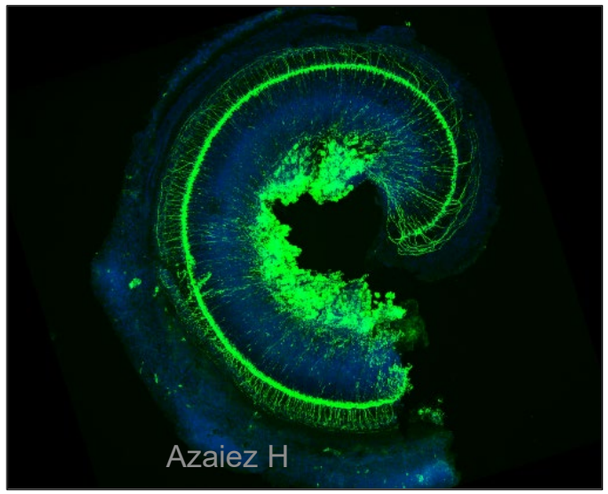
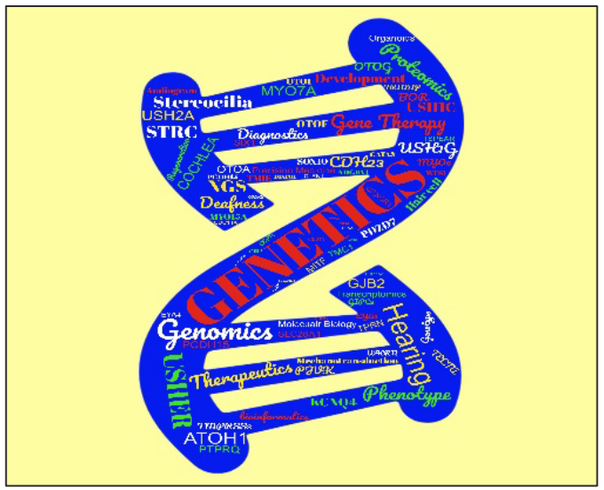
Precision Audiology: The Clinical Relevance of Genetic Testing in Hearing Loss

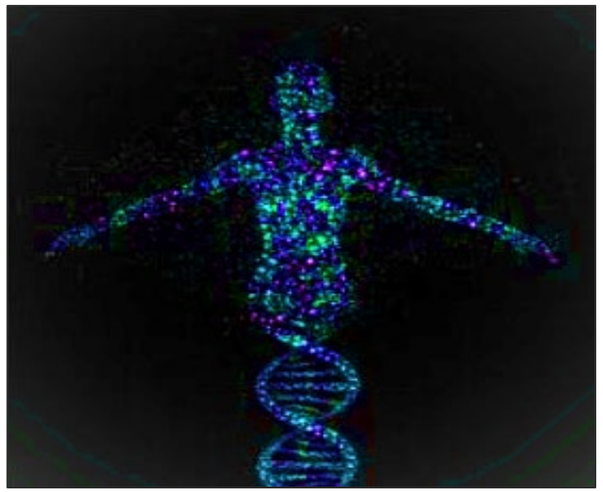
Hela Azaiez, MS, PhD

Department of Medical and Molecular Genetics
Indiana University School of Medicine

CAA2026, Moncton, Canada









Gene Therapy for Hearing Loss!!

FDA-Approved Drug



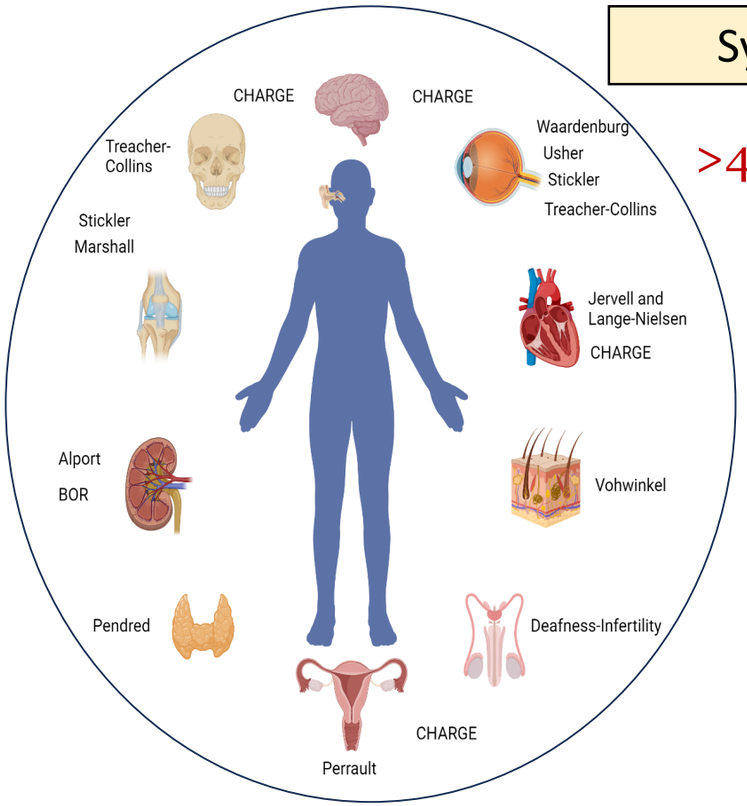
Drugs.com

Etiology of Hearing Loss

Congenital Hearing Loss – 1 in 1000

- Environmental causes (20%)
- Genetic causes (50% - 80%)
  - Syndromic (30%)
  - Nonsyndromic (70%)

