Usher Syndrome Research

Update to Families

Gwenaëlle Géléoc, PhD
Assistant Professor
I- What is Usher Syndrome?

II- Advances in understanding the patho-physiology of Usher Syndrome

III- Treatment: Strategies for restoring function
I - What is Usher Syndrome?

- Usher syndrome causes hearing loss and retinitis pigmentosa (RP) which is responsible for night-blindness and progressive loss of peripheral vision.
- Many people with Usher also suffer from balance problems.
- Usher Syndrome is the most common condition that affects hearing and vision.
- 16-40,000 people with Usher syndrome in the US.
- Clinically and genetically heterogeneous.
- Three clinical USH types: Type 1, Type 2 and Type 3 distinguished by their severity and age when signs and symptoms appear.
I - What is Usher Syndrome?

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<th>Gene/protein</th>
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<td></td>
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<td>−/−</td>
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<td>Calcium and Integrin-binding protein</td>
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**Usher proteins function:**

- Transmembrane
- Adhesion
- Signalling
- Scaffolding
- Transport
- Development and synapse formation
I - What is Usher Syndrome?

Cosgrove and Zallocchi, 2014, IJBCB
I - What is Usher Syndrome?

IE: Usher proteins play structural and developmental roles in stereocilia and ribbon synapses.
I- What is Usher Syndrome?

RE: Usher proteins are associated with the connecting cilium, outer limiting membrane and ribbon synapse of photoreceptors.

Cosgrove and Zallocchi, 2014, IJBCB
The Usher Interactome

USH1
The Usher Interactome

USH2

USH2A
USHerin

USH2B?
USH2C
VLGR1b

USH2A
PDZD7

USH2C
Whirlin

F - Actin

USH2J
Myosin XVa
II- Advances in understanding the pathophysiology of Usher Syndrome

Christine Petit
“Gathering basic knowledge towards the development of therapeutic approaches.”

Uwe Wolfrum
“Decoding of Usher Syndrome protein networks reveals insights in the molecular basis of the disease.”

Monte Westerfield
“Defective protein complex assembly produces ER stress that causes cell death in Usher Syndrome.”
II- Advances in understanding the patho-physiology of Usher Syndrome

Auditory and Balance Testing

Cellular/Molecular work

Imaging

Physiology

Voltage dependent currents
Voltage step
Mechano-transduction current
Displacement steps
Voltage response

Scrambled CDH23
siRNA CDH23
Scrambled PCDH15
siRNA PCDH15
Scrambled VLGR1
siRNA VLGR1

harm
harmΔ7
PDZ1
c control
selection

CDH23(+68)
CDH23ΔC(+68)
CDH23(-68)
selection
II- Advances in understanding the patho-physiology of Usher Syndrome

Christine Petit

“Gathering basic knowledge towards the development of therapeutic approaches.”

Genetics of auditory mechano-electrical transduction
II- Advances in understanding the patho-physiology of Usher Syndrome

Christine Petit

“Gathering basic knowledge towards the development of therapeutic approaches.”
## Advances in understanding the patho-physiology of Usher Syndrome

**Lateral link complex**

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<th>protocadherin-15, cadherin-23</th>
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<tr>
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<td>harmonin</td>
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<tr>
<td>actin-binding</td>
<td>myosin-VIla, sans</td>
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<tr>
<td>others</td>
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**Tip-link complex (MET machinery)**

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<tr>
<td>submembranous*</td>
<td>harmonin b, sans, harmonin a (?)</td>
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<tr>
<td>actin-binding</td>
<td>harmonin b, myosin-VIla, myosin-Ic (?)</td>
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<tr>
<td>others</td>
<td>TMHS, TMC1/2 (MET channel ?)</td>
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</table>

**Ankle link complex**

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<tr>
<th>link</th>
<th>VLGR1, usherin **</th>
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<tr>
<td>submembranous</td>
<td>whirlin, PDZD7</td>
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<td>actin-binding</td>
<td>myosin-VIla</td>
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<tr>
<td>others</td>
<td>vezatin (transmembrane protein)</td>
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**Top connector complex**

<table>
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<td>submembranous</td>
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<tr>
<td>actin-binding</td>
<td>unknown</td>
</tr>
<tr>
<td>others</td>
<td>stereocilin (extracellular protein?)</td>
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**Kinocilial link complex**

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<td>others</td>
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**Shaft connector complex**

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<tr>
<th>link</th>
<th>PTPRQ **</th>
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<tr>
<td>submembranous</td>
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<td>actin-binding</td>
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II- Advances in understanding the patho-physiology of Usher Syndrome

Christine Petit

“Gathering basic knowledge towards the development of therapeutic approaches.”

Bicycle, 1980s; Raleigh; Component count: 893. Thames & Hudson.
II- Advances in understanding the patho-physiology of Usher Syndrome

Uwe Wolfrum

“Decoding of Usher Syndrome protein networks reveals insights in the molecular basis of the disease.”

