Why do we care?

- > 360 million people worldwide have disabling hearing loss.
- ~10% are children.
- Most in low- and middle-income countries. Hearing loss contributes to the socioeconomic gap.
- > half are avoidable through primary prevention.
- Prevalence among US adolescents increased by about 30% from roughly 1990 to 2005.
- Reasons remain unknown
- Risk factors include noise exposure, smoking, and cardiovascular disease.

Review Resources

Websites:
- Hearing loss simulation
- Hereditary hearing loss compendium
  [http://hereditaryhearingloss.org/](http://hereditaryhearingloss.org/)

Articles:
- Konings et al., 2009. Genetic studies on noise-induced hearing loss, Ear & Hearing, 30:151-159
- Okano and Kelley, 2012. Stem cell therapy for the inner ear, Trends in Amplification, 16:4-17

Conductive hearing loss: Interference with sound transmission

Sensorineural hearing loss: Hair cells as primary targets

Hair cell damage is most common, is permanent, and often asymmetric.
Clinical definitions

**Mild**

**Moderate**

**Moderately Severe**

**Severe**

**Profound**

Causes of sensorineural hearing loss changes with age

- By age 70, 1:2

Perceptual deficits of SNHL:

**Threshold shift and recruitment**

**Effect on tuning curves**

More subtle auditory deficits: An exciting, emerging research area

Auditory Neuropathy and Dyssynchrony

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>(Central) Auditory Processing Disorder</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abnormal ABR but normal OAEs</td>
<td>Normal ABR, OAE, tonal audiogram</td>
</tr>
<tr>
<td>Normal to severe hearing loss</td>
<td>Poor speech discrimination</td>
</tr>
<tr>
<td>Poor speech discrimination</td>
<td>Difficulty with pattern recognition</td>
</tr>
</tbody>
</table>

Mechanisms:

- Selective IHC loss or dysfunction
- Synaptic dysfunction (otoferlin)
- Type I SGN loss

Auditory Neuropathy or Dyssynchrony

- Abnormal (central) auditory pathways
- Neurological dysfunction (e.g. tumors)

Acquired hearing loss

**Temporary threshold shift (TTS)**

- Recovery of hearing thresholds within days/weeks
- Morphological correlates include:
  - 'mild' stereocilia defects (tip links broken, rootlet deterioration, loss of stiffness)
  - Lateral wall/strial cellular defects
  - Auditory nerve fiber retraction/swollen terminals; possible neuron loss
- Recovery suggests active repair processes

**Permanent threshold shift (PTS)**

- Non-recoverable threshold elevation
- Morphological correlates include:
  - Severe stereocilia defects (broken, disorganized, splayed)
  - Sensory hair cell death - within days/weeks
  - Auditory nerve (spiral ganglion) cell death - often months/years after noise

5% of school aged children (Musiek et al., 1990)
Conventional wisdom vs recent findings

- Can there be PTS without hair cell loss or obvious damage?  
  - Cyclodextrin experiment example

Recent findings: Neuron damage in absence of hair cell loss

Adapted from Kujawa and Liberman, 2009

Mechanisms of acquired hearing loss

Pathways and categories are not independent

Other contributors

Modified from Kidd and Bao, 2011

Mechanisms of gene-based hearing loss

Nonsyndromic, Autosomal Dominant (DFNA), 13% >50 mapped loci

Syndromic, 30%

Nonsyndromic, Autosomal Recessive (DFNB), 55% >60 mapped loci

Mitochondrial, X-linked, Multilocus, 2%

Genetic hearing loss is extremely heterogeneous, affecting many cell types

- Wide variety of known or predicted gene functions
- Assortment of different primary pathologies (animal model studies)
- Seminal findings regarding cellular and molecular bases for cochlear function
A potential genetic “hotspot”: Gjb2, Connexin 26

1 in 30 in general population are carriers
Accounts for 30-50% of age-related hearing loss

Is Cx26 required for K+ recycling to endolymph?

Cx26 null mutant is neonatal lethal.
Conditional KO (supporting cells)
- Severe to profound hearing loss
- Decreased EP in adult, consistent with K+ recycling
- But... EP normal in early postnatal animals. It decreases only after hair cell death.
- Suggests other functions of Cx26.
- Possibly intercellular signaling with small molecules like IP3.