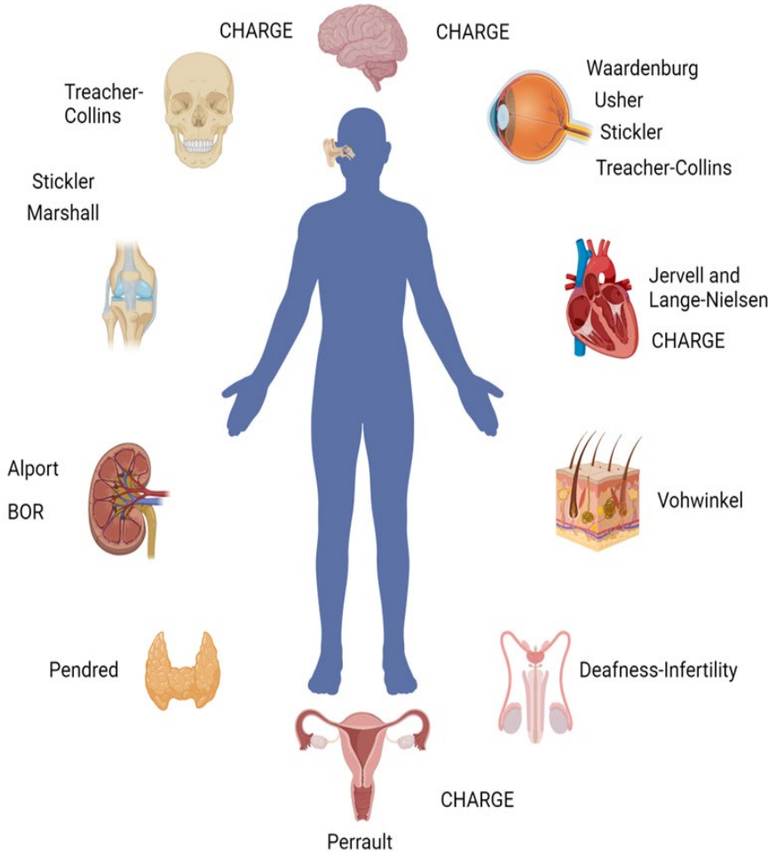
>400 different syndromes



Azaiez H.

Nonsyndromic Hearing Loss "mimics"

- Some syndromic forms of HL may initially appear nonsyndromic, because extra-auditory features are not yet evident on history or physical examination.
- Examples: Usher syndrome, Deafness Infertility syndrome, Jervell and Lange Nielsen syndrome, Pendred Syndrome, HDRS, etc.



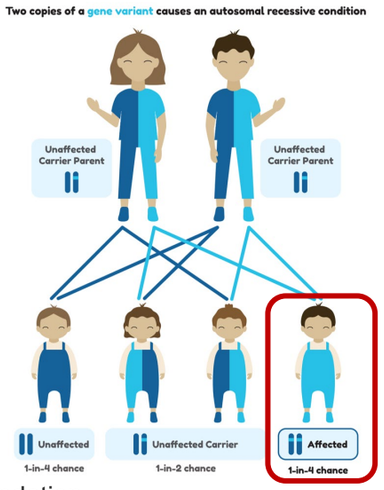
Key Point

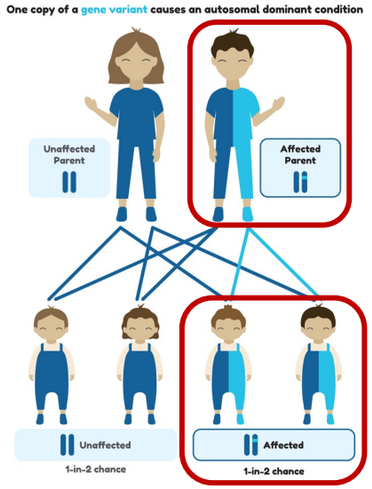
Up to **~20%** of children who initially present isolated HL may later be recognized as having syndromic HL

