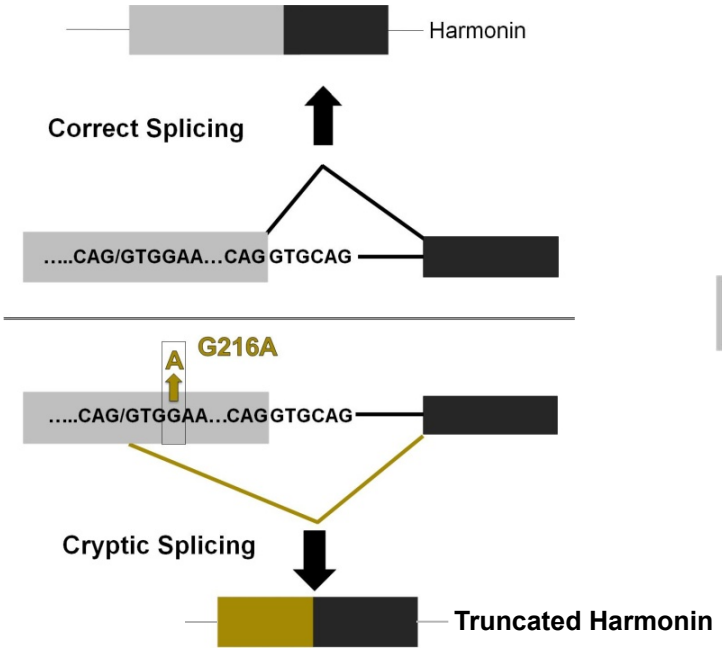
216AA mice express both wt and mutant harmonin

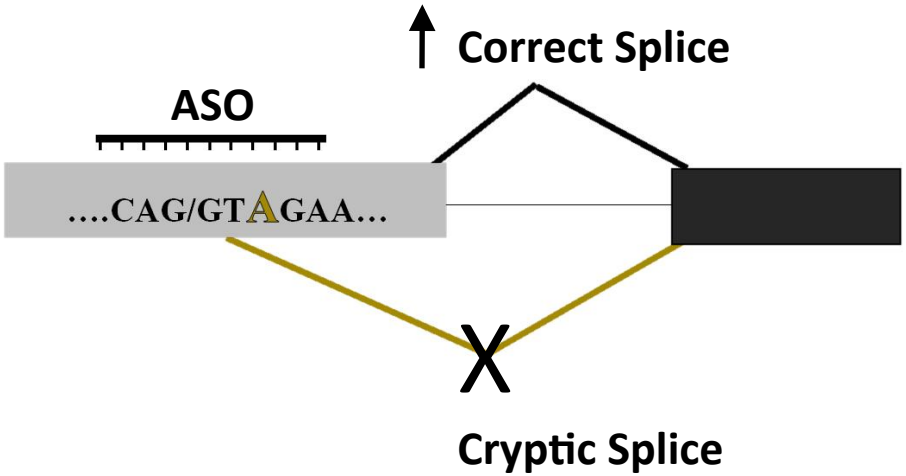


Can we detect both wt and mutant Ush1c mRNA?

Lentz unpublished data

Can we modulate the use of the mutant and wild type splice sites by treating with antisense oligonucleotides (ASOs)?





ASO targeted to 216 mutation prevents cryptic splicing and forces correct splicing

Antisense Oligonucleotides (ASOs)

