Gene-based therapy strategies for human Usher syndrome

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Human Usher syndrome (USH)

- USH is the most common form of combined hereditary deaf-blindness.
- Prevalence: 5-10/100,000
- Autosomal recessive disorder
- Symptoms:
  - Hearing impairment
  - Vestibular dysfunction
  - Vision loss – Retinitis pigmentosa
- 3 clinical types (USH1–3) are based on severity, age of onset and progression of symptoms.
- There are at least 13 genetic heterogeneous subtypes.
- 10 USH causing genes are identified, so far.

Human Usher syndrome (USH), genes

<table>
<thead>
<tr>
<th>Type</th>
<th>Gene locus</th>
<th>Gene</th>
<th>Protein</th>
<th>Function</th>
<th>Mouse model</th>
</tr>
</thead>
<tbody>
<tr>
<td>1A/1B</td>
<td>11q33.5</td>
<td>MYO7A</td>
<td>myosin VIIa</td>
<td>molecular motor</td>
<td>Shaker-1 (sh1)</td>
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<tr>
<td>1C</td>
<td>11q15.1</td>
<td>USH1C</td>
<td>harmonin</td>
<td>scaffold protein</td>
<td>Deaf circle (dc)</td>
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<td>1D</td>
<td>10q21-t22</td>
<td>CDC123</td>
<td>cadherin 23</td>
<td>cell-cell adhesion</td>
<td>Waltzer (w)</td>
</tr>
<tr>
<td>1E</td>
<td>17q21</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<tr>
<td>1F</td>
<td>10q11.2-t21</td>
<td>PCDH15</td>
<td>protocadherin 15</td>
<td>cell-cell adhesion</td>
<td>Semi wildtype (wt)</td>
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<td>1G</td>
<td>17q24-25</td>
<td>USH1G</td>
<td>-</td>
<td>scaffold protein</td>
<td>Jackson shaker (js)</td>
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<tr>
<td>1H</td>
<td>15q22-23</td>
<td>USH1H</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<tr>
<td>2A</td>
<td>1q41</td>
<td>USH2A</td>
<td>USH2A (usherin)</td>
<td>ECM, cell adhesion</td>
<td>-</td>
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<tr>
<td>2C</td>
<td>5q14.3-t21</td>
<td>VLGR1b</td>
<td>GPR98/VLGR1b</td>
<td>7-transmembrane receptor</td>
<td>Mass1 (frings)</td>
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<td>9q32</td>
<td>DFNB31</td>
<td>whirlin</td>
<td>scaffold protein</td>
<td>Whirl (wi); k.o.</td>
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<td>PDZD7</td>
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<td>3A</td>
<td>3q21-25</td>
<td>USH3A</td>
<td>clarin-1</td>
<td>cell adhesion</td>
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<tr>
<td>3B</td>
<td>20q</td>
<td>-</td>
<td>-</td>
<td>-</td>
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</tbody>
</table>

Diversity of Usher syndrome proteins

USH type 1
- Molecular motor
- Scaffold protein

USH type 2
- ECM component
- Cell adhesion protein
- Cell adhesion protein/7-transmembrane receptor
- Scaffold protein
- Scaffold protein

USH type 3
- Cell adhesion protein

USH protein interactome Feb. 2009 *

*Selected proteins

Molecular & cell biology of ciliated sensory cells

- Identification of novel molecules of the cytoskeleton in ciliated sensory cells.
- Centrins in sensory cells.
- Intracellular translocations in sensory cells
- Protein networks related to the Usher syndrome
- Pathomechanisms in seno-neuronal degenerations.

Evaluation of therapeutic strategies
USH protein networks are integrated and linked to the cytoskeleton by the USH scaffold proteins: harmonin, whirlin and SANS. In the ear, USH networks participate in kinocilia/stereocilia differentiation during hair cell development, but also in ribbon synapse function, and in signal transduction. In photoreceptor cells, USH networks are found at the synapse and in the cilary region. They may contribute to the intracellular transport and to the ciliary import and delivery. Defects in one protein of these networks may cause dysfunction of the entire networks leading to USH.

Conclusions
Defects in one protein of these networks may cause dysfunction of the entire networks leading to USH.

UW
In the ear, USH networks participate in kinocilia/stereocilia differentiation during hair cell development, but also in ribbon synapse function, and in signal transduction.

Reiners et al. 2006
Therapy strategies for the Usher Syndrome
Ear: USH is an developmental defect – prenatal diagnoses and molecular genetic treatment are necessary.
- Accessibility is very difficult.
- Cochlea implants are efficient.

Eye:
The is progression in retina implant development, but they are still at early development stages.
Retina provides very good accessibility for molecules/agents - neuroprotection and molecular genetic interventions.
~ Gene based therapies

Gene addition - replacement
Viral gene addition - replacement:
Lentivirus:
- single stranded RNA-Virus, member of Retrovirus family,
- maximum insert size: 7.5 kb,
- integration into genome, long term expression,
- low immune response, infect both dividing and non-dividing cells
Adeno-associated virus (AAV):
- DNA virus, but virus of Parovirus family, maximum insert size: 7 kb;
- no/low rate integration (preferentially chromosome 19) into genome;
- long term expression, non pathogenic, no immune response;
- infect both dividing and non-dividing cells;
- depend on a helper virus to replicate (Dependovirus).
Non-viral gene addition - replacement by nanoparticle:
- unlimited size for delivery, very poor integration;
- no immunological problems: expression termination;
- low efficiency in transfection of both dividing and non-dividing cells
- alternative "carriers": liposomes, DNA-protein conjugates ...