Genetic Hearing Loss

Congenital Hearing Loss – 1 in 1000

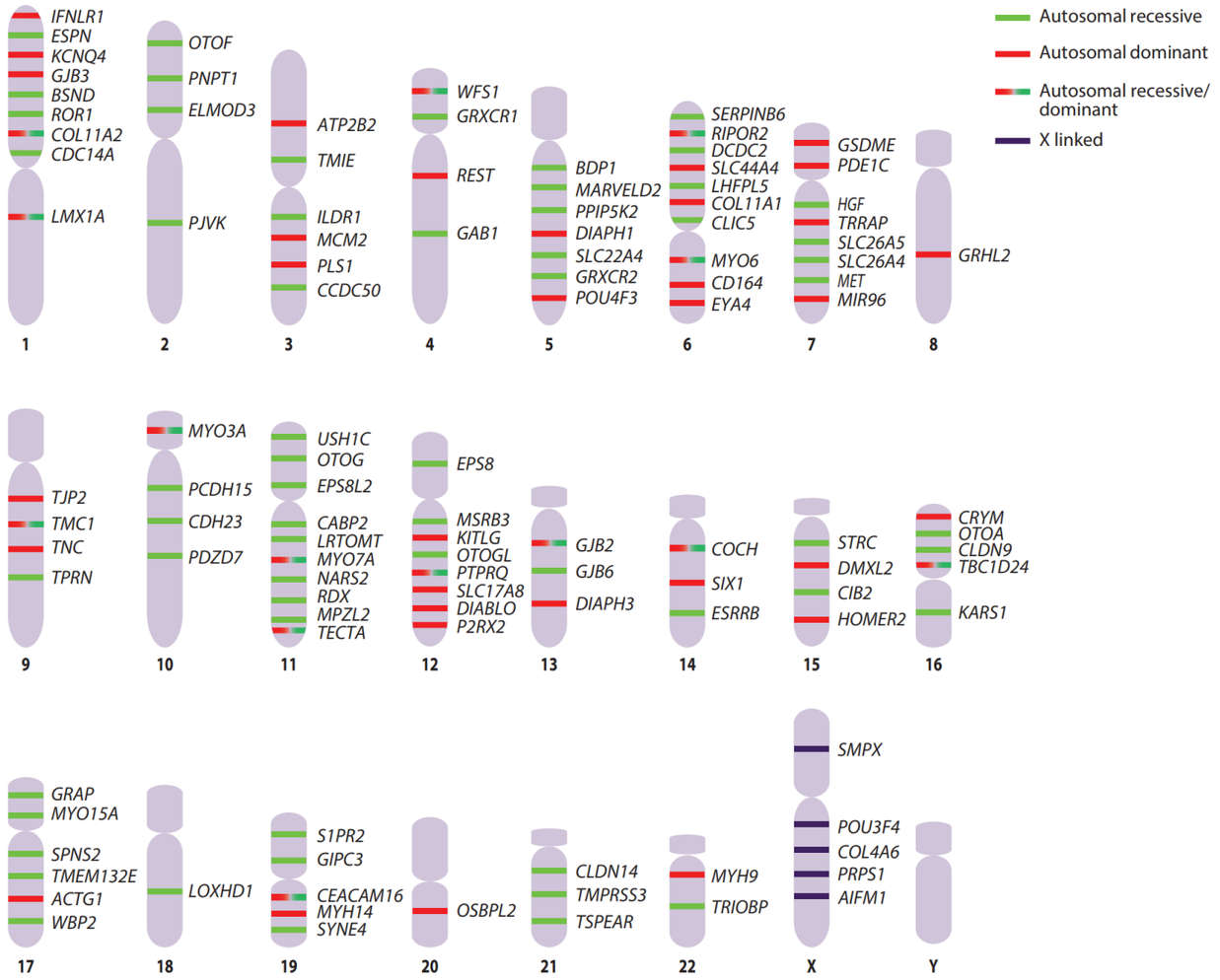
- Environmental causes (20%)
- Genetic causes (50% - 80%)
  - Syndromic (30%)
  - Nonsyndromic (70%)
    - Recessive (**~80%**) DFNB
    - Dominant (**~19%**) DFNA
    - X-linked (**<2%**) DFNX
    - Mitochondrial (**<1%**)