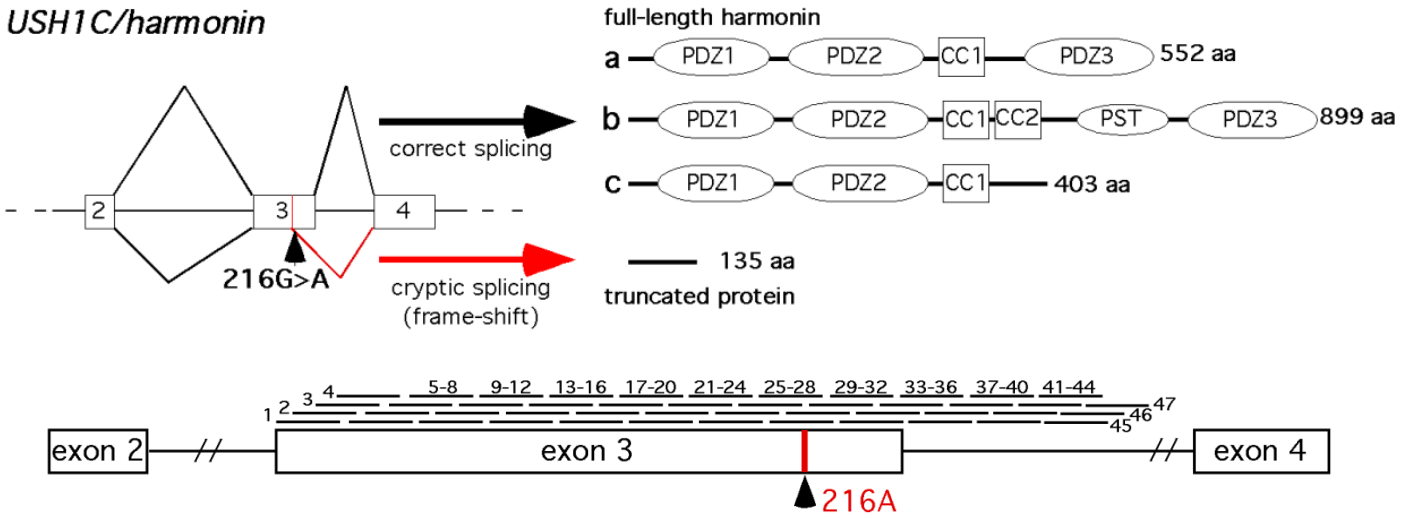
ASOs are small molecules that are

- designed to have high affinity for their RNA target through unique sequence base pairing

- resistant to degradation, which allows for high potency and specificity, and low toxicity

ASO-based therapeutics have been FDA approved for other conditions and toxicity studies have proven the ASO chemistry is not toxicity for humans

ASO design & optimization



1. **Tested 50 different ASOs in cell lines transfected with 216A minigenes**
   Selected an ASO that showed the largest increase in correct splicing and the largest decrease in cryptic splicing

2. **Tested that ASO in patient cell lines and cells from 216AA mice**
   Ush-ASO corrected cryptic splicing and increased correct splicing

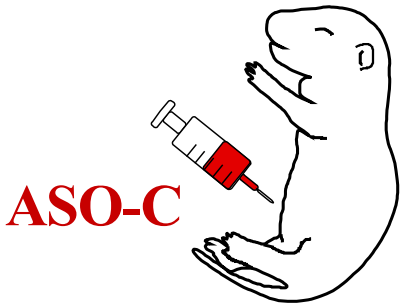
3. **Tested the Ush-ASO in Adult mice**
   Ush-ASO corrected cryptic splicing and increased correct splicing in a dose dependent manor

Treatment Model with ASOs

Usher pup

Injection Time
(Postnatal Day)

P3-5, P10 or P16-18



Usher pup

Behavior

Vestibular function (Open-field chamber)

Physiology

Hearing function (ABRs, preyer reflex)

Histology

Hair cell morphology (Immunohistochemistry)

Molecular

***Ush1c* and Harmonin Expression**

Lentz & Hastings

Treatment of 216AA mice with Ush-ASO

Vestibular Function-

Single treatment between postnatal day 5 (P5) – P13 rescues vestibular function

Hearing function-

Single treatment at P5 restores hearing at low – mid frequencies similar to wild type levels

Histology-

Single treatment at P5 rescues harmonin expression in the hair cell bundle and at the synapse; partially restores hair bundle structure; and rescues hair cells at the apex and middle turn

Molecular-

Single treatment at P5 decreases cryptic splicing and increases wt expression of *Ush1c* and harmonin in the ear

Conclusions

ASOs targeted to the 216A mutation rescue gene and protein expression in patient and mouse cell lines

A single systemic injection in 216AA mice-

- **Cures vestibular dysfunction**
- **Rescues hearing at low and mid frequencies**
- **Partially restores hair bundle morphology and decreases hair cell loss**
- **Partially rescues *Ush1c* and harmonin expression in the ear**

Future Directions

Further develop an ASO treatment regiment in our mouse model to prevent deafness and vestibular defects with the goal of providing pre-clinical data that would lay the framework for clinical trials in patients

Test the ASO for the treatment of blindness in our mouse model

Continue studies in the ear and eye to understand how the 216A mutation causes deafness and blindness

ACKNOWLEDGEMENTS

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