Treatments of the Future for Usher Syndrome: the future is now

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I have nothing to disclose
Early Identification of babies with hearing loss

More and more deaf and hard of hearing babies are being identified early

<table>
<thead>
<tr>
<th>Year</th>
<th>Babies</th>
</tr>
</thead>
<tbody>
<tr>
<td>2000</td>
<td>855</td>
</tr>
<tr>
<td>2005</td>
<td>2,634</td>
</tr>
<tr>
<td>2016</td>
<td>6,337</td>
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</table>

[https://www.cdc.gov/ncbddd/hearingloss/ehdi-data.html](https://www.cdc.gov/ncbddd/hearingloss/ehdi-data.html)  10.10.18
Fun facts about DNA

-A human has 20,000 genes
-67 billion miles of DNA in each person
-99.6% of a person’s DNA is identical to all other people
-99% of DNA does not directly code for proteins (but the rest is not junk...)

*Enhancers, promoters, silencers, insulators
*Codes for tRNA, rRNA, miRNA
*Structural elements of chromosomes--
Sex chromosomes

Autosomes
The DNA from one cell stretches 7.5 feet.

All of the DNA in your body would stretch from here to the moon 300,000 times.

http://www.accessexcellence.org/AB/GG/
How DNA is stored
The Central Dogma
<table>
<thead>
<tr>
<th>DNA level</th>
<th>mRNA level</th>
<th>protein level</th>
</tr>
</thead>
<tbody>
<tr>
<td>TTC</td>
<td>TTT</td>
<td>Lys</td>
</tr>
<tr>
<td>ATC</td>
<td>TCC</td>
<td>Arg</td>
</tr>
<tr>
<td>TGC</td>
<td>AGG</td>
<td>Thr</td>
</tr>
<tr>
<td>AAG</td>
<td>AAA</td>
<td>Lys</td>
</tr>
<tr>
<td>UAG</td>
<td>ACG</td>
<td></td>
</tr>
<tr>
<td>Lys</td>
<td>STOP</td>
<td></td>
</tr>
</tbody>
</table>

**Point mutations**

- **Silent**
- **Nonsense**
- **Missense**
  - Conservative
  - Non-conservative
## USH Genes and When Identified

<table>
<thead>
<tr>
<th>Locus</th>
<th>Gene</th>
<th>Year</th>
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<tbody>
<tr>
<td>USH1B</td>
<td>MYO7A</td>
<td>1995</td>
</tr>
<tr>
<td>USH1C</td>
<td>USH1C</td>
<td>2000</td>
</tr>
<tr>
<td>USH1D</td>
<td>CDH23</td>
<td>2001</td>
</tr>
<tr>
<td>USH1E</td>
<td></td>
<td>1997</td>
</tr>
<tr>
<td>USH1F</td>
<td>PCDH15</td>
<td>2001</td>
</tr>
<tr>
<td>USH1G</td>
<td>SANS</td>
<td>2003</td>
</tr>
<tr>
<td>USH1H</td>
<td></td>
<td>2009</td>
</tr>
<tr>
<td>USH2A</td>
<td>USH2A</td>
<td>1998</td>
</tr>
<tr>
<td>USH2C</td>
<td>ADGRC1/VLGR1/GPR98</td>
<td>2004</td>
</tr>
<tr>
<td>USH2D</td>
<td>WHRN</td>
<td>2007</td>
</tr>
<tr>
<td>USH3A</td>
<td>CLRN1</td>
<td>2001</td>
</tr>
</tbody>
</table>
Other possible USH genes

- **CIB2**: Probably just non-syndromic hearing loss 2018
- **PDZD7**: Probably only non-syndromic hearing loss 2015
- **HARS**: Found in Old Order Amish. 80 other genes
Usher Syndrome

- 9 genes identified: 5/9 Ush1, 3/5 Ush2, one Ush3
- Trafficking, scaffolding, development and maturation
- Cells are terminally differentiated
Seven steps to treatment for an Inherited Disease (Bill Kimberling)

- Find the disease gene
- Correlate genotype with phenotype
- Find or develop animal models
- Elucidate the disease mechanism
- Find or develop an effective treatment in the animal model
- Screen the human population to identify people who might benefit
- Test the treatment in these people
  - Orphan diseases, small numbers
Clinical Trials.gov

- 17 studies listed
- Most are completed, not recruiting, or terminated
- Recruiting:
  - ProQR--QR-421a for USH2A
  - SCOTS2 (Stem Cell Ophthalmology Study II; bone marrow derived stem cells)
- UshStat..following patients already treated
Strategies for Gene therapy

- Correct
- Replace
- Modify
- Restore absent genetic function
- Override abnormal function
- Inhibit abnormal gene function
Techniques for Gene therapy

- Gene editing: CRISPR-Cas9, etc.
- Replacement genes attached to viral vectors
- Exon skipping; Oligosense nucleotides
- RNAi
- Inner ear organoids
- Stem cells
- Nanoparticles
- Small molecules
**1. Determine level of normal protein needed to improve cellular function**

Achieve ≥30% of normal hemoglobin levels to prevent cell sticking.

**2. Determine percentage of functioning cells needed to improve organ function**

Achieve function in ≥60% of cells to improve blood passage and avoid a vasculo-occlusive crisis.