DFN: DeaFNess

Hearing Loss is *Genetically* Heterogeneous



Taiber et al. 2022

Hereditary Hearing Loss Homepage

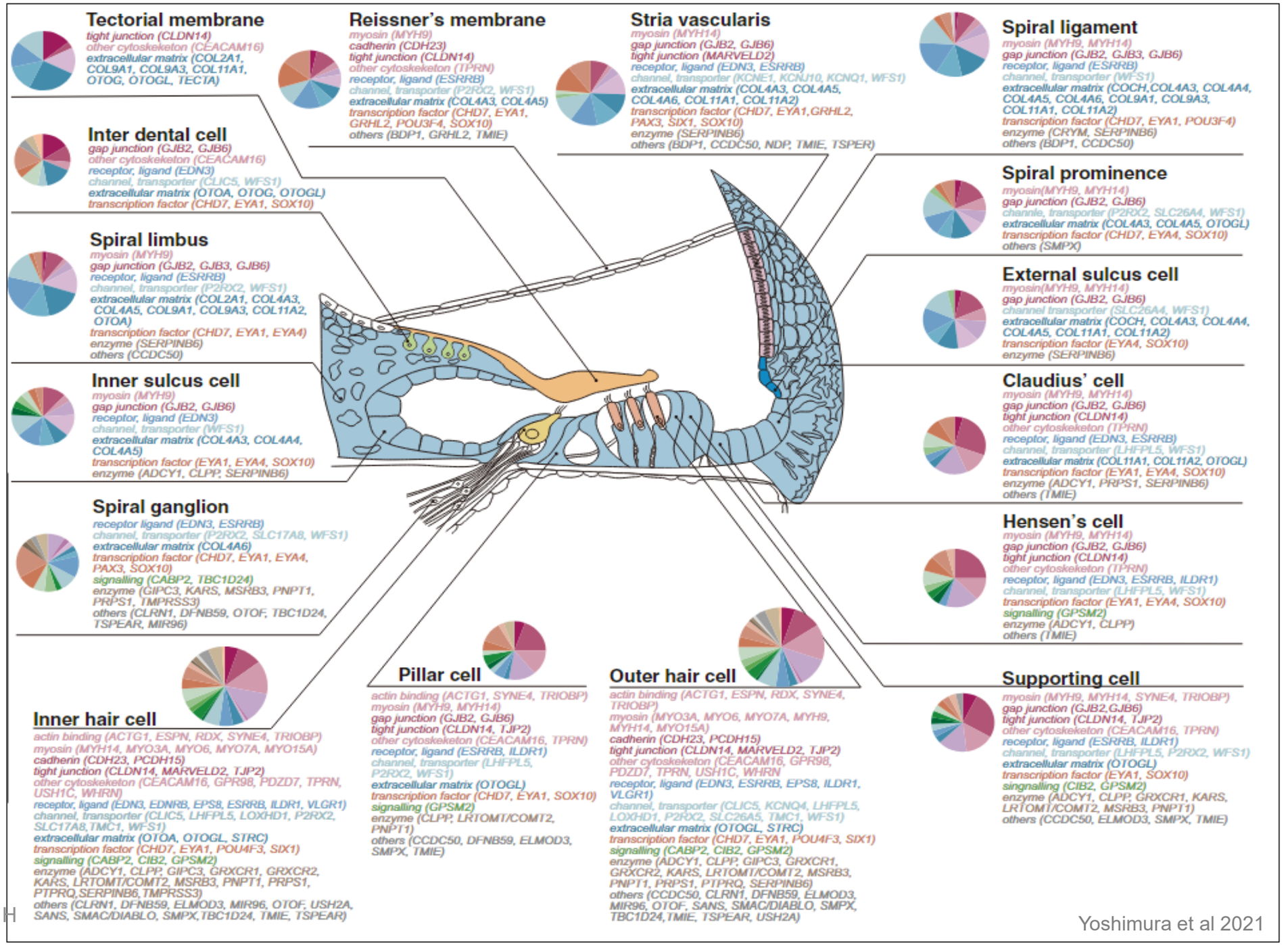
https://hereditaryhearingloss.org/

Home
About the HHL Homepage
Citing the HHL Homepage
Nonsyndromic Hearing Loss
Autosomal Dominant
Autosomal Recessive
Sex-Linked
Mitochondrial
Modifiers
Auditory Neuropathy
Otosclerosis
Syndromic Hearing Loss
Updates Log
Useful Links
Frequently Asked Questions
Contact

Autosomal Dominant Nonsyndromic Hearing Loss

Locus (OMIM)	Gene (OMIM)	Key Locus References (PubMed)	Key Gene References (PubMed)	Evidence (ClinGen)
DFNA1	*DIAPH1* (see note 1)	Leon et al., 1992	Lynch et al., 1997	Definitive
DFNA2A	*KCNQ4*	Coucke et al., 1994; Van Camp et al., 1997	Kubisch et al., 1999	Definitive
DFNA2B	*GJB3*	Xia et al., 1998	Xia et al., 1998	Disputed
DFNA3A	*GJB2*	Chaïb et al., 1994	Kelsell et al., 1997	not reviewed
DFNA3B	*GJB6* (see note 2)	Grifa et al., 1999	Grifa et al., 1999	not reviewed
DFNA4A	*MYH14*	Chen et al., 1995; Mirghomizadeh et al., 2002	Donaudy et al., 2004	Moderate
DFNA4B	*CEACAM16*	Yang et al., 2005	Zheng et al., 2011	Moderate

