7th International Pediatric Audiology Conference

Next-Gen Newborn Hearing Screening

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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

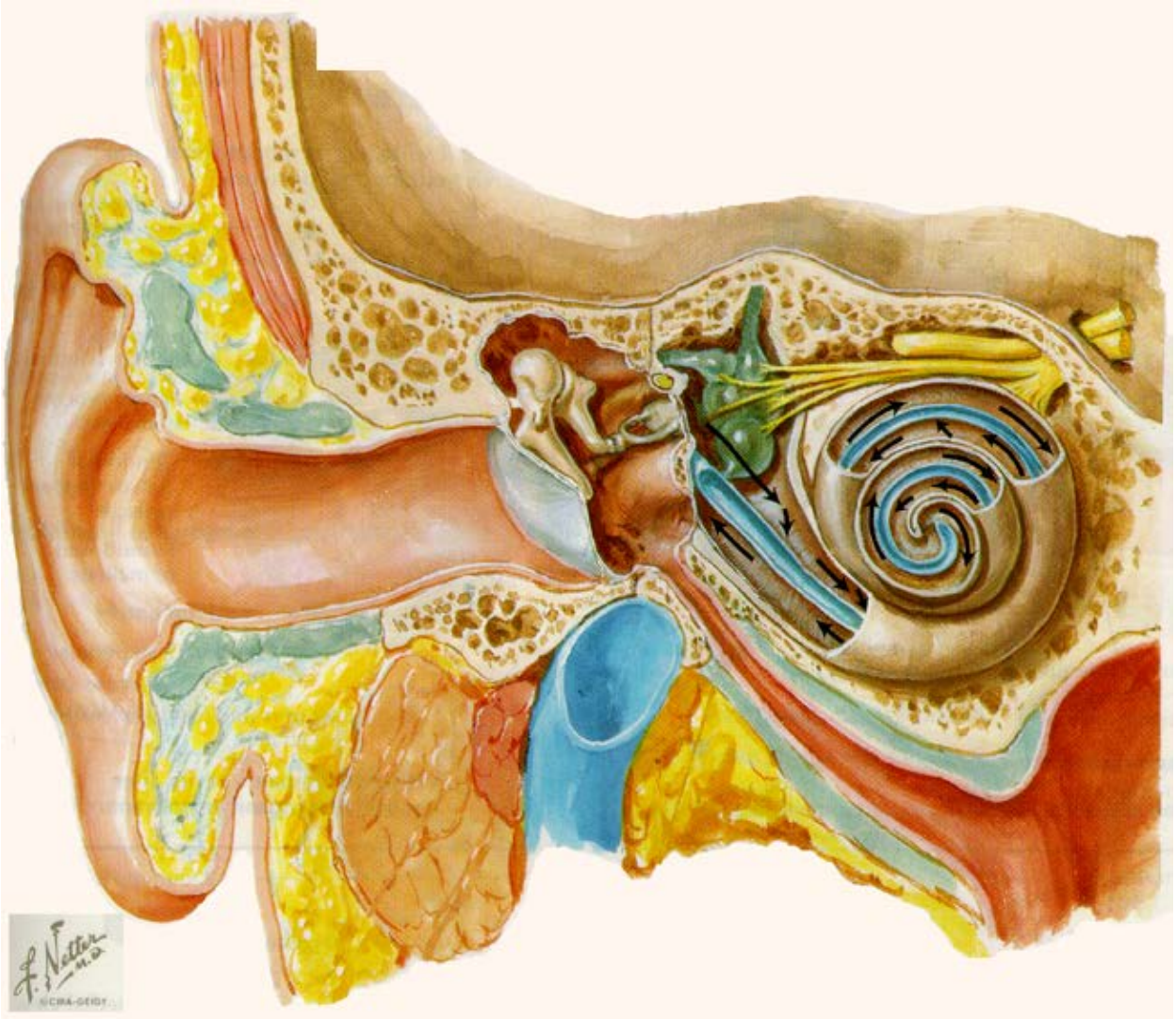
- Unilateral
- Bilateral

- 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

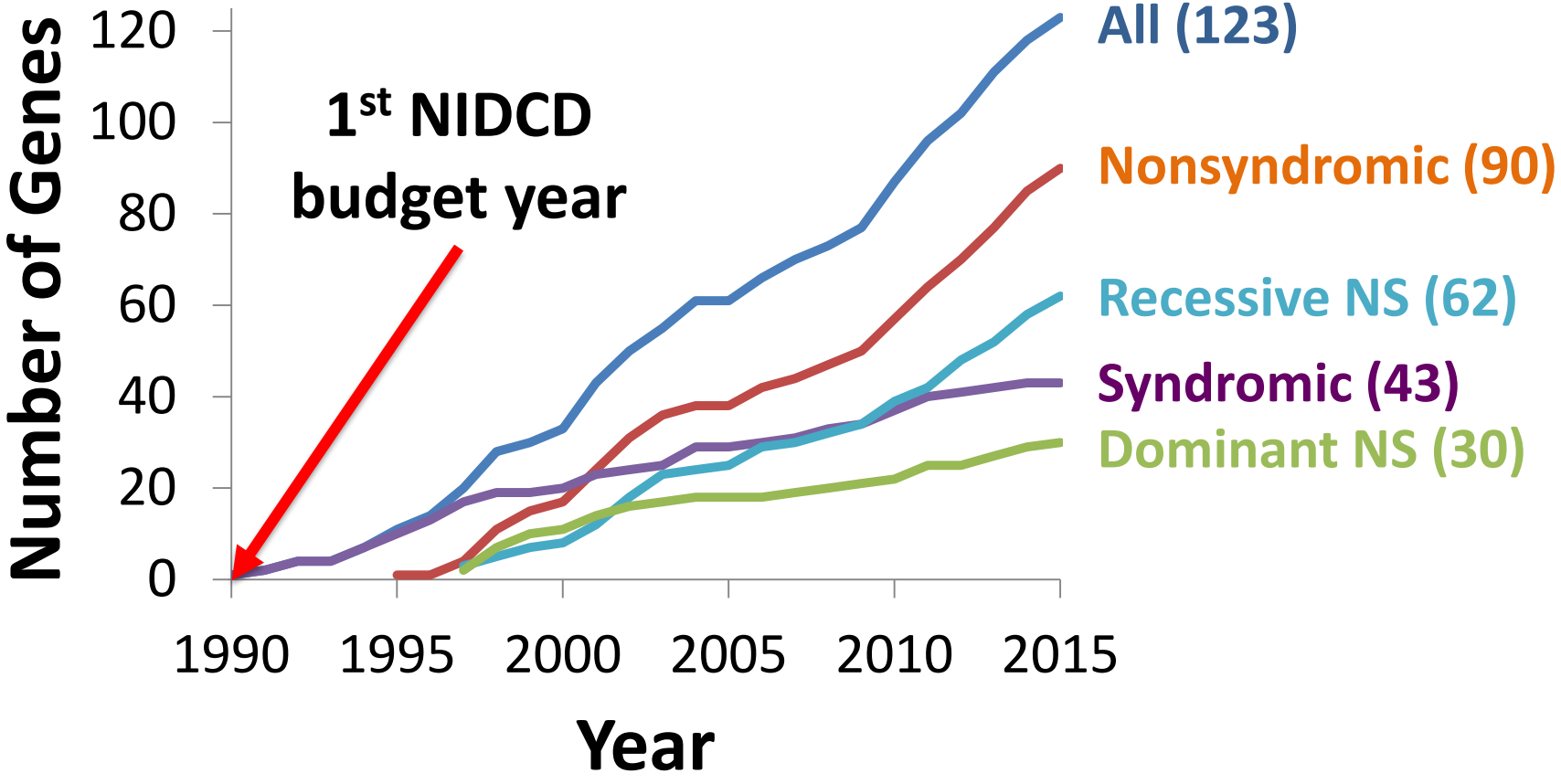
Syndromic (~30%)

