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
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**

216G>A mutation

**USH1C gene**

**mRNA splice variants**

**Harmonin isoforms**

Reiners et al. 2006
Gene Expression in USH1C Patients

**Wild-type Harmonin**
MDRKVAREFRHKVDFLIENNDAEKDLYDVLRLMYHTMDVAVLVGLDLKVINEPSRLPLFDAIRPLIPLKHKQVYEQDQTFRRESRKLKEVRDLRLPEEGLLSVRGLFCCGLFISSLIKGQADSVGLOVGDIVRINGYSIS8C8HEVIIILN1RTKVKTSIKVRHGLIPKSSPEPLTWQYDQFSESQGSGVGLSGPNRENEEKVFINOSRGLGCSISSGPQPKPGIFSIVKPGSLSAEVCLEIGDOIVEVNGVFSNLHKEADVNKLNSRLTISISVAAAGRFMTDRERLAEARQRELQELMLMQKRLAMESNKLQEQQEMERQRKKEIAQKAAEENERYKEMEQIVEEEEKKQWEDWSKKEQILLPITITAEVHPVLRPKPYDQGVREPELADDLDGTELQDQDFRKYEEGFDYSMPFTFPQIQMGKVRLLRRIKKEGBDLALAGEGVDSPI

**Truncated 216 A mRNA Translation**
MDRKVAREFRHKVDFLIENNDAEKDLYDVLRLMYHTMDVAVLVGLDLKVINEPSRLPLFDAIRPLIPLKHQEAEGGASGPSAPRRPPPECAPWGVLWLALHPHQHQRSSGRQRRAPGRGRDRDPQNIFHLLLYP

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

*Ush1c* genotype

Lentz et al 2007
216AA mice have vestibular dysfunction and are deaf

No Auditory-evoked Brainstem Response (ABR)

Circling and head tossing behavior

Lentz et al. 2010
216AA mice have abnormal bundles and missing hair cells

Abnormal bundles

Disorganized OHC rows

Missing IHC & OHCs
216AA mice have progressive photoreceptor degeneration

Wild Type 216AA

[Images of histological sections showing progressive photoreceptor degeneration in 216AA mice compared to Wild Type.]

Mean # Nuclei in ONL

6.5 months

1 year

p=0.00064

Region of Retina

Superior Inferior
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% rhodopsin)

Test flash $\xrightarrow{\text{ISI (sec)}}$ Probe flash

**Deactivation of Rhodopsin Kinetics**

<table>
<thead>
<tr>
<th></th>
<th>6 months old</th>
<th>12 months old</th>
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<tbody>
<tr>
<td>Test</td>
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- **Panel A**: 22 Cd S/m²
- **Panel B**: 88 Cd S/m²
- **Panel C**: 200 Cd S/m²

Amplitude (µV)

- WT 12 months
- 1 sec ISI
- 2 sec ISI
- 4 sec ISI
- 8 sec ISI

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

Lentz unpublished data
Paired flash protocol
Bleach 30-40 % Rhodopsin

Test flash $\rightarrow$ Probe flash
3000 lux
2 min

Mutant a-wave not recovered after 30 min

Visual Cycle Kinetics

*WT/Het*

*Mutant*
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
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

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**Behavior**
Vestibular function (Open-field chamber)

**Physiology**
Hearing function (ABRs, preyer reflex)

**Histology**
Hair cell morphology (Immunohistochemistry)

**Molecular**
Ush1c and Harmonin Expression

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ASO-Ush

Injection Time (Postnatal Day)

P3-5, P10 or P16-18

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ASO-C

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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 regimen 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.
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