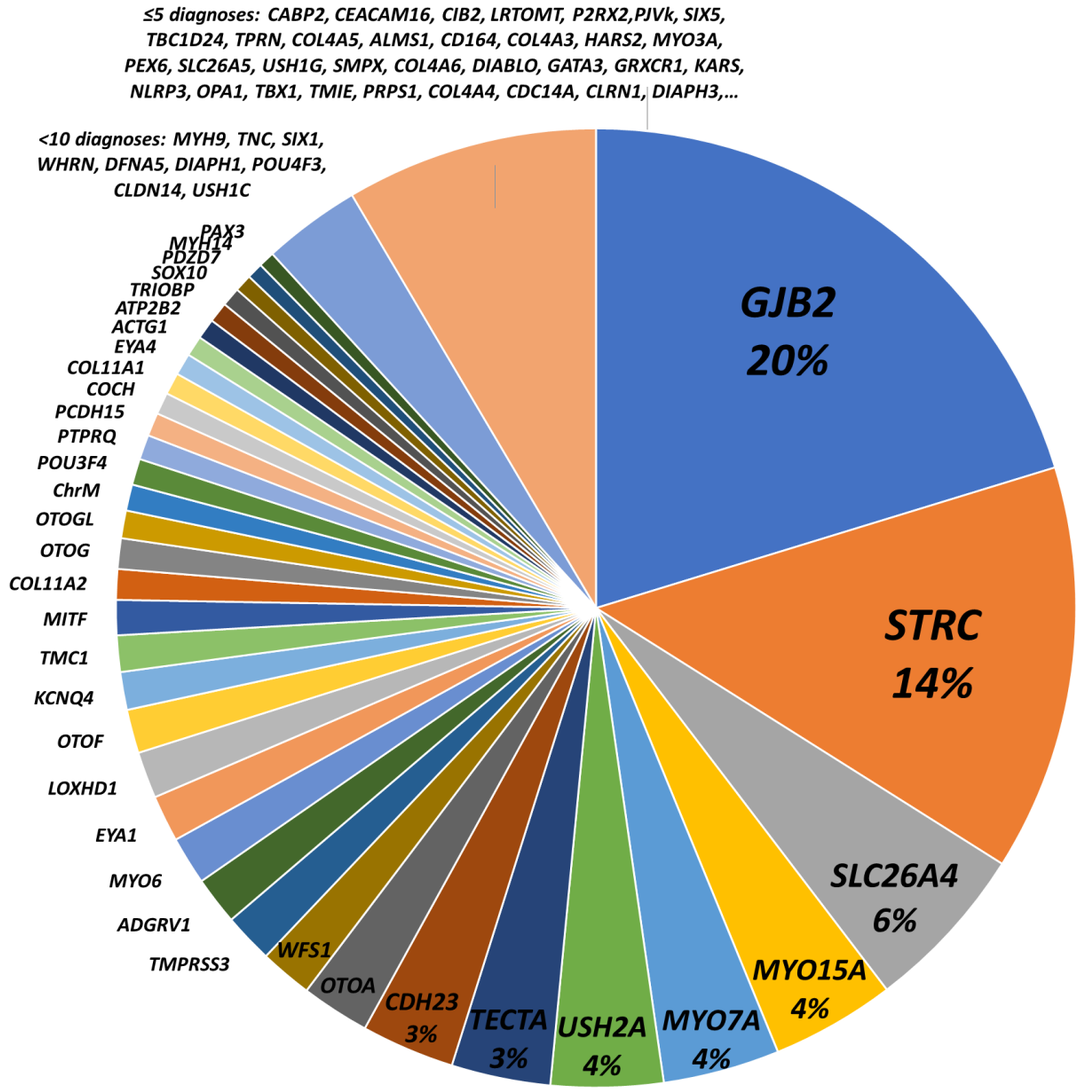
Azaiez H



Azaiez H

Yoshimura et al 2021

Genetic Etiology of Hearing Loss

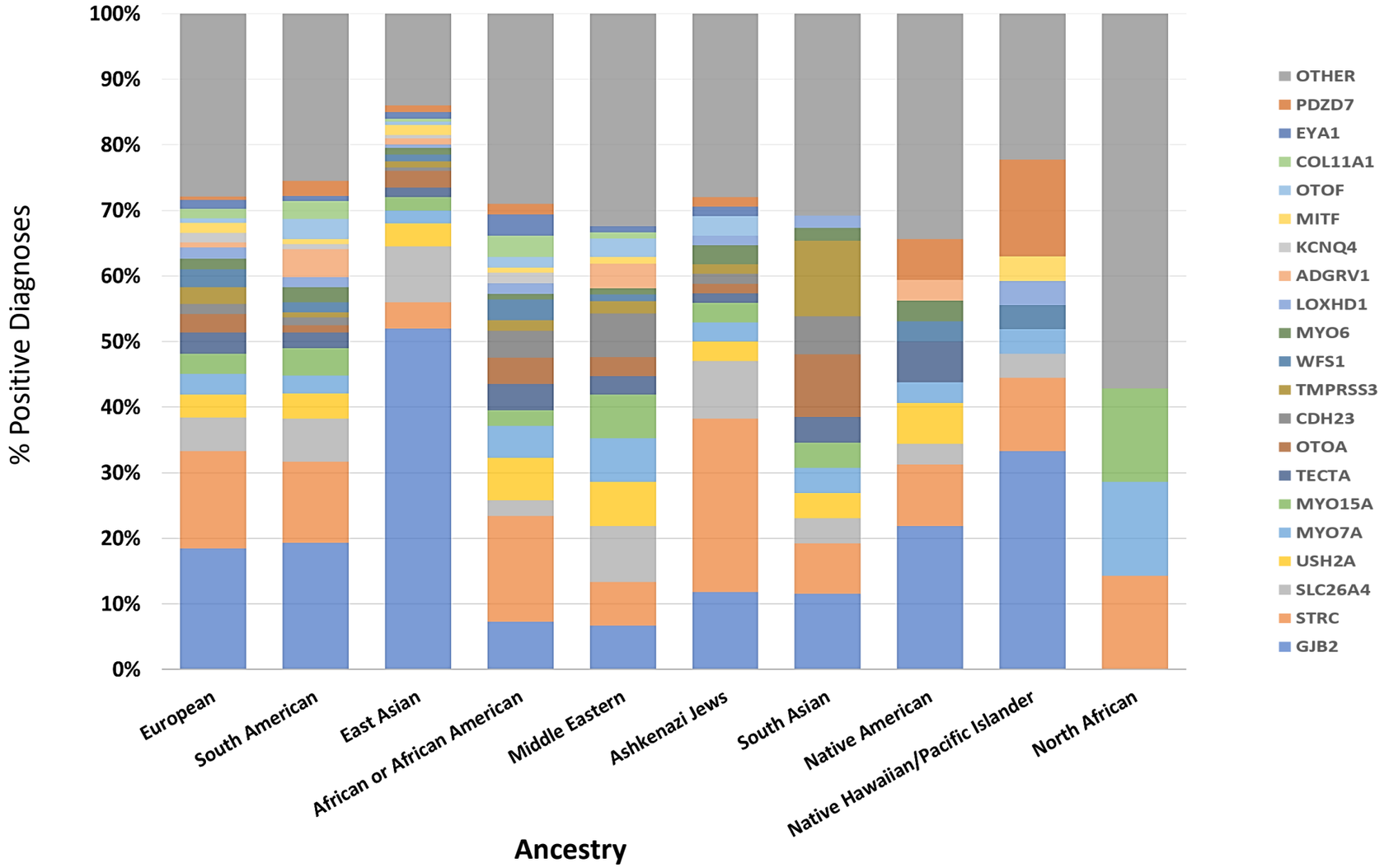


- *GJB2* is the most frequent cause of hearing loss
- *STRC* is the second most common cause of HL
- 6 genes account for 50% of all cases
- 20 genes account for 75% of all cases

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Molecular Otolaryngology and Renal Research Laboratories (MORL)
https://morl.lab.uiowa.edu/