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



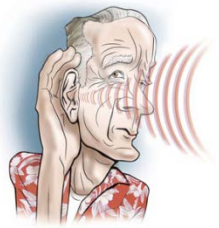
Clinical Utility of Genetic Testing

Newborn screening

Early intervention

Prenatal testing

Risk assessment



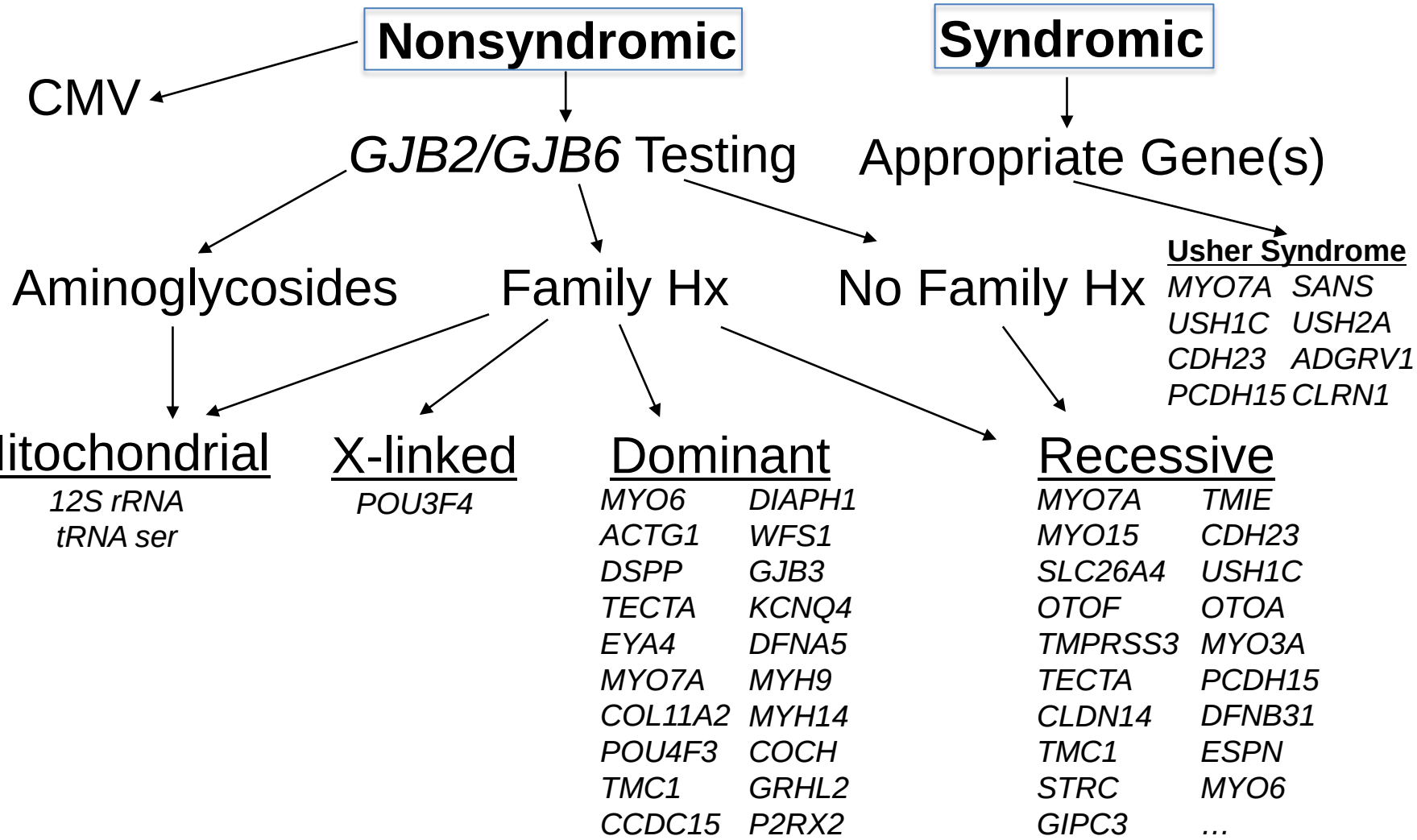
Life-long management

As Gene Test Menu Grows, Who Gets to Choose?

