Next-Gen Newborn Hearing Screening

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Manchester Centre for Audiology and Deafness (ManCAD)
Next-Gen Diagnostics and Newborn Screening for Hearing Loss

- Why is genetic testing so important for hearing impairments?
- What is the landscape of genetic testing for hearing loss?
- What is the status of Universal Newborn Screening?
- What is the future of testing and screening?

How do we do it....and get it right?
Hearing Loss Across the Lifespan

Most common sensory deficit in humans leading to speech and language delay, challenges in school, work and relationships, isolation and depression in the elderly with critical time for habilitation during the first few months of life.

Newborns and Children (in the U.S.)

- ~3 in 1000 newborns born with permanent hearing loss (HL)
- One of most common birth defects in the United States
- Majority of hearing impaired children born into families with little or no experience with HL—Deaf X Deaf matings result in >90% all hearing offspring, highlighting unparalleled heterogeneity in etiology (genetic, environmental and gene X env)
- ~50% of children with HL from racial/ethnic minority populations
- ~1 in 2 cases in babies due to genetic causes
- ~1 in 4 cases in babies due to maternal infections during pregnancy, complications after birth, and head trauma
- ~1 in 100 cases in children by school age → 2.5M (5.4%) with mild or unilateral HL
**Hearing Loss Across the Lifespan**

**Age Related Hearing Impairment (ARHI)**
- ~1 in 3 individuals over age 65 with hearing impairment significant enough to impair speech perception
- By 2030 over 20% of the U.S. population will be >76 years old (U.S. Census Bureau)
- More prevalent in males than females
- Complex trait with genetic and environmental factors contributing to onset and progression
- Little progress to date in understanding the underlying molecular basis

But, stay tuned...
- Recent genetic methods empowered by big data resources from the human genome project, such as genome wide association studies (GWAS), provide an approach to deciphering complex traits and are being applied to ARHI.
Types of Hearing Loss

- Unilateral
- Bilateral
- Conductive
  - Outer ear
  - Middle ear
- Sensorineural
  - Inner ear
- Mild
- Moderate
- Profound
- Stable
- Progressive
- Syndromic
- Nonsyndromic
Heterogeneous Causes of Hearing Loss

Environmental
- Drug
- Noise
- Trauma
- Infection
- Malnutrition

Genetic

Syndromic (~30%)
- Alport, BOR, CHARGE, JLN, Norrie, Pendred, Perrault, Stickler, Treacher Collins, Usher, Waardenburg, Wolfram

Nonsyndromic (~70%)
- Autosomal dominant (DFNA, 22%)
- Autosomal recessive (DFNB, 77%)
- X-linked (DFNX, 1%)
- Y-linked (DFNY)
- Mitochondrial
Known Hearing Loss Genes

- All (123)
- Nonsyndromic (90)
- Recessive NS (62)
- Syndromic (43)
- Dominant NS (30)

Number of Genes vs. Year

1st NIDCD budget year
Clinical Utility of Genetic Testing

Newborn screening → Early intervention

Prenatal testing ← Risk assessment → Life-long management
As Gene Test Menu Grows, Who Gets to Choose?

*The New York Times*

Wednesday, July 21, 2004
Hearing Loss Screening Protocol

- **Nonsyndromic**
  - CMV
  - GJB2/GJB6 Testing
  - Aminoglycosides
  - Family Hx
  - No Family Hx

- **Syndromic**
  - Appropriate Gene(s)
  - Usher Syndrome
    - MYO7A
    - SANS
    - USH1C
    - USH2A
    - CDH23
    - ADGRV1
    - PCDH15
    - CLRN1

- **Mitochondrial**
  - 12S rRNA
  - tRNA ser

- **X-linked**
  - POU3F4

- **Dominant**
  - MYO6
  - ACTG1
  - DSPP
  - TECTA
  - EYA4
  - MYO7A
  - COL11A2
  - POU4F3
  - TMC1
  - CCDC15

- **Recessive**
  - MYO7A
  - MYO15
  - SLC26A4
  - OTOF
  - TMEM33
  - TECTA
  - CLDN14
  - TMC1
  - STRC
  - GIPC3
  - CMV

- **Testing**
  - Appropriate Gene(s)
  - mitotic recombination
  - gene expression

- **Clinical Manifestations**
  - Family history
  - Clinical features
  - Diagnostic testing