Scale Revealed Variability in Genetic Spectrum

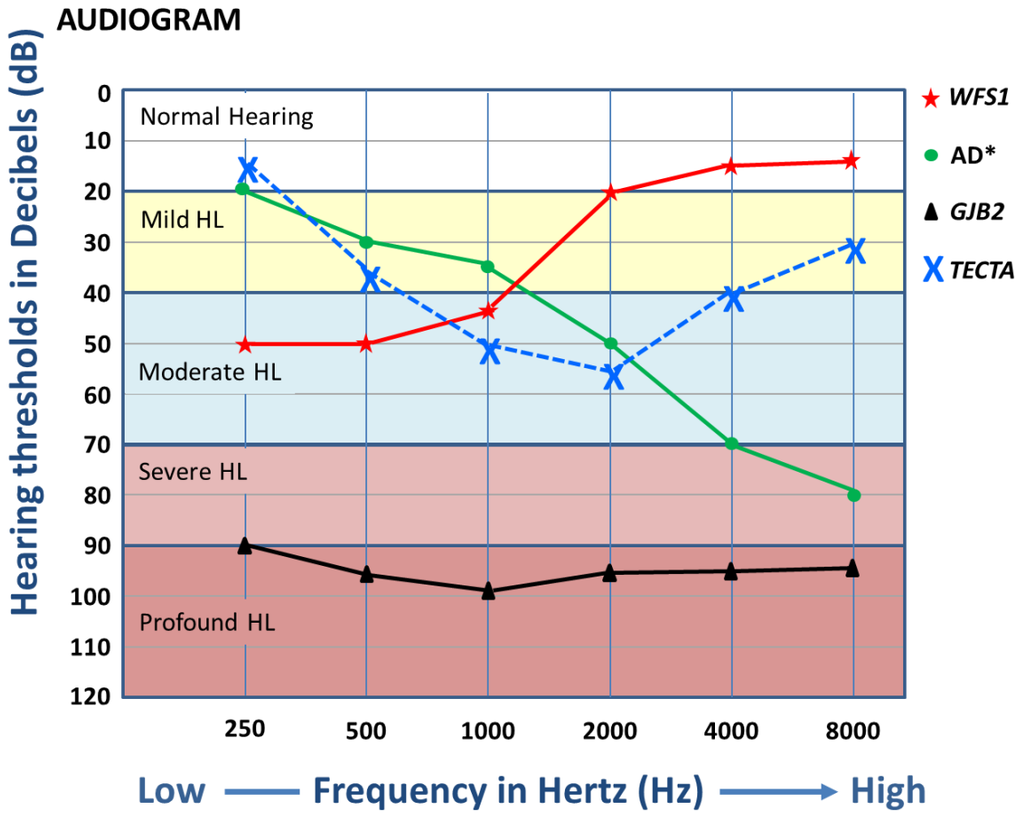
❖ Genetic Spectrum is Ancestry Dependent



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https://morl.lab.uiowa.edu/

Hearing Loss is *Clinically* Heterogeneous

Hearing Loss

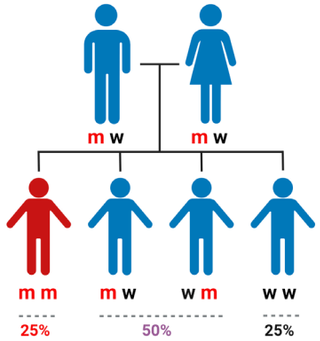


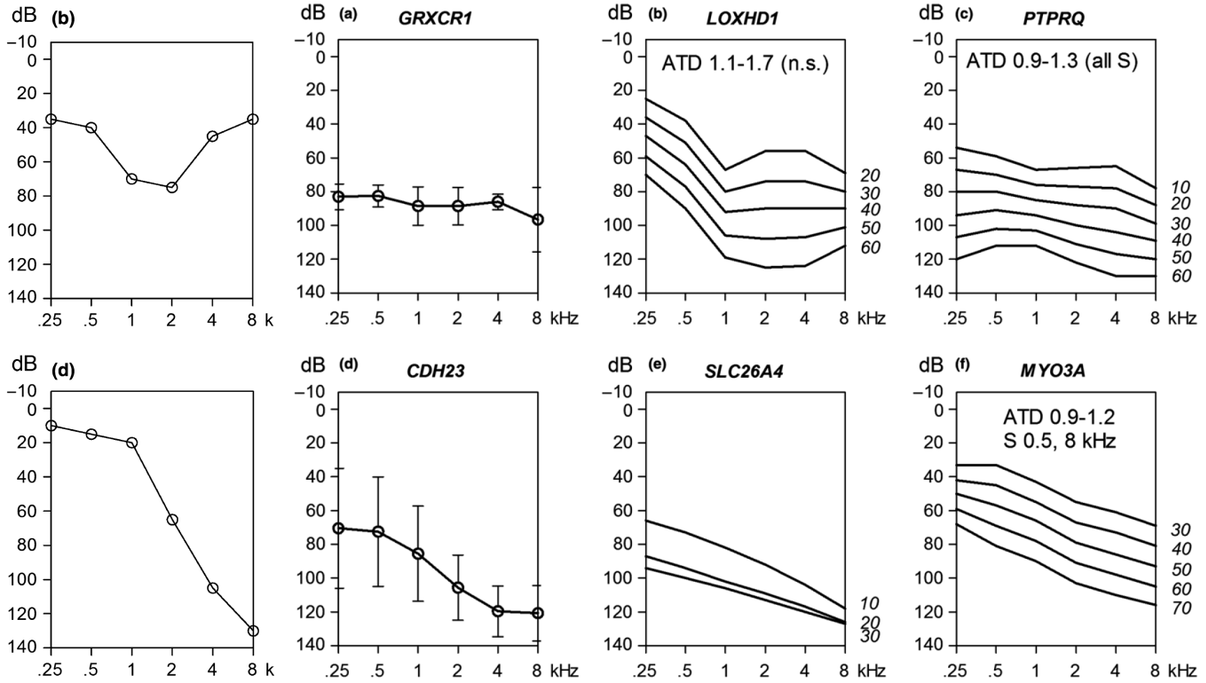
- Onset
- Severity
- Frequencies Affected
- Symmetry
- Progression

- **Wide Range of clinical presentations**

Autosomal Recessive Nonsyndromic HL

- **ARNSHL-associated genes: 88** (*as of 10/2026*)
- **Onset:** usually prelingual
- **Severity:** usually severe-to-profound across all frequencies. *However*,…

