Advances in Drug Therapy for Usher Syndrome

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Overall goal

Usher syndrome update and current hypotheses

New therapeutic approaches

Read-through small molecules

Antisense oligonucleotides (ASOs)

Future Directions
Overall Goal for the scientific and medical Usher community

- Develop tools that provide a medical benefit to every individual with Usher syndrome
Usher Syndrome Update

• Prevalence
  – Autosomal, recessive, genetic disease = males and females are equally affected; and you have to inherit 2 copies of a mutation (1 copy from mom and 1 copy from dad)
  – Leading genetic cause of deaf-blindness
  – ~ 1 in 20,000 individuals worldwide
  – 1 in 6,000 in 2 pediatric populations (hearing impaired children)
  – Both rare and common
Usher Syndrome Update

- Significant progress has been made to identify genes and mutations that cause Usher
- 3 clinical types – USH1, USH2, USH3
- 1 new clinical type – atypical USH (CEP250); early onset hearing impairment, mild RP
- Usher genes
  - 16 loci (different places in our genome)
  - 13 causative genes and 1 modifier gene (PDZD7) have been identified
# Usher syndrome – Types and Genes

<table>
<thead>
<tr>
<th>Type</th>
<th>Presentation</th>
<th>Locus</th>
<th>Gene</th>
<th>Protein</th>
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</thead>
<tbody>
<tr>
<td><strong>USH1</strong></td>
<td><strong>35-45%</strong></td>
<td>Congenital, severe-profound HI Vestibular Areflexia Adolescent onset RP</td>
<td>USH1B</td>
<td>MYO7A</td>
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<td></td>
<td></td>
<td></td>
<td>USH1C</td>
<td>Harmonin</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>CDH23</td>
<td>Cadherin 23</td>
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<td></td>
<td>USH1F</td>
<td>Protocadherin 15</td>
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<td></td>
<td>USH1G</td>
<td>Sans</td>
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<td></td>
<td>USH1J</td>
<td>Calcium- and integrin-binding protein 2</td>
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<tr>
<td><strong>USH2</strong></td>
<td><strong>55-65%</strong></td>
<td>Congenital, mild-severe HI Late adolescent-early adult onset RP</td>
<td>USH2A</td>
<td>USH2A</td>
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<tr>
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<td>ADGRV1 (GPR98)</td>
<td>G protein-coupled receptor 98</td>
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<td></td>
<td></td>
<td></td>
<td>USH2B</td>
<td>GTPb</td>
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<td></td>
<td></td>
<td></td>
<td>USH2D</td>
<td>Whirlin</td>
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<tr>
<td><strong>USH3</strong></td>
<td><strong>5%</strong></td>
<td>Post-lingual, progressive HI Adult onset RP Variable Vestibular Responses</td>
<td>USH3A</td>
<td>CLRN1</td>
</tr>
<tr>
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<td></td>
<td></td>
<td>HARS</td>
<td>Histidyl-tRNA synthetase</td>
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</table>
HYPOTHESIS: mutations in Usher genes result in defects in hair cell development and photoreceptor maintenance
Gene (code) → RNA (message) → Proteins (perform functions)

Gene

RNA

Proteins
Mutation types

**Nonsense** – a mutation that prematurely stops the making of a protein

**Missense** – a mutation that changes the code of the protein by 1 amino acid

**Splicing** – a mutation that alters the processing of the RNA used to code for a protein

**Insertions/Deletions** (INDELS) – insertions or deletions of part of a gene, which then alters the code of a protein

**HYPOTHESIS**: New data suggest a strong genotype–phenotype correlation that is based on mutation type.
Genotype – Phenotype Correlation

- Relationship between gene/mutation (genotype) and symptoms (phenotype)

- For at least 4 USH1 genes (MYO7A, CDH23, PCD15 and USH1C), there appears to be a genotype-phenotype correlation in patients. Nonsense/indel/splicing mutations that result in essentially no protein being made cause USH1; whereas missense or splicing mutations that result in a small amount of protein cause hearing loss alone and not RP.

- All USH genes make multiple forms of their encoded protein. Single gene makes several mRNAs (splicing), which encode several forms of a protein. These different forms of a protein have different functions in hair cells and photoreceptors. Different types of mutations in different areas of the gene may affect all of the forms of a protein or only some, and this may contribute to the differences in phenotypes.

- Natural History Studies aim to determine a genotype-phenotype correlation. Understanding natural history will allow us to be able to predict timing and severity of symptoms; which is needed to conduct clinical trials because it tells us when to give therapy and how to determine if the therapy is working.
New Therapeutic Strategies

• Target deafness and/or blindness in general
  – Treatment for any type of Usher
    ➢ Stem cell therapy to replace cochlear and/or retinal cells
      • Skin or blood sample, turn them into stem cells, then coax them into becoming retinal cells
    ➢ Optogenetics (gene therapy)
      • Deliver genes that give light sensitivity to different cells of the retina

• Target a particular gene
  – Treatment would target one type of Usher regardless of mutation
    ➢ Gene replacement therapy – deliver a normal copy of a gene to replace the one with a mutation
      • Viral mediated – USH1B (clinical trial); USH1C; USH2A (dual vector); USH3A
      • Nanotechnoloty – USH2A
New Therapeutic Strategies