**End Determination**

Achieve ≥30% of normal hemoglobin levels in ≥60% of cells to improve blood passage and avoid a vasculo-occlusive crisis.
Viral vectors for inner ear gene therapy

<table>
<thead>
<tr>
<th>Feature</th>
<th>Adenovirus</th>
<th>Lentivirus</th>
<th>AAV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transfection Efficiency</td>
<td>Close to 100%</td>
<td>~30%</td>
<td>30-40%</td>
</tr>
<tr>
<td>Host genome integration</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Packaging Capacity</td>
<td>8-34kb</td>
<td>8.5kb</td>
<td>4kb</td>
</tr>
<tr>
<td>Protein Expression Level</td>
<td>High</td>
<td>medium</td>
<td>low</td>
</tr>
<tr>
<td>Ease of Scaling-up/Amplification</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Ease of High Viral Titer (≥10^10 vp/ml)</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
</tr>
</tbody>
</table>
USH Gene therapy for Hearing/Vestibular Loss

- Ush1C (Harmonin). Lentz et al (2013) used antisense oligonucleotides for correction of splicing, correcting defective mRNA.

- Using same model of Ush1c, Pan et al (2017) delivered 2 splice forms of harmonin (a1 and b1) were delivered with AAV2/Anc80 vector via the round window in mice.

- USH3 (Clarin-1)

- Ush1G (sans) AAV8 with sans cDNA; partial restoration of hearing and balance (Empotoz et al, 2017).

- HRN (whirlin, USH2D) AAV8-Whirlin cDNA via round window (Chien et al, 206) and the PSCC (Isgrig et al, 2017).
**USH1C Viral Gene Therapy**

### Full-length harmonin

- **a** PDZ1 - PDZ2 - CC1 - PDZ3 552 aa
- **b** PDZ1 - PDZ2 - CC1KCC2 - PST - PDZ3 899 aa
- **c** PDZ1 - PDZ2 - CC1 - 403 aa

**Cryptic splicing (frameshift)**

135 aa

**Truncated protein**

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**16 kHz audiograms**

- **A**
  
- **B**

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*Lentz et al. 2005, 2010, Tian et al. 2010*
Recent USH3 work

- Alagramam et al, 2016. Small molecule stabilized hearing in a mouse model of *Clrn1*
- Dulon et al, 2018. Identified clarin-1 as a key organizer of IHC ribbon synapses. Used AAV mediated *Clrn1* transfer into hair cells durably improving hearing in *Clrn1* conditional k/o
Eye is accessible, immune-privileged, has a tight-ocular barrier, and can be non-invasively monitored.

First gene therapy trial for USH was carried out with lentiviral delivery of MYO7A for USH1B injected into the subretinal space of mice (Hashimoto et al, 2007). A phase I/II clinical trial of LV-MYO7A (UshStat; SAR421869) has been underway since 2012.

In 2017, voretigene neparvovec-rzyl (Luxturna, Spark Therapeutics) received FDA approval and became the first gene therapy targeting a disease caused by specific gene mutations to be approved in the United States. Bennett et al have recently (2016) reported on durability and safety of injection in contralateral eye in children with RPE65-mediated blindness.
December 2017: First retinal gene therapy is approved

On December 19, 2017, the U.S. Food and Drug Administration approved a new gene therapy (AAV2-hRPE65v2Luxturna), manufactured by Spark Therapeutics in Philadelphia.

Luxturna is the first gene therapy approved in the United States that’s directly administered into the eye, targeting diseases caused by mutations in the gene RPE65. Mutations in this gene can produce Leber’s congenital amaurosis or retinitis pigmentosa, both rare but potentially blinding diseases.
December 2017: First retinal gene therapy is approved

https://www.youtube.com/watch?v=jTVW-E5Cw2U

https://www.youtube.com/watch?v=IAo9Jdqrdo
Therapeutics

- ProQR - RNA-based therapies
  - Leber’s Congenital amaurosis (LCA10)
  - USH2A
    - Exon 13 skipping strategy; goal is to end up with a shortened but functional protein
    - Second mutation, PE40 in USH2A
- Editas – CRISPR-based therapeutics
- Eloxx – USH2A; read through strategy
  - LCA10; eliminate mutation in CEP290
- jCyte – stem cells
  - Retinitis pigmentosa
- Frequency Therapeutics – progenitor cell activation
  - Sudden Hearing Loss; noise related hearing loss
ProQR Therapeutics

- STELLAR trial; Phase 1/2
- QR-421a
- Exon 13 skipping can be induced with an oligonucleotide to mask the splice site in an intron
- With exon skipping, a more functional RNA is produced, leading to some degree of functional protein
- One of 3 doses into one eye, or sham procedure
- Mass Eye and Ear; Univ. of Michigan; Casey Eye (Oregon); Retina Foundation of the Southwest (Dallas); UZ Gent (Belgium); Centre de maladies rares CHNO des Quinze Vingts (France)
Exon Skipping Technology

DNA

Transcription

Mutation

Antisense oligo

pre-mRNA

mRNA

Translation

Functional protein
Eloxx

- Eukaryotic ribosomal selective glycoside (ERSG) compounds designed to treat premature stop codon diseases.

- Read-through therapeutic development is focused on extending mRNA half-life and increasing protein synthesis by enabling the cytoplasmic ribosome to read through premature stop codons to produce full-length proteins, a process known as translation.
Nonsense Read-through technology

From PTC therapeutics Ataluren for DMD
Challenges to gene therapy

- Multiple types of mutations; point mutations, expansion of exons, deletions
- Multiple protein expressions. For example, there are three isoforms of harmonin (USH1C). Harmonin b is localized to the stereocilia; harmonin a is localized at hair cell synapses
Summary

- Ush1B…UshStat…….being analyzed
- Ush2A.....ProQR….exon 13 skipping…..recruiting
- Ush2A…..Eloxx….read through…in development
- Ush3…….small molecules….in the lab
- Ush1f….zebrafish…in the lab…looking at the retina
- Ush1c…..mouse model for both ear and eye; pig model in development
Join the USH Trust!
Thank You!