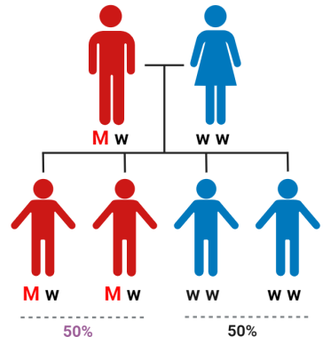


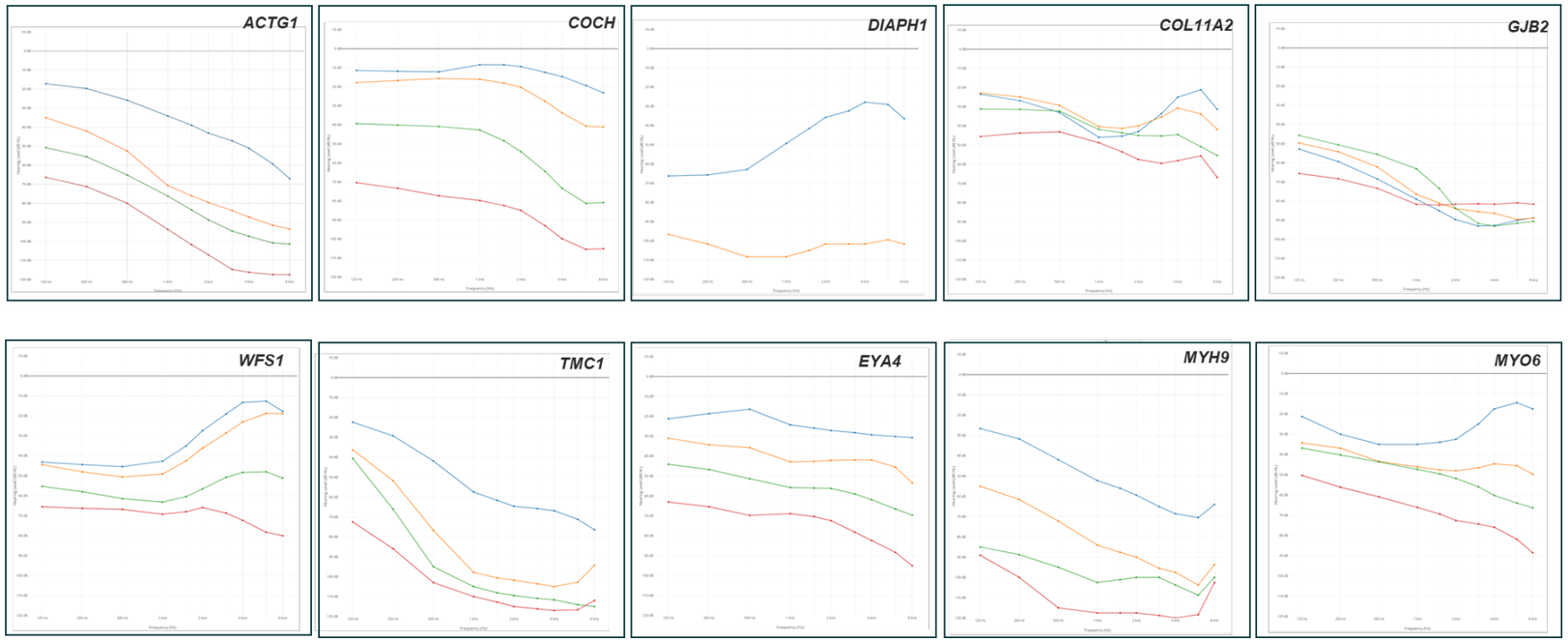


Oonk et al. 2016

Autosomal Dominant Nonsyndromic HL

- **ADNSHL-associated genes: 65** (*as of 10/2026*)
- **Onset:** usually postlingual
- **Severity:** usually progressive, affecting high frequencies first