- **Differential Diagnosis**
  - Syndromic
  - Nonsyndromic

- **Genetic Testing**
  - Whole-exome sequencing
  - Targeted panel

- **Prevention**
  - Counseling
  - Carrier detection
  - Prenatal diagnosis

- **Gene Panels**
  - SNPs
  - Genotype-phenotype correlation

- **Management**
  - Hearing aids
  - Cochlear implants
  - Speech therapy

- **Further Reading**
  - American Academy of Otolaryngology-Head and Neck Surgery
  - American Speech-Language-Hearing Association

- **Acknowledgments**
  - Sponsors
  - Contributors

- **References**
  - PubMed
  - ClinicalTrials.gov
  - GenBank

- **Contact Information**
  - Medical Geneticist
  - Genetic Counselor

- **Copyright**
  - 2023

- **Disclaimer**
  - Information is for educational purposes only.
  - Professional medical advice should be sought.

- **Note**
  - This information is not exhaustive.
  - Updates and revisions are encouraged.

- **Contact for Updates**
  - Medical Geneticist
  - Genetic Counselor

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  - Website
  - Social Media

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  - Sponsors
  - Partnerships

- **Thank You**
  - Supporters
  - Contributors
Panel Inclusion Criteria for Nonsyndromic or “Apparent” Nonsyndromic Hearing Loss Genes

163 hearing loss genes

Is hearing loss syndromic?

Yes

Is hearing loss the presenting feature?

No

Exclude 73 genes (45%)

Evidence level 0 or 1

Yes

What is the gene-disease association evidence level?

Evidence level 2 or 3

Include 90 genes (55%)
<table>
<thead>
<tr>
<th>Genes</th>
<th>Genes</th>
<th>Genes</th>
<th>Genes</th>
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<td>GPSM2</td>
<td>OTOG</td>
<td>ADGRV1</td>
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<td>GRXCR1</td>
<td>P2RX2</td>
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<td>KARS</td>
<td>PRPS1</td>
<td>HARS</td>
</tr>
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<td>KCNQ4</td>
<td>RDX</td>
<td>MYO7A</td>
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<td>SERPINB6</td>
<td>MYO7A</td>
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<td>SLC17A8</td>
<td>USH1C</td>
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<td>LRTOMT</td>
<td>STRC</td>
<td>USH1G</td>
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<tr>
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<td>MARVELD2</td>
<td>SYNE4</td>
<td>USH2A</td>
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<td>MIR96</td>
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<td>SLC26A4</td>
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<td>MSRB3</td>
<td>TECTA</td>
<td>OTOA</td>
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<td>MTRNR1</td>
<td>TIMM8A</td>
<td>DFNB59</td>
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<td>DIAPH1</td>
<td>MTTS1</td>
<td>TMC1</td>
<td>CLPP</td>
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<tr>
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<td>MYH14</td>
<td>TMIE</td>
<td>HARS2</td>
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<tr>
<td>ESRRB</td>
<td>MYH9</td>
<td>TPRN</td>
<td>HSD17B4</td>
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<td>MYO15A</td>
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<td>LARS2</td>
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<td>KCNE1</td>
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<td>MYO6</td>
<td>TPSPEAR</td>
<td>KNCQ1</td>
</tr>
<tr>
<td>GJB6</td>
<td>OTOF</td>
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</tbody>
</table>

Subpanels available (Usher, Waardenburg, BOR, JLNS, Pendred, AN, WFS1, Mito, etc.)

Courtesy of LMM, Rehm
OtoGenome Detection Rates (n=959)

- **Positive**
- **Inconclusive**
- **Negative**

- **Usher**
  - Pathogenic/Likely Pathogenic
  - Biallelic Recessive

- **VUS only**
- **1 Path**
- **10%**

- **20%**
- **59%**

- **Benign/Likely Benign**

- **23%**

- **GJB2-related hearing loss excluded in most cases**
- **~7% of cases have pathogenic variants in Usher genes, most unaware of their risk for retinitis pigmentosum**

Courtesy of LMM, Rehm
Genetic Etiology of 218 OtoGenome Positive Cases

![Diagram showing the percentage of cases for various genes](Diagram)

- STRC: 23.7%
- GJB2: 22.7%
- SLC26A4: 8.1%
- Usher Genes: 7.1%
- USH2A: 5.2%
- USH1G: 2.4%

Courtesy of LMM, Shen
Deletions Detected by NGS

Copy number variants confirmed by digital droplet PCR

Courtesy of LMM, Rehm
Hearing Loss Variants in over 2000 Cases Tested at the Laboratory for Molecular Medicine