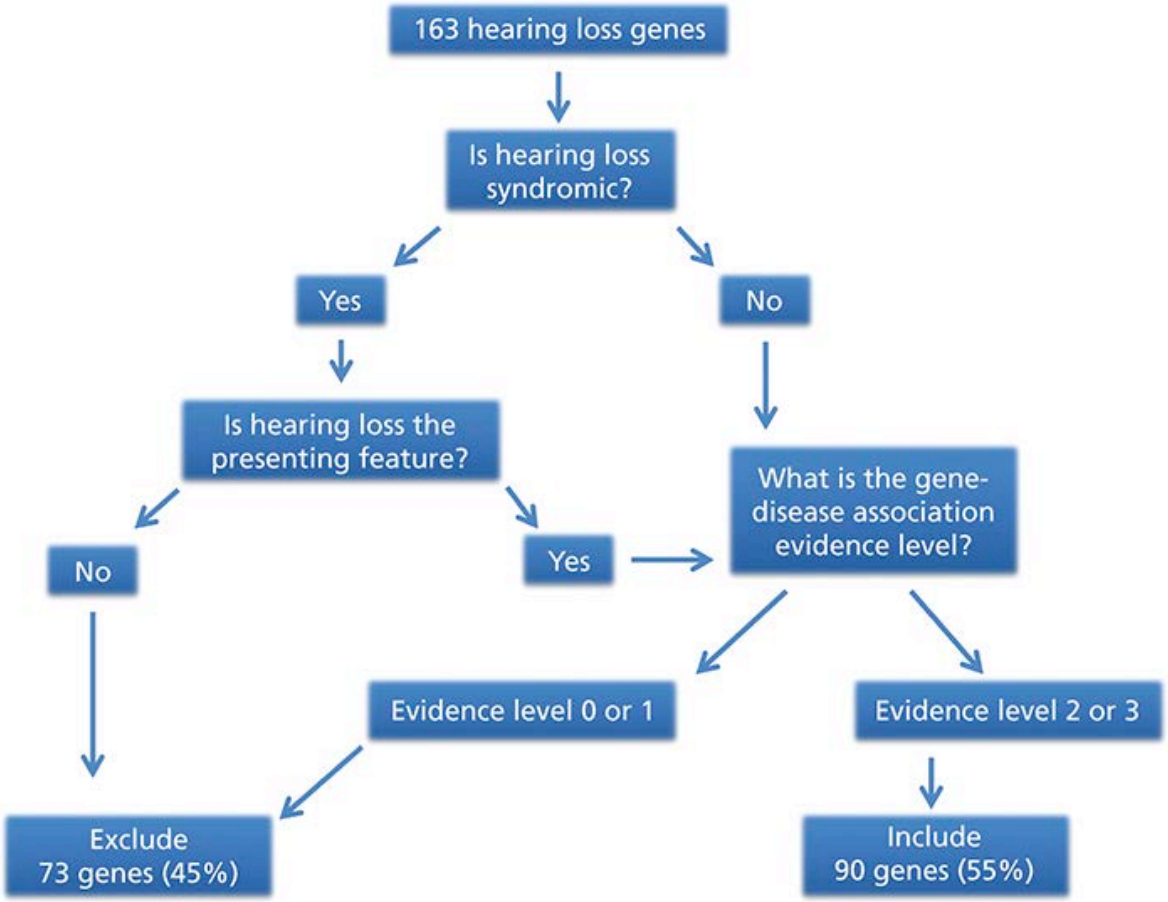
The New York Times

Wednesday, July 21, 2004

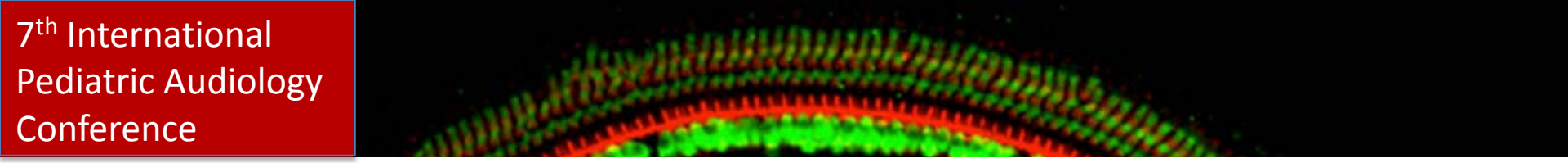
Hearing Loss Screening Protocol



Panel Inclusion Criteria for Nonsyndromic or "Apparent" Nonsyndromic Hearing Loss Genes



Abou Tayoun et al., Genetics in Medicine (2015)



ACTG1
ATP6V1
BSND
CATSPER2
CACNA1D
CCDC50
CEACAM16
CLDN14
COCH
COL11A2
CRYM
DFNA5
DIABLO
DFNA5
DIAPH1
ESPN
ESRRB
EYA4
GIPC3
GJB2
GJB6

GPSM2
GRHL2
GRXCR1
HGF
ILDR1
KARS
KCNQ4
LHFPL5
LOXHD1
LRTOMT
MARVELD2
MIR96
MSRB3
MTRNR1
MTTS1
MYH14
MYH9
MYO15A
MYO3A
MYO6
OTOF

OTOG
OTOGL
P2RX2
POU3F4
POU4F3
PRPS1
RDX
SERPINB6
SLC17A8
SMPX
STRC
SYNE4
TBC1D24
TECTA
TIMM8A
TMC1
TMIE
TMPRSS3
TPRN
TRIOBP
TPSPEAR