• Target a particular mutation type
  – Treatment of any Usher type caused by a nonsense mutation
    ➢ Translational read-through inducing drugs – USH1C

• Target a particular mutation in a particular gene
  – Treatment for one type of Usher caused by one specific mutation
    ➢ Antisense oligonucleotides – USH1C (USH1C c.216G>A)
    ➢ Small Molecule Chaperone Therapy – USH3A (CLRN1 p.N48K)
Therapeutic Strategies under development for USH1C

There are 2 strategies in the preclinical testing phase in the research laboratory-

1. Translational read-through inducing drugs (TRIDs)
2. Antisense Oligonucleotides (ASOs)
**USH1C gene and Harmonin Protein Isoforms**

**c.216G>A Splicing mutation (ASO)**

**Nonsense mutation (TRID)**

**USH1C gene**

**mRNA splice variants**

**Harmonin isoforms**

Reiners et al 2006
Translational read-through inducing drugs (TRIDs)

TRIDs – type of aminoglycosides (antibiotics)

Target ribozymes, which are the proteins responsible for translating mRNA into proteins; site of protein synthesis.

Insert a random amino acid at the nonsense mutation site to prevent stopping translation and a truncated protein.
Translational read-through inducing drugs (TRIDs)

Currently being tested in the research laboratory on nonsense mutations in the USH1C gene, but would work on any nonsense mutation in any USH gene

~ 12% of USH mutations are nonsense mutations

2 different TRIDS have been tested to correct the p.R31X mutation in USH1C in the eyes of laboratory animals; and full length Harmonin protein was detected (Goldmann et al, 2012)

**Status**: developing a better model to test for efficacy
- animal model with retinal degeneration
- patient cell lines
Development of Antisense oligonucleotide therapy for USH1C in Louisiana

- Nearly all type 1 Usher in Louisiana is caused by the c. 216G>A mutation in *USH1C*

**USH1C gene expression in USH1C Patients**

- Truncated mRNA
- Truncated protein

**Ush1c216 genotype**

- USH1C Patient
- USH1C Patient
- USH1C Patient

**c.216G>A** mutation in *USH1C* gene
Put in the human 216A mutation into the Mouse \textit{Ush1c} gene (knock-in)

\textbf{Cochlea}

\textbf{Retina}

\text{Lentz et al. 2007}
USH1C 216AA Mice

Abnormal/no ABRs

Circling and head tossing behavior

Deaf

Vestibular Defects

Reduced electroretinograms (ERGs) & photoreceptor loss

Visual Dysfunction & Degeneration

Lentz et al 2010
Antisense Oligonucleotides (ASOs)

- ASOs - Short, modified RNA molecules
- Targets complementary RNA in cell
- USH-ASO targets USH1C 216A-RNA to correct splicing
- Treatment for USH1C caused by the \textit{USH1C} c.216G>A mutation

\textbf{USH1C/harmonin}

<table>
<thead>
<tr>
<th>a</th>
<th>PDZ1</th>
<th>PDZ2</th>
<th>CC1</th>
<th>PDZ3</th>
<th>552 aa</th>
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<tbody>
<tr>
<td>b</td>
<td>PDZ1</td>
<td>PDZ2</td>
<td>CC1</td>
<td>CC2</td>
<td>PST</td>
</tr>
<tr>
<td>c</td>
<td>PDZ1</td>
<td>PDZ2</td>
<td>CC1</td>
<td></td>
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</tbody>
</table>

- full-length harmonin
- cryptic splicing (frame-shift)
- 135 aa truncated protein
Laboratory Treatment Model with ASOs

- **Behavior**
  - Vestibular function (Open-field, swimming)

- **Physiology**
  - Hearing function (ABR, DPOAE)
  - Visual function (ERG)

- **Structure**
  - Hair cell morphology (IHC)
  - Photoreceptor cell morphology (IHC)

- **Molecular**
  - Ush1c and Harmonin expression
ASO treatment improves balance in Usher mice

Open-field Chamber to measure vestibular function

Lentz et al 2013
ASO treatment improves hearing in Usher mice

Example Hearing Audiograms (ABRs)

Lentz et al 2013
Development of ASO Therapy for USH1C

- Treatment for the Acadian USH1C c.216G>A mutation
- Treatment of Usher mice improves hearing and balance (Lentz et al 2013); currently testing for vision improvements

Status:

- preclinical animal testing in progress
  - testing effects of different doses
  - testing effects of timing of doses
  - testing for how long improvements last
- Natural clinical history in patients
Progress with success or promise in the development of treatments for Usher syndrome

**USH1B** - Ush-Stat clinical trial: gene replacement therapy

**USH1C** – ASOs, TRIDs, gene replacement therapy

**USH2A** – Gene therapy (viral, nanotechnology)

**USH3A** – Gene therapy, small molecule chaperone therapy
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USH1C.216G>A

2000
USH1C.216G>A causes Usher syndrome type 1C
Bitner-Glindzics et al
Verpy et al

2005
Knocked-in 216A

2010
Deaf, vestibular defects
Retinal degeneration
Lentz et al

2013
Rescue of deafness, vestibular defects
Lentz et al

Further develop Ush-ASO: treat deafness/blindness in Usher mice