**Reported Variant Classification**

- Pathogenic: 223
- Likely Pathogenic: 121
- Unknown Significance: 462
- Likely Benign: 580
- Benign: 899
- Total: 2285

82.4% (673/817) of variants only detected in one family

**Variant Distribution**

- **SLC26A4**
  - 1001+1G>A

- **USH2A**
  - 2299delG

- **GJB2**
  - V37I
  - M34T

- **GJB2 35delG**

Courtesy of LMM, Rehm and Amir
Next-Generation Sequencing Tests for Hearing Loss
<table>
<thead>
<tr>
<th>Panel</th>
<th>Institution</th>
<th>Genes</th>
<th>Diagnostic yield</th>
<th>Capture</th>
<th>Sequencing</th>
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<tr>
<td>Belgium</td>
<td>U Antwerp</td>
<td>79</td>
<td>25-30%</td>
<td>TruSeq</td>
<td>Illumina</td>
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<tr>
<td>CUHK-HL</td>
<td>CUHK</td>
<td>252</td>
<td>57%</td>
<td>SureSelect</td>
<td>Illumina</td>
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<tr>
<td>Israel-MidEast</td>
<td>Tel Aviv</td>
<td>246</td>
<td>56%</td>
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<td>Illumina</td>
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<tr>
<td>Italy+Qatar</td>
<td>U Trieste</td>
<td>96</td>
<td>33%</td>
<td>AmpliSeq</td>
<td>Ion PGM</td>
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<tr>
<td>Japan</td>
<td>Shinshu U</td>
<td>63</td>
<td>30%</td>
<td>AmpliSeq</td>
<td>IonTorrent</td>
</tr>
<tr>
<td>KFSH&amp;RC</td>
<td>KFSH</td>
<td>90</td>
<td>54%</td>
<td>AmpliSeq</td>
<td>Ion PGM</td>
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<tr>
<td>Medical Exome</td>
<td>Ege U</td>
<td>102/2761</td>
<td>72.4%</td>
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<td>MiamiOtoGenes</td>
<td>U Miami</td>
<td>146</td>
<td>50%</td>
<td>SureSelect</td>
<td>Illumina</td>
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<tr>
<td>OtoGenetics</td>
<td>Commercial</td>
<td>131</td>
<td>42-52%</td>
<td>NimbleGen</td>
<td>Illumina</td>
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<tr>
<td>OtoGenome</td>
<td>LMM/Harvard</td>
<td>87</td>
<td>23%</td>
<td>SureSelect</td>
<td>Illumina</td>
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<tr>
<td>OtoSCOPE</td>
<td>MORL/U Iowa</td>
<td>89</td>
<td>39%</td>
<td>SureSelect</td>
<td>Illumina</td>
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<tr>
<td>Syndromic</td>
<td>U Brasilia</td>
<td>52</td>
<td>10%</td>
<td>AmpliSeq</td>
<td>Ion PGM</td>
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<tr>
<td>TRS-204</td>
<td>Seoul National</td>
<td>204</td>
<td>55%</td>
<td>?</td>
<td>Illumina</td>
</tr>
<tr>
<td>Whole Exome</td>
<td>Rotterdam</td>
<td>120/20k</td>
<td>70%</td>
<td>?</td>
<td>?</td>
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</tbody>
</table>
Explanation for Variable Diagnostic Yields

- Inclusion or exclusion of \textit{GJB2}-positive patients
- Stringency of variant classification and interpretation
- Small sample sizes in some reported studies
- Family history / simplex vs. multiplex
- Specific clinical features
- Ethnicity (consanguinity, availability of population data)
Factors Influencing Diagnostic Yield for Hearing Loss

Sloan-Heggen et al., Human Genetics (2016)
Newborn Hearing Screening

- Standard of care in the U.S. with greater than 98% of newborns screened before leaving the birth hospital
- ~1.6% documented not passing final hearing screening
- ~45% not passing hearing screening lost to follow up or documentation for diagnosis
- Prevalence of documented HL ~1.4% per 1000 screened
- ~88% documented referrals of those with HL to Early Hearing Detection and Intervention (EHDI)
- ~50% of states have language in legislation or regulations that include coverage for early intervention services to children with mild or unilateral HL
- Two common screening methods: otoacoustic emissions (OAEs) and auditory brainstem response (ABR), detecting HL in the frequency region important for speech recognition
DID YOU HEAR?