ADGRV1
CDH23
CIB2
CLRN1
DFNB31
HARS
MYO7A
PCDH15
USH1C
USH1G
USH2A
— Usher

SLC26A4 — Pendred

OTOA
DFNB59
— AN

CLPP
HARS2
HSD17B4
LARS2
— Perrault

KCNE1
KCNQ1
— JLNS

OtoGenome

EDN3
EDNRB
MITF
PAX3
SNAI2
SOX10
— Waardenburg

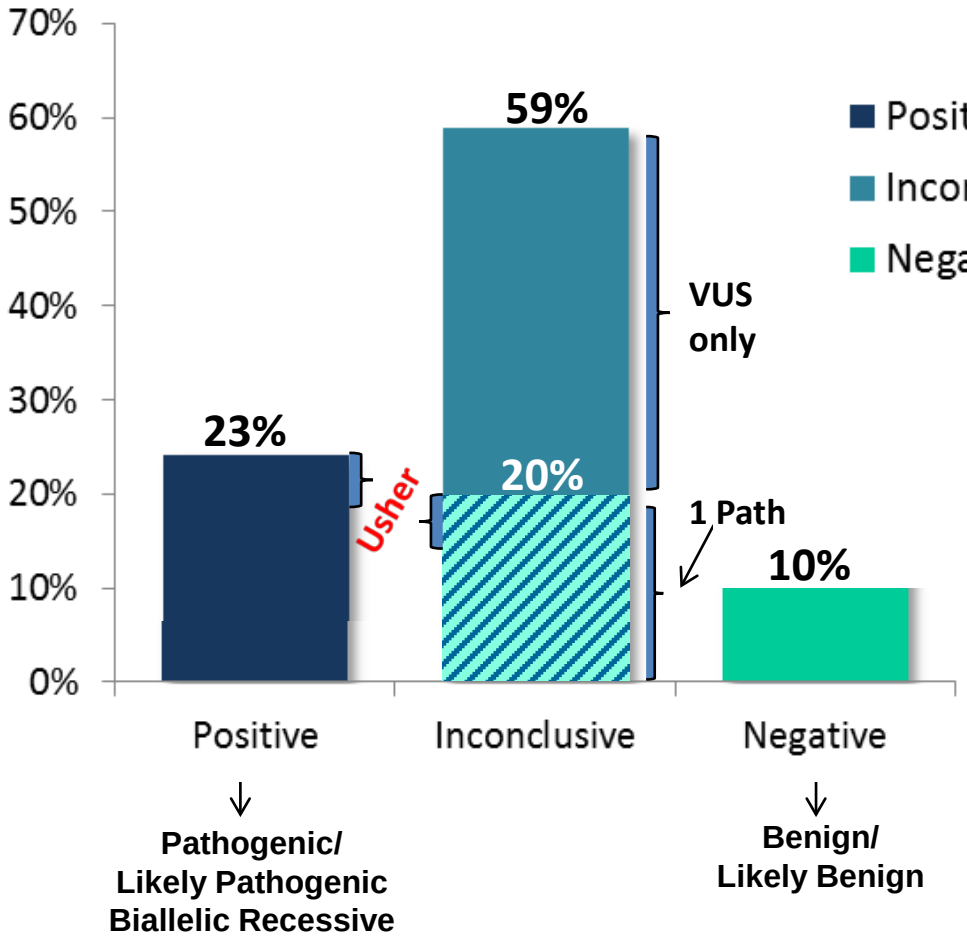
EYA1
SIX1
— BOR

WFS1 — Wolfram

CATSPER2 — DIS

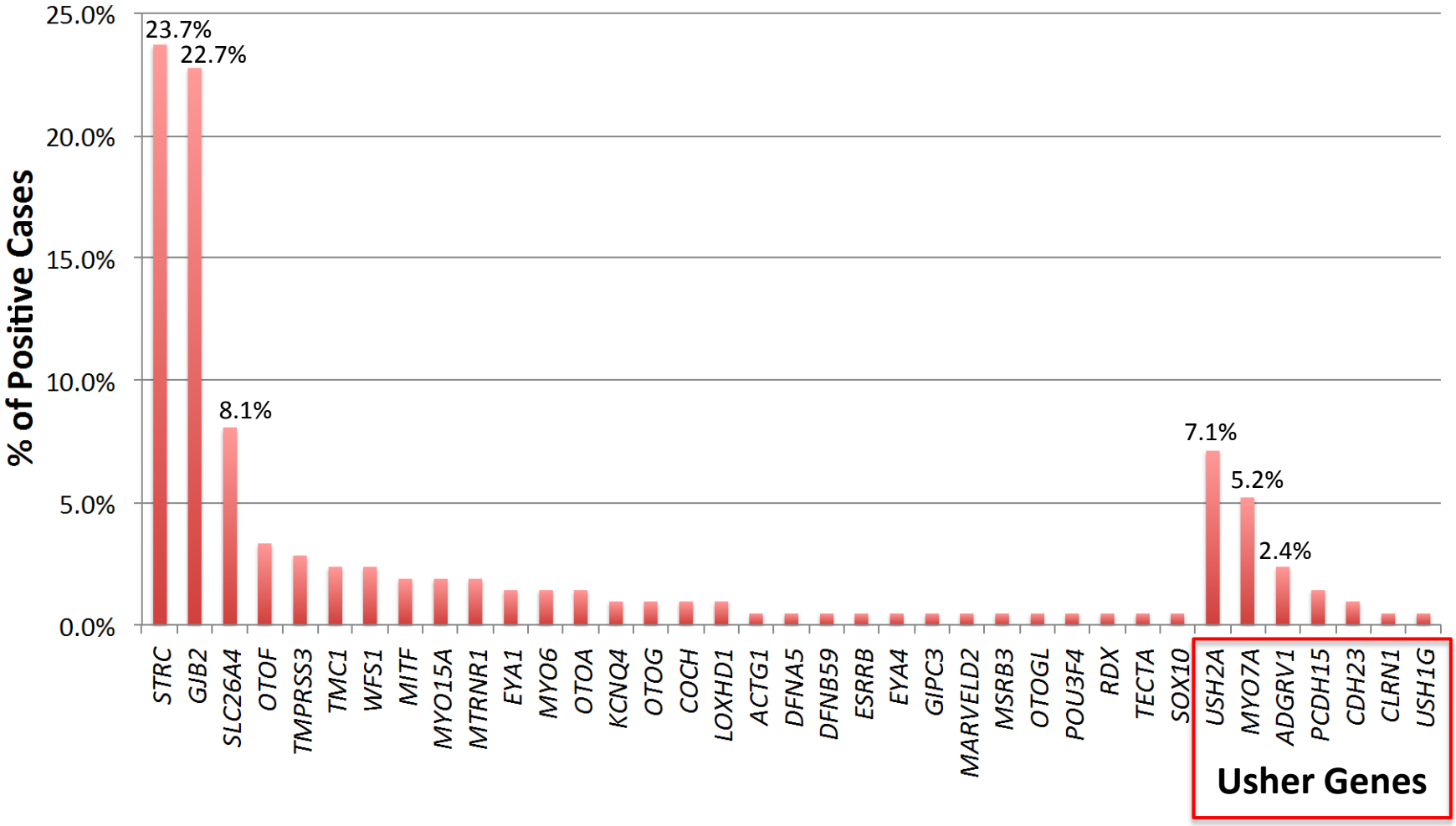
Subpanels available (Usher, Waardenburg, BOR, JLNS, Pendred, AN, WFS1, Mito, etc.)

OtoGenome Detection Rates (n=959)



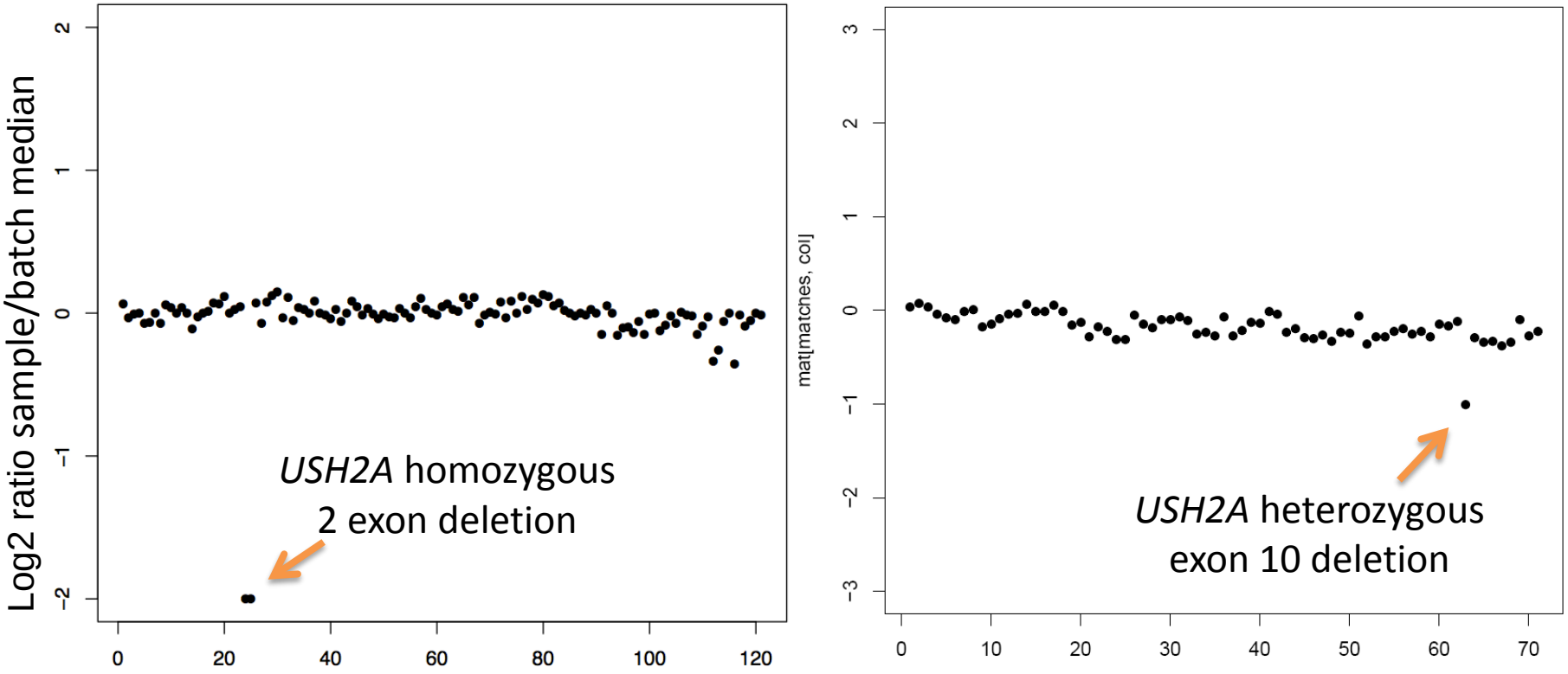
- *GJB2*-related hearing loss excluded in most cases
- ~7% of cases have pathogenic variants in Usher genes, most unaware of their risk for retinitis pigmentosum