- Large contribution to our understanding of USH1/USH2 interactome in the eye and the ear (ex: Interfering with Sans/Ush2A interaction leads to UHS1G)
- Recently demonstrated that USH1G (Sans) contributes to periciliary protein networks in the retina
- Now his lab has identified a new binding partner that is important for endocytosis (process by which cells absorb molecules by engulfing them)

MAGi2/Sans are present at the base of the primary cilia and both play a role in ciliogenesis.
II- Advances in understanding the patho-physiology of Usher Syndrome

Monte Westerfield

“Defective protein complex assembly produces ER stress that causes cell death in Usher Syndrome”

Ush1 proteins are shown to pre-assemble in the endoplasmic reticulum. Disruption in any one of the Ush1 protein partially disrupts trafficking of the complex as well as Ush2 proteins. This defect leads to ER stress and apoptosis (cell death).
III- Treatment: Strategies for restoring function

- Reinsertion of the missing link
  - a- Engineering AAV vectors: *Luk Vandenberghe*
  - b- Gene Therapy for Usher Syndrome Interaction partners. *Jeff Holt*

- Correction of translation
  - a- Antisense Oligonucleotides *Jennifer Lentz*
  - b- Translation read-through: *Kirsten Nagel Wolfrum*
III- Treatment: Strategies for restoring function

Reinsertion of the missing link

Engineering AAV vectors: Luk Vandenberghe

**Novel adeno-associated viral vectors for retinal gene therapy**


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AAV: non-pathogenic, highly stable viral vector (carrier)

At least 30 virus of different serotypes have been identified

Important goal: Develop vectors for which the **host immune response is limited**.

Subretinal injection of adeno-associated viral vector (AAV) with different types of AAV, each of which have distinct specificity for the various retinal cell types. Peter Mallen
III- Treatment: Strategies for restoring function

Reinsertion of the missing link

- *Gene Therapy for Usher Syndrome Interaction partners, Jeff Holt*
III- Treatment: Strategies for restoring function

Correction of translation

Jennifer lentz (USH1C)

“Antisense oligonucleotides effectively treat Usher Syndrome in mice.”

Frame shift mutation found in French-Acadian USH1C patients
Results in a severely truncated protein and affects all harmonin isoforms
III- Treatment: Strategies for restoring function

Correction of translation
Correction of translation

Rescue of hearing and vestibular function by antisense oligonucleotides in a mouse model of human deafness


USH1C

Correct splicing

216G>A

Cryptic splicing (frameshift)

Full-length harmonin

a - PDZ1 - PDZ2 - CC1 - PDZ3 - 552 aa

b - PDZ1 - PDZ2 - CC1 - CC2 - PST - PDZ3 - 899 aa

c - PDZ1 - PDZ2 - CC1 - 403 aa

- 135 aa

Truncated protein

1 month  2 months  3 months
TRIDS: Drugs that target in-frame nonsense mutations (premature stop) - Most studied: Aminoglycosides

**Correction of translation**

Kirsten Nagel Wolfrum
“Translation read-through to treat hereditary retinopathy”

III- Treatment: Strategies for restoring function

pR155X USH1C in-frame nonsense mutation (harmomin: red)
Recovered harmonin protein expression after translational read-through

III- Treatment: Strategies for restoring function

Recovered harmonin protein expression after translational read-through
Recovered functional harmonin

### III- Treatment: Strategies for restoring function

**Restored harmonin\_b3 actin bundling capacity:**

Also now study with other Usher causing non-sense mutations:
- USH1F (PCDH15)
- USH2A (Usherin)
- USH2C (GPR98)
- USH3A (Clarin 1)

*Goldmann et al. (2012) EMBO Mol Med 4:1186-99*
III- Treatment: Strategies for restoring function

Sound Strategies for Hearing Restoration

Gwenaëlle S. G. Géléoc and Jeffrey R. Holt*

Background: Sensorineural hearing loss is the most common sensory deficit in the world, with nearly 300 million affected individuals. The problem is multifactorial and can arise from damage or death of the primary sensory cells or the inner-ear neurons that relay auditory information to the brain. The inner-ear sensory cells and neurons can be damaged by environmental insult (such as exposure to infectious agents, drugs such as aminoglycoside antibiotics or chemotherapeutics, or overexposure to loud sounds) or by a host of genetic factors. Because mature inner ears lack the capacity for self-repair, the cellular damage is permanent. In addition to acquired hearing loss, more than 300 genetic loci have been linked to hereditary hearing loss, with about 70 of the causative genes identified. Eighty percent of genetic hearing loss is recessive, with the rest inherited as a dominant trait. More subtle genetic defects that cause a predisposition toward age-related hearing loss are poorly understood. Unfortunately, there is no cure for acquired, inherited, or age-related hearing loss.

Advances: Efforts to restore and repair damaged inner-ear cells have intensified over the past 10 years. Thus far, a major thrust has been to adapt three biological strategies for use in the inner ear: gene therapy, stem-cell therapy, and molecular therapy. Using these approaches, researchers have restored sensory function at the cellular level in animal models of human hearing loss. A few reports suggest functional recovery at the systems and behavioral levels, although caveats remain.

Outlook: As the population continues to age and expand, so will the number of patients who suffer from clinically serious hearing loss. As such, the need for a deeper and comprehensive understanding of hearing-loss therapies is more pressing than ever. Although the pace of progress is accelerating and clinical trials are on the horizon, it is clear that there are still a number of hurdles to overcome. Overall, there are reasons to be both cautious and optimistic as we attempt to repair and regenerate one of nature’s most exquisite mechanosensory devices: the human inner ear.
The many faces of Usher Syndrome Research