98% of newborns in the U.S. are screened for hearing loss before they leave the hospital.

Research improves the quality of life of people with hearing loss, starting with the day they are born.

Biomedical discoveries supported by the National Institute on Deafness and Other Communication Disorders (NIDCD) laid the foundation for states to take action to ensure children are screened and treated early for hearing loss.

- NIDCD research demonstrates the need for both newborn hearing screening and early intervention, which is crucial for speech and language development.
- NIDCD research leads to two gold-standard tests for hearing loss in infants.
- NIDCD research finds genetic causes of profound hearing loss and deafness, which account for more than half of all cases.
- NIDCD research explores intervention strategies for children with hearing loss.
- NIDCD research develops and improves technology for hearing devices such as hearing aids and cochlear implants.
- NIDCD research reveals the basic mechanisms of how we hear.

DID YOU KNOW?

12,000 babies are born deaf or hard of hearing each year in the United States.
From before 1993:

- Only newborns at high risk are screened, which misses 50% of children who are eventually diagnosed with severe hearing impairments.

- Only 8% of babies with congenital hearing loss are diagnosed by their first birthday.

- 47% of children with congenital hearing loss are not diagnosed until their third birthday or later.

1993:

- About 1 in 10 newborns are screened for hearing loss.

1997:

- The NIH convenes an expert panel, which recommends standard newborn hearing screening methods for state programs.

1999:

- President Clinton signs the Newborn and Infant Hearing Screening and Intervention Act, authorizing support for statewide screening programs.

2010:

- President Obama signs the Early Hearing Detection and Intervention Act of 2010, expanding funding to include diagnostic services.

2010:

- 98% of newborns are screened for hearing loss before they leave the hospital.

3.8 million newborns are screened annually.
Next-Gen Newborn Screening for Hearing: a paradigm for expanded genetic screening

- It makes a difference for treatment and management to know the precise diagnosis—the foundation of precision medicine.

- Hereditary hearing loss displays unparalleled genetic heterogeneity and offers an opportunity to simulate a complex genetic disorder in the context of hundreds of single gene defects.
Precise Diagnosis Impacts Care

- *GJB2*—benefit from cochlear implantation
- *PJVK*—more harm than good from amplification
- Others with optimized habilitation?
- Syndromic disorders that can be indistinguishable from nonsyndromic disorders at birth:
  - *Alport*
  - *Branchio-oto-renal*
  - *Jervell and Lange-Nielson*
  - *Pendred*
  - *Usher*
### When will screening take place?

<table>
<thead>
<tr>
<th>At Birth (~4 million newborns)</th>
<th>Failed Audiometry (2-8 per 100 infants)</th>
<th>Confirmed Hearing Impairment</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>All newborns tested</td>
<td>Less infants tested</td>
</tr>
<tr>
<td></td>
<td>Reduce work up</td>
<td>Reduce work up</td>
</tr>
<tr>
<td></td>
<td>Use blood spots</td>
<td>Use blood spots?</td>
</tr>
<tr>
<td></td>
<td>Identify those at risk</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Less infants tested</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Reduce work up</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Specific testing</td>
</tr>
<tr>
<td></td>
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<td>High parental interest</td>
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<tr>
<td>-</td>
<td>Genotype-phenotype?</td>
<td>Genotype-phenotype?</td>
</tr>
<tr>
<td></td>
<td>False positives</td>
<td>False positives?</td>
</tr>
<tr>
<td></td>
<td>Detect many carriers</td>
<td>Detect many carriers</td>
</tr>
<tr>
<td></td>
<td>Parental interest low</td>
<td>Parental interest low?</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Miss some at risk</td>
</tr>
<tr>
<td></td>
<td></td>
<td>New specimen</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Genotype-phenotype?</td>
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<tr>
<td></td>
<td></td>
<td>Miss some at risk</td>
</tr>
<tr>
<td></td>
<td></td>
<td>New specimen?</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Delay in intervention</td>
</tr>
</tbody>
</table>
Hearing Loss from Cytomegalovirus (CMV)

- 4,000,000 live births per year in U.S.