Genetic Etiology of 218 OtoGenome Positive Cases



Courtesy of LMM, Shen

Deletions Detected by NGS

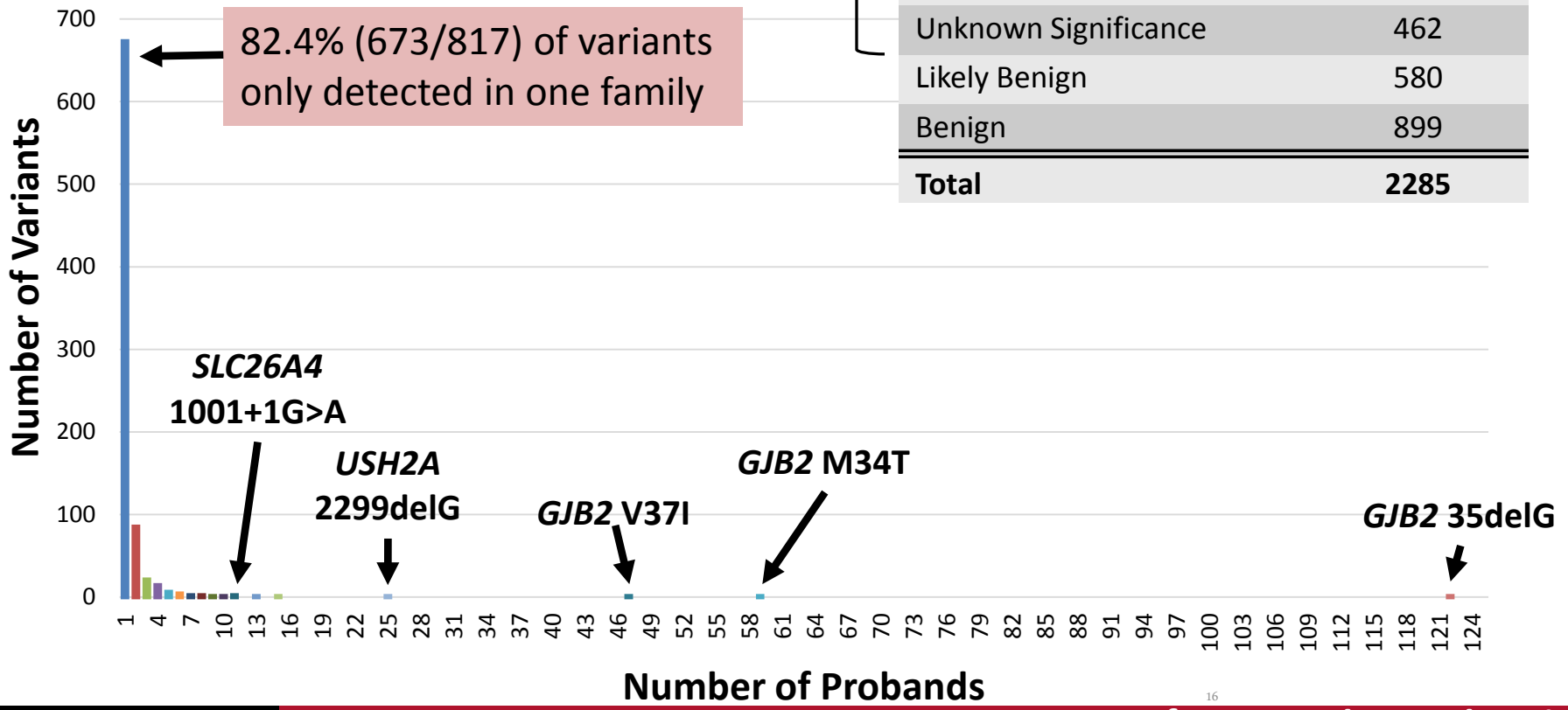


Copy number variants confirmed by digital droplet PCR

Hearing Loss Variants in over 2000 Cases Tested at the Laboratory for Molecular Medicine

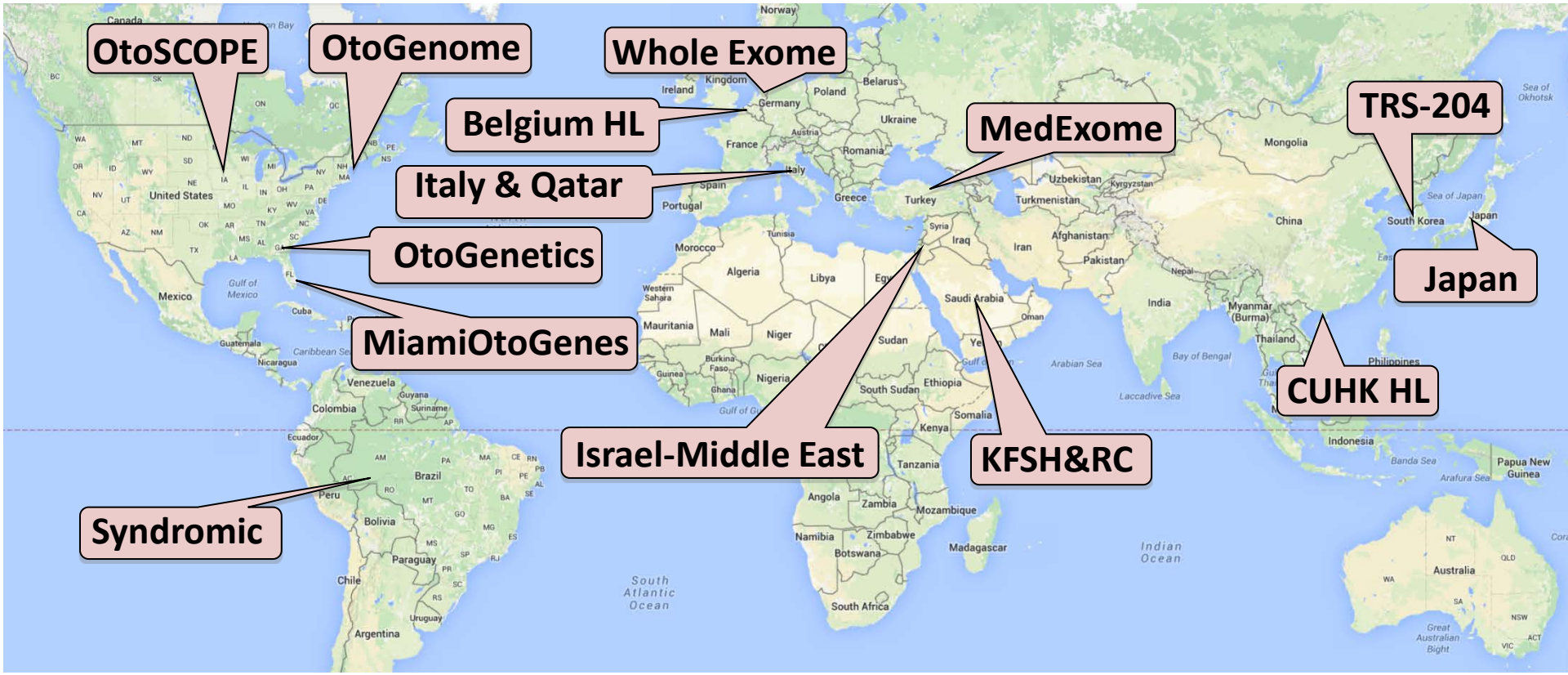
817 variants

Reported Variant Classification	
Pathogenic	223
Likely Pathogenic	121
Unknown Significance	462
Likely Benign	580
Benign	899
Total	**2285**