Connexins form hemichannels or gap junctions

- permit transfer of ions and small molecules (<1.2 kDa)
- family of related genes:
  - Cx26 DFNA3, DFNB1
  - Cx30 DFNA3, DFNB1
  - Cx31 DFNA2

Cx29 and Cx32 (Schwann cells - peripheral neuropathy with assoc’d deafness)

GJB2 (Cx26) - current questions

- What underlies phenotype variation between families?
- What underlies phenotype variation within families?
  - Modifying genes (background)
  - Environment
- What causes hair cell death in Cx26 conditional KO?
  - Disturbed ion homostasis
  - Small molecule signaling defects via lost junctions or hemichannels (IP3)
  - New evidence that OHC motility altered

Cochlear implant... demo movie and patients if there is time

Regeneration in non-mammalian vertebrates: Structural Recovery

noise trauma: 1.5 kHz at ~115 dB for 48 hrs

no noise

0D

6D

10D

Corwin and Cotanche, Science 240:1772, 1988
**Regeneration in non-mammalian vertebrates: Functional recovery**

Noise exposure: 0.9 kHz, 120 dB SPL, 48 hr
- Greatest ABR shift at exposure frequency
- Complete recovery within 3 weeks
- Repeatable
- Indicates stem/progenitor cell persists into adulthood in chick

Adler et al., Hearing Res. 71:214-224, 1993

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**Regeneration in non-mammalian vertebrates: Mechanisms**

Two mechanisms:
- **Early – Transdifferentiation**
  - Occurs in absence of DNA synthesis
  - Cell number decreases
  - Difficult to maintain mosaic
- **Late – Proliferation**
  - DNA synthesis required
  - Cell number constant
  - Mosaic maintained

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**Regeneration in mammals: Lessons from developmental programs**

A central hypothesis of regenerative medicine: Regeneration will mimic development.

Can we manipulate expression of key transcription factors?

- Atoh1 and lateral inhibition

A model of lateral inhibition:
- Cells communicate via notch signaling (both directions)
- Atoh1 is upregulated in some
- Atoh1 inhibits Notch receptor and upregulates ligands
- Notch activation suppresses Atoh1
- The result is an epithelial mosaic. One hair cell surrounded by supporting cells.

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**Regeneration by… Block of lateral inhibition**

Mizutari et al., Neuron, 2013

Are cells functionally normal? Why did this require pre-injury? Why only the base? Why incomplete?

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**Regeneration by… Atoh1 gene therapy**

Izumikawa et al., Nature Med, 2005

a-c: three examples of ears treated with Ad.Atoh1, 2 months post-infection

Why was the outcome variable? Are cells functionally normal?
Open questions

- **Regeneration in non-mammals**
  - Is the trigger diffusible or contact mediated?
  - Does the trigger cause loss of inhibitory control and/or activation of positive control?
  - What controls subtype specification of IHC/OHC-like cells?
  - Why signals Notch/Delta upregulation after injury?

- **Regeneration in mammals**
  - Why limited to early postnatal animals?
  - Why does it occur mostly in apex?
  - Atoh1 overexpression variable and inefficient. Are there other genes that block differentiation? Positive co-factors?
  - What genes/factors are involved in inhibiting supporting cell proliferation?

Other approaches:

**Intrinsic inner ear progenitors**

- Cochlea shows some ability to regenerate (embryos) and presence of Nestin+ stem cells.
- Caveat: intrinsic progenitors are lost after P7.

**Exogenous stem cells**

- So far accomplished with mouse ESCs or iPSCs
- Stepwise protocol: Ectodermal EB, Wnt suppression, FGF treatment, and factors from stromal cells

Other approaches: Exogenous stem cells

- Reprogramming possible (genes and small molecules)
- Can survive and reinnervate hair cells
- How to organize as ganglion and maintain tonotopy in CNS?

Reprogram ESCs with Neurog1
Tailor firing with Neurotrophins
Align on nanofibers

Supplementary Material

**An example of nonsyndromic dominant hearing loss**

- Typically later in onset (postlingual) with varied severity
- Often residual cochlear function
- Mutational mechanism: haploinsufficiency (dosage) or gain of function (e.g., dominant negative)
- Potential therapy: add back normal gene function; block of dominant negative; regeneration/replacement of hair cells/spiral ganglion cells
- >50 DFNA loci mapped
An example of nonsyndromic recessive hearing loss

- typically prelingual and profound
- mutational mechanism: loss of function
- potential therapy: add back normal gene function; regeneration/replacement of hair cells/spiral ganglion cells
- >60 DFNB loci mapped