Gaps between the bedside and the bench: Perspectives from the bench

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Sponsored by the Office of the Director National Institutes of Health, the National Human Genome Research Institute, the National Institute of Child Health & Development, the National Institute on Deafness & Other Communication Disorders, the National Eye Institute, the Usher 1F Collaborative, and the Megan and Vision for a Cure Foundations
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Case study 1: Positive results validate candidate genes

Case study 2: Negative results reveal incorrect diagnoses

Mind the gaps

Undiagnosed Diseases Network
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Undiagnosed Diseases Network
Usher syndrome - the leading cause of deafblindness

- Prevalence ≈ 1 per 6,000 births in the US
  (more common than ALS or Huntington’s Disease)

- Congenital deafness (~4% of deaf have Usher)
  Sensorineural hearing loss
  Vestibular dysfunction

- Retinitis pigmentosa
  Loss of rod photoreceptors
  Progressive tunnel vision as cones die
<table>
<thead>
<tr>
<th>Type</th>
<th>Human</th>
<th>Protein: potential function</th>
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<tbody>
<tr>
<td>USH1B</td>
<td>MYO7A</td>
<td>MyosinV11A: motor activity</td>
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<tr>
<td>USH1C</td>
<td>USH1C</td>
<td>Harmonin: scaffold</td>
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<td>USH1D</td>
<td>CAD23</td>
<td>Cadherin: calcium dependent adhesion</td>
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<td>USH1E</td>
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<td>PCDH15</td>
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<tr>
<td>USH1H</td>
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<td>USH1K</td>
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<td>GPR98</td>
<td>Vlgr1: G-protein coupled receptor, signaling</td>
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<td>USH3A</td>
<td>CLRN1</td>
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<tr>
<td>USH3B</td>
<td>HARS</td>
<td>Histidyl-tRNA Synthetase</td>
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</table>
Genetic counseling is important for Usher patients
Gene discovery is important for Usher patients
Exome sequencing of undiagnosed patients identifies mutations in PDZD7, a gene of unknown function.
Zebrafish Pdzd7a is localized with other Usher proteins

Eye

Ear

Pdzd7 + ac-tubulin

(Jennifer Phillips)
Stereocilia are defective after *pdzd7a* knockdown

Control

*pdzd7a* MO

(Bernardo Blanco)
PDZD7 mutations are heterozygous in patients with known Usher gene mutations.
Caspase labeling of dying cells

*pdzd7a interacts with ush2a & gpr98 in photoreceptor cell death*

(Ebermann et al., 2010)
PDZD7 binds to USH2A & GRP98 proteins

HEK293T cells

V5-USH2A  V5-GPR98  V5-PDZD7

36 kD  28 kD  36 kD

FLAG-tag precipitates + anti-V5

(Hanno Bolz & Inga Ebermann)
PDZD7 forms a quaternary complex of USH 2 proteins

(Chen et al., 2014)
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*PDZD7* causes disease
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*PDZD7* causes disease

Where are the missing homozygous and compound heterozygous patients?

- embryonic lethal? (model organism data suggest not)
- patient pool too small?
  - limited access to patient data?
  - lack of communication (or sharing) among clinicians?
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  • Joubert syndrome

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Undiagnosed Diseases Network
Consanguineous family with deafness

(Solaf Elsayed & Hanno Bolz)
Mapping homozygosity by descent identifies no good candidates
Whole exome sequencing for homozygous SNPs identifies mutation in $AHI1$, a gene responsible for Joubert syndrome.

Patients

II:3 & II:5
- p.Arg1066* (homozygous)

I:1 & I:2
- p.Arg1066* (heterozygous)

Wildtype
Joubert syndrome - a severe ciliopathy disease

• Underdevelopment of the cerebellum and brainstem*
• Impaired intellectual development, seizures
• Retinitis pigmentosa
• Developmental abnormalities
• Kidney and liver abnormalities
Homozygous patients have normal CNS MRIs

(Raoul Heller & Hanno Bolz)
Nonsense mutation truncates the protein-protein interaction domain of \textit{AHI1}
Targeting upstream in zebrafish gene blocks expression

Cluster of severe disease causing mutations

p.Arg1066*

Protein-protein interaction domain
Upstream targeting produces strong ciliopathy phenotype

uninjected control

SPL8

(Jennifer Phillips)
3' targeting truncates the protein

Cluster of severe disease causing mutations

bp

400 500

Ctrl SPL8

p.Arg1066*

e23i23

4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29

SPL8
e23i23

(Jennifer Phillips)
Truncated protein has no apparent phenotype

uninjected control

e23i23

SPL8

(Jennifer Phillips)
Nonsense *AHI1* mutation is not linked to deafness

(Solaf Elsayed, Raoul Heller & Hanno Bolz)
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Mind the gaps (perspective from the bench)
  • Barriers to accessing patient data
    • Sociological: clinical vs basic research attitudes
    • Limited access to clinical records: de-identified vs IRB
  • Limited patient data: horde vs share variant & phenotypic data

Undiagnosed Diseases Network
Seven clinical sites, a coordinating center, two DNA sequencing cores, a metabolomics core, a model organisms screening center, and a central biorepository.
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