Courtesy of LMM, Rehm and Amir

Next-Generation Sequencing Tests for Hearing Loss

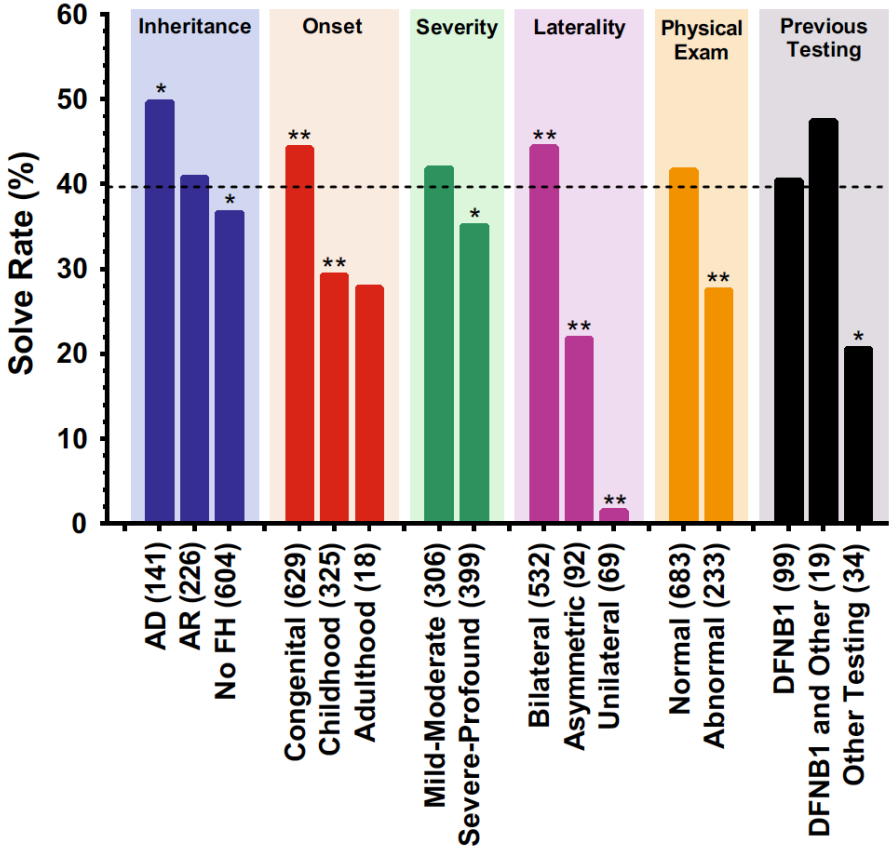


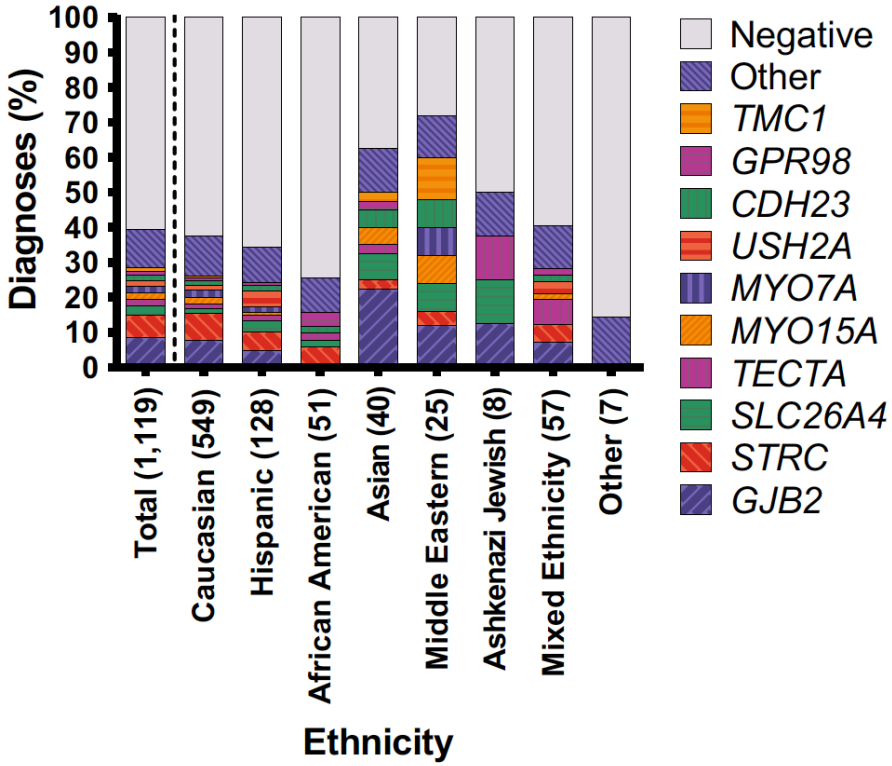
Panel	Institution	Genes	Diagnostic yield	Capture	Sequencing
Belgium	U Antwerp	79	25-30%	TruSeq	Illumina
CUHK-HL	CUHK	252	57%	SureSelect	Illumina
Israel-MidEast	Tel Aviv	246	56%	SureSelect	Illumina
Italy+Qatar	U Trieste	96	33%	AmpliSeq	Ion PGM
Japan	Shinshu U	63	30%	AmpliSeq	IonTorrent
KFSH&RC	KFSH	90	54%	AmpliSeq	Ion PGM
Medical Exome	Ege U	102/2761	72.4%	TruSight	Illumina
MiamiOtoGenes	U Miami	146	50%	SureSelect	Illumina
OtoGenetics	Commercial	131	42-52%	NimbleGen	Illumina
OtoGenome	**LMM/Harvard**	**87**	**23%**	**SureSelect**	**Illumina**
OtoSCOPE	MORL/U Iowa	89	39%	SureSelect	Illumina
Syndromic	U Brasilia	52	10%	AmpliSeq	Ion PGM
TRS-204	Seoul National	204	55%	?	Illumina
Whole Exome	Rotterdam	120/20k	70%	?	?

Explanation for Variable Diagnostic Yields

- Inclusion or exclusion of *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