Gene addition via rAAVs in retinitis pigmentosa/LCA2 patients

The New England Journal of Medicine, 2008
Brief report
Safety and Efficacy of Gene Transfer for Leber’s Congenital Amaurosis
Albert M. Maguire .... and Jean Bennett, Philadelphia, U.S.A.

The New England Journal of Medicine, 2008
Brief report
Effect of Gene Therapy on Visual Function in Leber’s Congenital Amaurosis

Human Gene Therapy, 2008
Phase I Trial of Leber Congenital Amaurosis due to RPE65 Mutations by Ocular Subretinal Injection of Adeno-Associated Virus Gene Vector: Short-Term Results
William W. Hauswirth .... and Samuel G. Jacobson, Florida and Pennsylvania, U.S.A.

Gene addition - replacement

Vector Host cells/ efficiency Gene expression Integration into genome Immune- response
Viral
Lenti- virus dividing and non-dividing cells/ high efficiency long term/ years yes low
rAVV dividing and non-dividing cells/ high efficiency long term/ years no/ ? under discussion no
Non-viral
DNA only dividing and non-dividing cells very low efficiency short term no no
PEG nanoparticle dividing and non-dividing cells/ high efficiency up to 3 months analyzed stays episomal no

Gene addition projects for USH genes

USH1B (myosinVIIa) - Consortium – Welp/Brown families
David Williams, San LA, U.S.A. - Lentivirus successful transfer into mouse RPE
Alberto Aurichio, Univ. of Naples, Italy - recombinant aden-associated virus (modified rAVV5)

USH1C (harmonin) – Consortium planned Suchert family
Uwe Wolfrum, Mainz,* Germany; - recombinant adeno-associated virus (rAVV5); PEG nanoparticle

USH2A - Consortium ?
W. Kimberling, Omaha, Peter Francis, Portland, U.S.A.* ...

USH3A (clarin-1) - Consortium – Alexander family - “Hope for Vision”
John Flannery, Berkeley, U.S.A.*; Eva-Marja Sankila, Helsinki, Finland; Kris Palczewski, Cleveland; David A. Saperstein, Seattle, U.S.A./QLT Inc., Vancouver, Canada ...
- recombinant aden-associated virus (rAVV5)

*Cooperation with W.W. Hauswirth, Gainesville, U.S.A.
**Strategies for treatment of USH1C in the retina – Wolfrum Team Mainz**

- **gene addition** using recombinant adeno-associated virus (rAAV), Collaboration: W.W. Hauswirth, Gainesville, USA
- **gene addition** using polyethylene glycol (PEG) nanoparticles, Collaboration: M. Naash, Oklahoma City, USA
- **gene repair** by homologous recombination with zinc finger nucleases (ZFN) delivered by nanoparticles, Collaboration: D. Carroll, Utah, USA
- **translational readthrough** with modified aminoglycosides, Collaboration: T. Bassov, Haifa, Israel; T. Ben-Yosef, Haifa, Israel

**Harmonin (USH1C)-based protein network**

- USH2 proteins
- USH1 cadherins
- USH1 proteins
- filamin A
- β-catenin
- F-actin

**Gene addition via recombinant adeno-associated viruses (rAAV)**

- photoreceptor cell specific

rAAV5 Opsin:: GFP harmonin a1 delivered by subretinal injected into Ush1c -/- mouse retina

**Non-viral gene addition by DNA PEG nanoparticles**

Interaction of PEG-maleimide with CK30 to form polycation CK30PEG that compacts DNA vector to form charged-neutral nanoparticles. Lysine counter ion determines shape of the particles.

Copernicus Therapeutics,
Cleveland, Ohio, USA.
Mol. characteristics of DNA PEG nanoparticles

- PEG (Polyethyleneglycol), CK30 (30 lysins - cystein) + plasmid DNA
- ~ 8 nm; neutral charge; no toxicity; no immunoreactions; no cellular degradation; high in vivo transfections efficiency.
- Bind to cell surface exposed Nucleolin - internalization and transport into the nucleus (~ 15 min) & stays episomal.

Rod & Cone IRBP-1.3kb
All cells CBA-280bp

USH1C mutation p.R31X

Harmonin slice variants
USH1C mutation p.R31X

ZFN expression in nuclei of HEK cells.

Aminoglycoside/PTC124 pharmacogenetic therapy

- Aminoglycosides are broad-spectrum antibiotics and inhibit prokaryotic protein synthesis.
- In eukaryotic cells, they interfere with protein synthesis and suppress of nonsense mutations (= readthrough).
- PTC124 is a synthetic compound with readthrough activity.
Aminoglycoside/PTC124 mediated readthrough leads to expression of full length harmonin

Toxicity of aminoglycosides in the retina

Toxicity of aminoglycoside/PTC124 in treated retina explants

Quantification of aminoglycoside/PTC124 induced apoptosis

Research prospects, gene-based therapy strategies for USH1C in the retina

- rAAV2/5 harmonin a1 and b4 – in primates
- Subretinal application of rAAV2/5 harmonin in Ush1C knock-out and knock-in mice
- Evaluation of nanoparticle mediated transfer of zinc finger nucleases and rescue plasmids.
- Evaluation of gene repair in vitro, in cells and in R31X transgenic mice.
- Validation of results obtained by modified aminoglycosides and PTC124 in R31X transgenic mice.
- Success – Transfer outcome to other USH genes and other hereditary retinopathies.

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