2% (80,000) pregnancies have maternal primary CMV
- 98% of pregnancies have no maternal CMV

- 60% not transmitted to fetus
- 40% (32,000) transmitted to fetus

10% (3,200) symptomatic at birth
- 59.3% have normal hearing
- 40.7% (1,302) have hearing loss

90% (28,800) asymptomatic at birth
- 7.4% (2,131) have hearing loss
- 92.6% have normal hearing

3,433 (0.086%) or 1 in 1,165 births has hearing loss from congenital CMV
- As many as 2/3 of these babies will pass newborn screening and develop hearing loss later
SEQaBOO

SEQuencing a Baby for an Optimal Outcome
SEQaBOO (BWH/BCH/MEEI)
Total Enrolled 214 (85/32/97)
Confirmed HL 74 (19/33/22)
Unconfirmed HL 141 (66/0/75)
Genetic Arm 93 (65/28/0)
Survey Arm 122 (20/5/97)

@ BCH
Infants seen at 1 m/o 148 (0/148/0)

1 m/o Hearing Test @ BCH/MEEI

Confirmed HL Offered Testing 34 (0/34/0)
Unconfirmed HL Not Enrolled 114 (0/114/0)

BCH+s
Genetic Arm 28 (0/28/0)
Survey Arm 5 (0/5/0)

BCH-s
Decline 1 (0/1/0)

Confirmed HL Genetic Arm 43 (15/28/0)
Confirmed HL Survey Arm 9 (4/5/0)

Confirmed HL Survey Arm (SOC) 22 (0/0/22)

Unconfirmed HL Genetic Arm 50 (50/0/0)
Unconfirmed HL Survey Arm 16 (16/0/0)

Unconfirmed HL Survey Arm (SOC) 75 (0/0/75)

7th International Pediatric Audiology Conference
## Comparisons of Traditional and Next-Generation Newborn Hearing Screening

<table>
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<tr>
<th>Hearing Screening</th>
<th>Traditional</th>
<th>Next-generation</th>
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<tbody>
<tr>
<td>Method</td>
<td>OAE and ABR</td>
<td>DPOAE and ABR plus WGS</td>
</tr>
<tr>
<td>Type</td>
<td>Phenotypic</td>
<td>Phenotypic + Genetic</td>
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<tr>
<td>Symptomatic</td>
<td>Yes</td>
<td>Not required</td>
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<tr>
<td>Prognostic</td>
<td>No</td>
<td>Maybe</td>
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<tr>
<td>Accurate recurrence risk estimate</td>
<td>No</td>
<td>Yes</td>
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<tr>
<td>Etiology</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Precision medicine</td>
<td>No</td>
<td>May inform</td>
</tr>
<tr>
<td>Cost</td>
<td>$10-50/baby, single purpose, stable</td>
<td>&lt;$10,000/baby, decreasing</td>
</tr>
<tr>
<td>Turnaround time</td>
<td>Immediately</td>
<td>Days</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>Unable to detect later-onset</td>
<td>Expected, validation ongoing</td>
</tr>
<tr>
<td>Specificity</td>
<td>Transient loss as false positives</td>
<td>Expected, validation ongoing</td>
</tr>
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</table>
Whole Genome Sequencing Projects Underway

• Discovery projects for additional genes involved in hearing loss
“We’ve gotten notification that we are funded ($100,000) as a supplement to our cooperative agreement with HRSA for the National Coordinating Center for the Regional NBS and Genetics Collaboratives to do the project I had discussed with you a few months ago. It is to first review etiologies of newborn hearing loss cases that are identified before age 5 but missed by NBS. We then would look at how best to pick up the kids with these later onset forms. There seem to me to be two main possibilities, though altering audiometric cut-offs might also detect some of them. The main options are: 1) screen kids audiometrically before school to identify those with hearing loss; 2) add a molecular test to NBS hearing screening to pick them up as a part of NBS. This option requires determining which genes have definitive or strong associations with hearing loss and then assessing the proportion of cases with those genes involved and with variants in them that would classify as pathogenic or likely pathogenic. There are other trade-offs with either of these such as the opportunity to introduce interventions earlier if found earlier to maximize the benefits of being found that the group would factor in to its thinking.”
With Appreciation to

Brigham and Women’s Hospital
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Jun Shen

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Margaret Kenna

Harvard University
Jennifer Hochschild

Massachusetts Eye and Ear Infirmary
Michael Cohen

Laboratory for Molecular Medicine
Ahmad Abou Tayoun
Sami Amr
Amy Hernandez
Andrea Murihead
Heidi Rehm

Harvard Medical School Center for Hereditary Deafness

Manchester Centre for Audiology and Deafness (ManCAD)