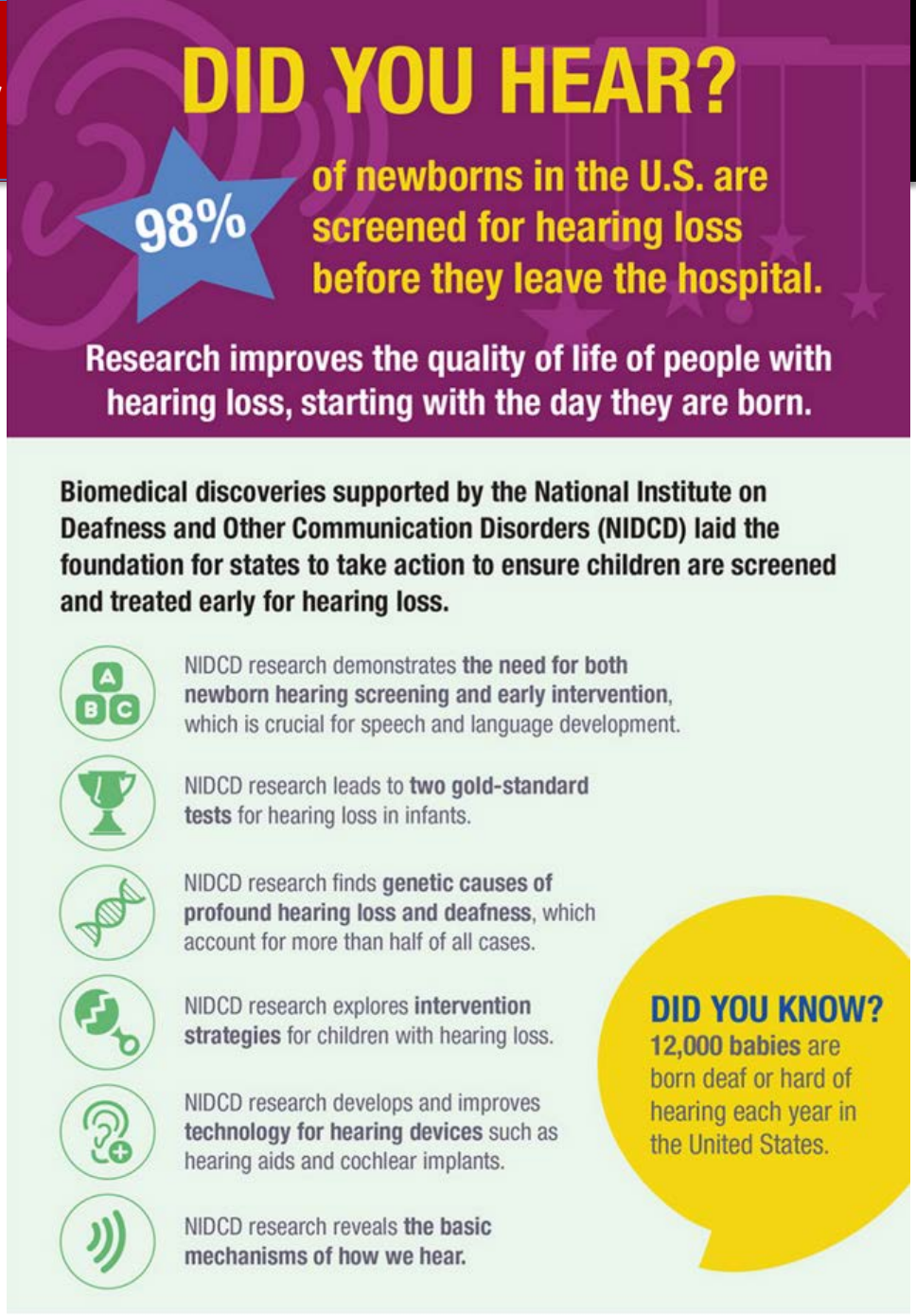


Newborn Hearing Screening

- 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

MedlinePlus Magazine Fall 2013

www.medlineplus.gov



NIDCD authorized on 10-28-88 by Public Law 100-553

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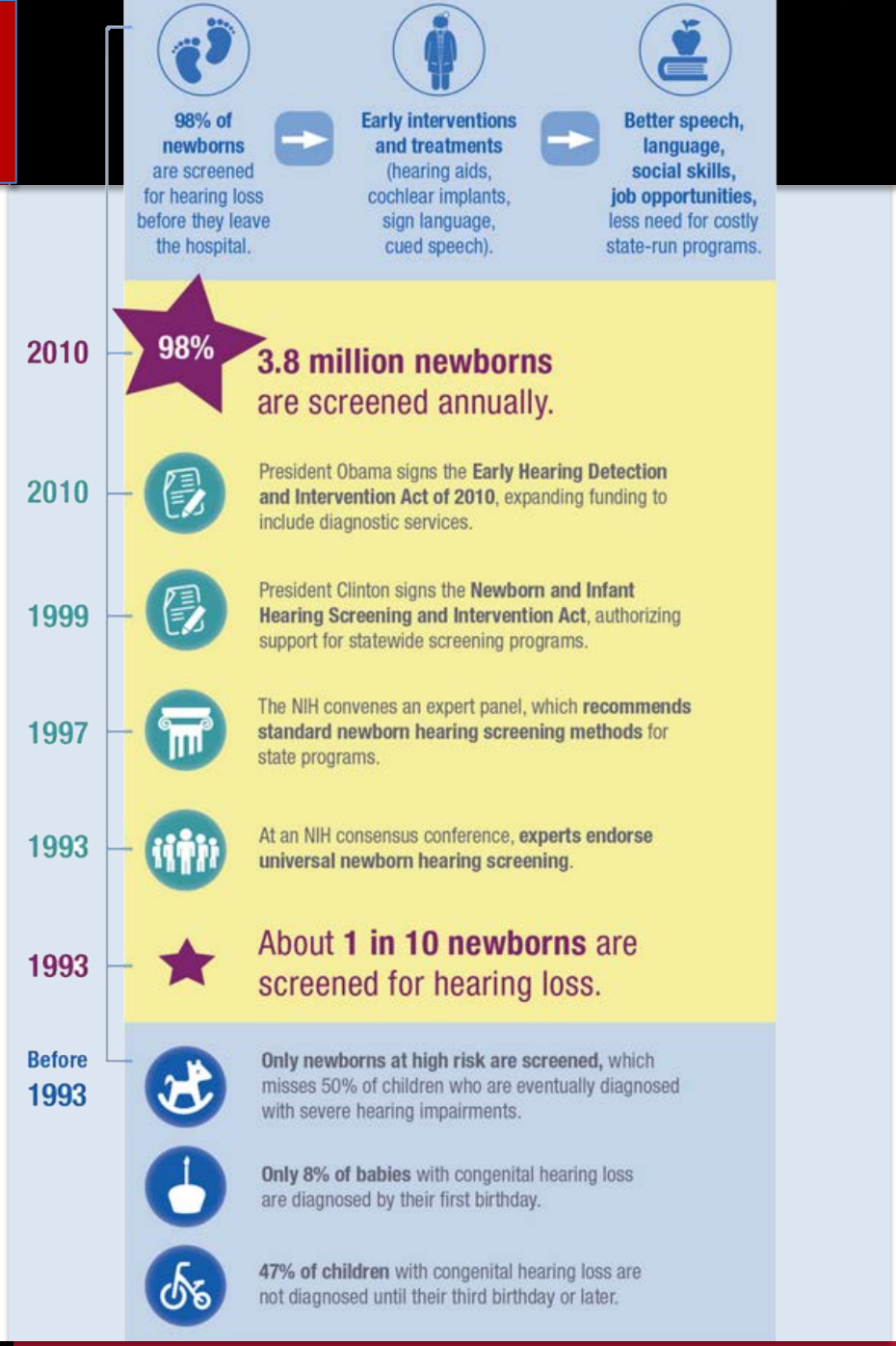


Next-Gen Newborn Screening for Hearing: a paradigm for expanded genetic screening

- 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.

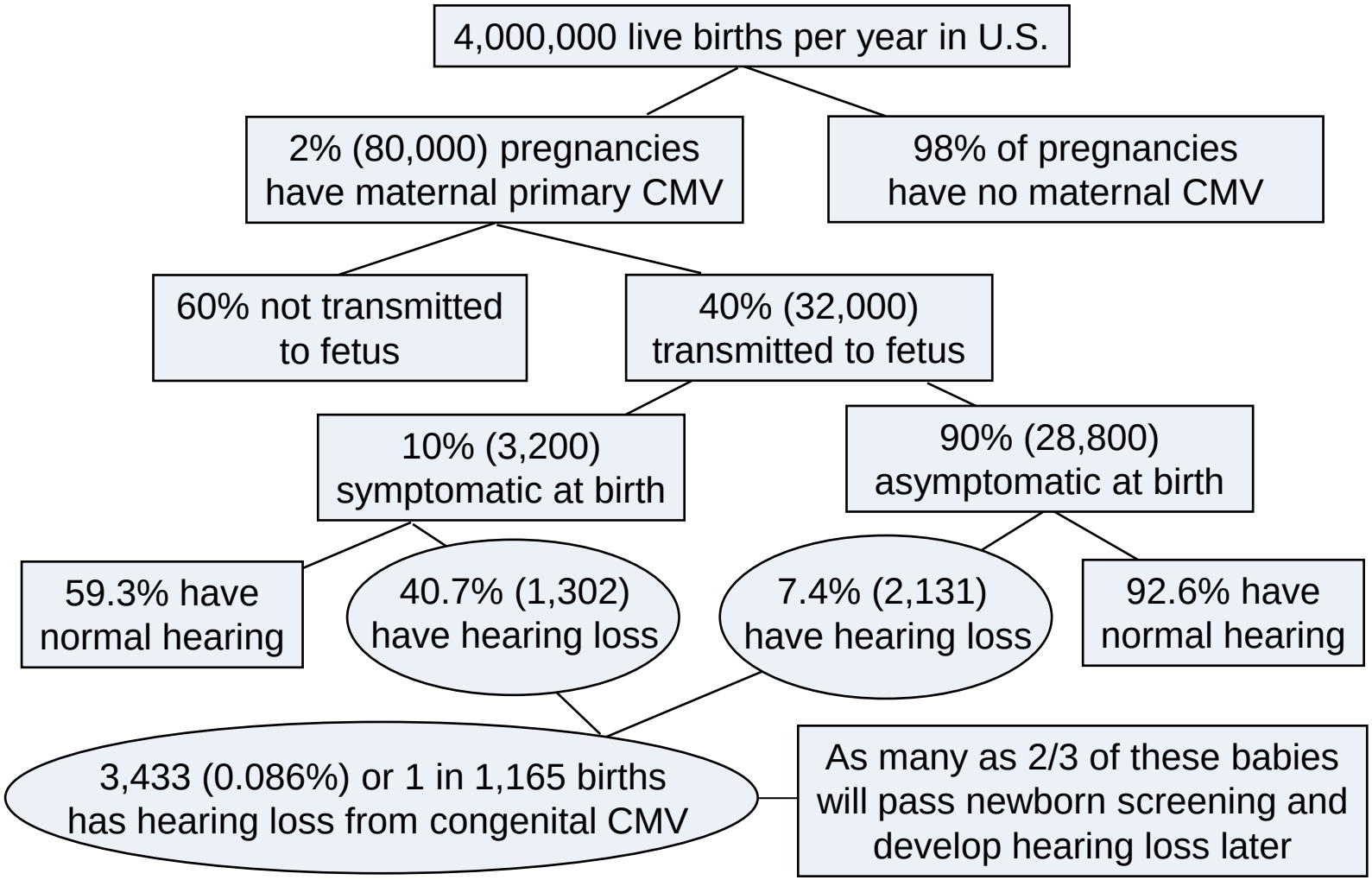
Precise Diagnosis Impacts Care

- *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?

	At Birth (~4 million newborns)	Failed Audiometry (2-8 per 100 infants)	Confirmed Hearing Impairment
+	All newborns tested Reduce work up Use blood spots Identify those at risk	Less infants tested Reduce work up Use blood spots?	Less infants tested Reduce work up Specific testing High parental interest
–	Genotype-phenotype? False positives Detect many carriers Parental interest low	Genotype-phenotype? False positives? Detect many carriers Parental interest low? Miss some at risk New specimen	Genotype-phenotype? Miss some at risk New specimen? Delay in intervention

Hearing Loss from Cytomegalovirus (CMV)



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SEQuencing a **B**aby for an **O**ptimal **O**utcome

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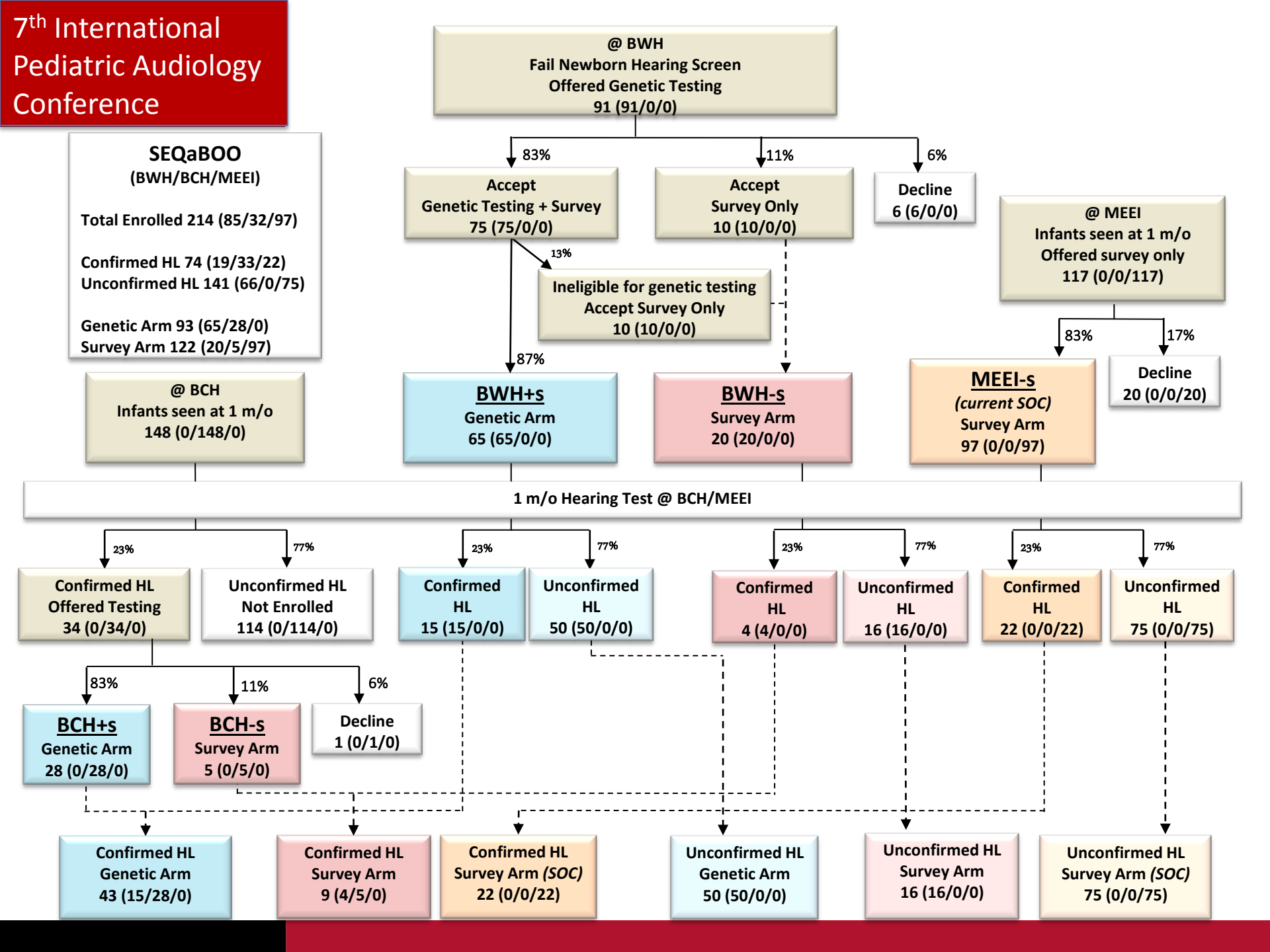

Comparisons of Traditional and Next-Generation Newborn Hearing Screening

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

Whole Genome Sequencing Projects Underway

- Discovery projects for additional genes involved in hearing loss

Genomics england

Gabriella Miller Kids First

PEDIATRIC RESEARCH PROGRAM

"Hot Off the Email" (Sept. 2016): from Michael Watson, Exec Director, ACMG

"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

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Massachusetts Eye and Ear Infirmary

Michael Cohen

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Ahmad Abou Tayoun
Sami Amr
Amy Hernandez
Andrea Murihead
Heidi Rehm

Harvard Medical School
Center for Hereditary Deafness

HMSCHD

Manchester Centre for
Audiology and Deafness (ManCAD)