Azaiez H

https://audiogene.eng.uiowa.edu/

Genetic evaluation for HL *IS* Standard of Care

- **Joint Committee on Infant Hearing (JCIH)**

  All infants suspected or confirmed deaf or hard of hearing

- **International Pediatric Otolaryngology Group (IPOG)**

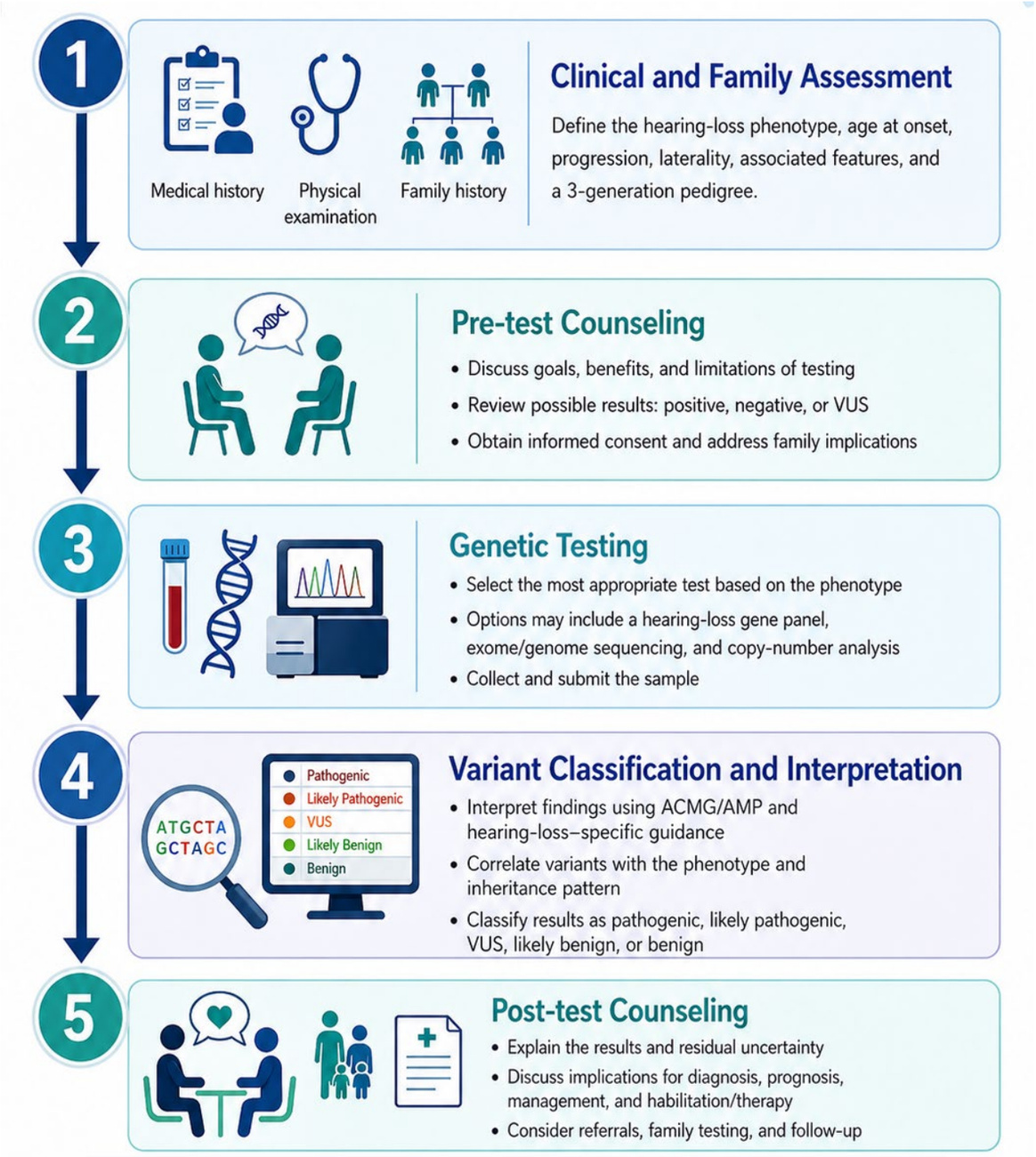
  Infants and children

- **American College of Medical Genetics and Genomics (ACMG)**

  All infants and children

  Adults: customized on age of onset and HL characteristics

Genetic Testing Workflow for Hearing Loss:
A stepwise approach



Azaiez H 2026 © Hearing First

Clinical and Family Assessment: Define the Phenotype

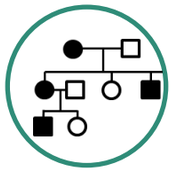
1. **Assessment**
2. Counseling
3. Testing
4. Interpretation & Results
5. Counseling



history



exam



pedigree

Core information to collect

Medical history: prenatal, neonatal, perinatal, and postnatal risk factors (CMV, NICU>5 days, ECMO, ventilation, ototoxic drugs, meningitis, trauma, etc).

Developmental clues: speech/language trajectory, vocal play, vestibular markers such as delayed sitting and walking.

Physical Evaluation

Audiologic profile: type, severity, configuration, laterality, symmetry, age at onset, progression.

Syndromic clues: vision, vestibular, craniofacial, renal, cardiac, neurologic, dental, skeletal, endocrine, metabolic, skin

Family History

3-generation pedigree.

Record hearing status, age at onset, audiograms when available, medical comorbidities, consanguinity, ancestry, and country or region of origin.

JCIH 2019; Li et al. 2022; Toriello, Reardon and Gorlin 2023.

Importance of Phenotypic and Familial Information

Genetic report

A child has a **variant of uncertain significance (VUS)** in a hearing-loss gene.

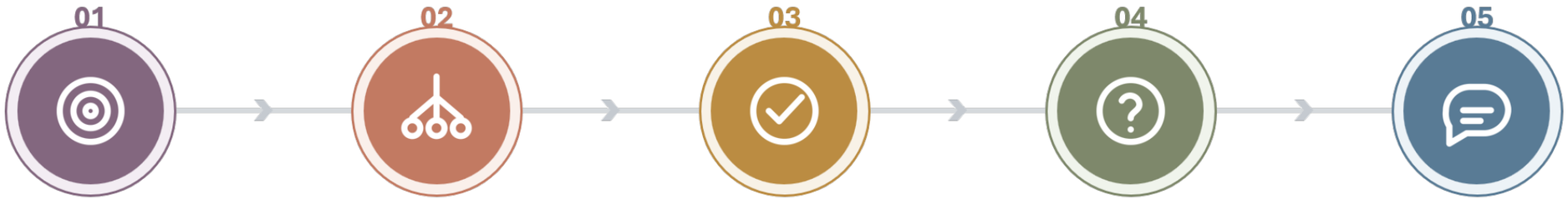
Question for the care team/lab:
Does this variant fit the child?

Missing phenotype	Why it matters
Age at onset unknown	Some genes are typically congenital; others are later-onset or progressive.
Audiogram not provided	Configuration and severity may or may not match the gene.
Progression unknown	Progressive versus stable hearing loss can change gene fit.
Laterality unknown	Bilateral versus unilateral disease may affect plausibility.
Family history absent	Segregation may help clarify whether the variant tracks with hearing loss.
Vestibular / vision concerns not documented	Could shift suspicion toward syndromic forms.
Speech-language trajectory unknown	Helps infer timing, functional impact, and clinical consistency.

Takeaway: When results are uncertain, clinical information can determine whether a variant remains of uncertain significance, becomes likely pathogenic, or becomes less likely to explain the hearing loss.

Pre-test Counseling: Set Expectations Before Testing

1. Assessment
2. **Counseling**
3. Testing
4. Interpretation & Results
5. Counseling



01

Purpose

Why is testing being considered?

02

Possible outcomes

What results could be reported?

03

Benefits

How could results guide care?

04

Limitations

What may remain unanswered?

05

Topics to address

What should families consider?

Genetic Testing Outcomes

POSITIVE

Identified P/LP variant linked to the patient's condition, and can guide treatment and management decisions

INCONCLUSIVE

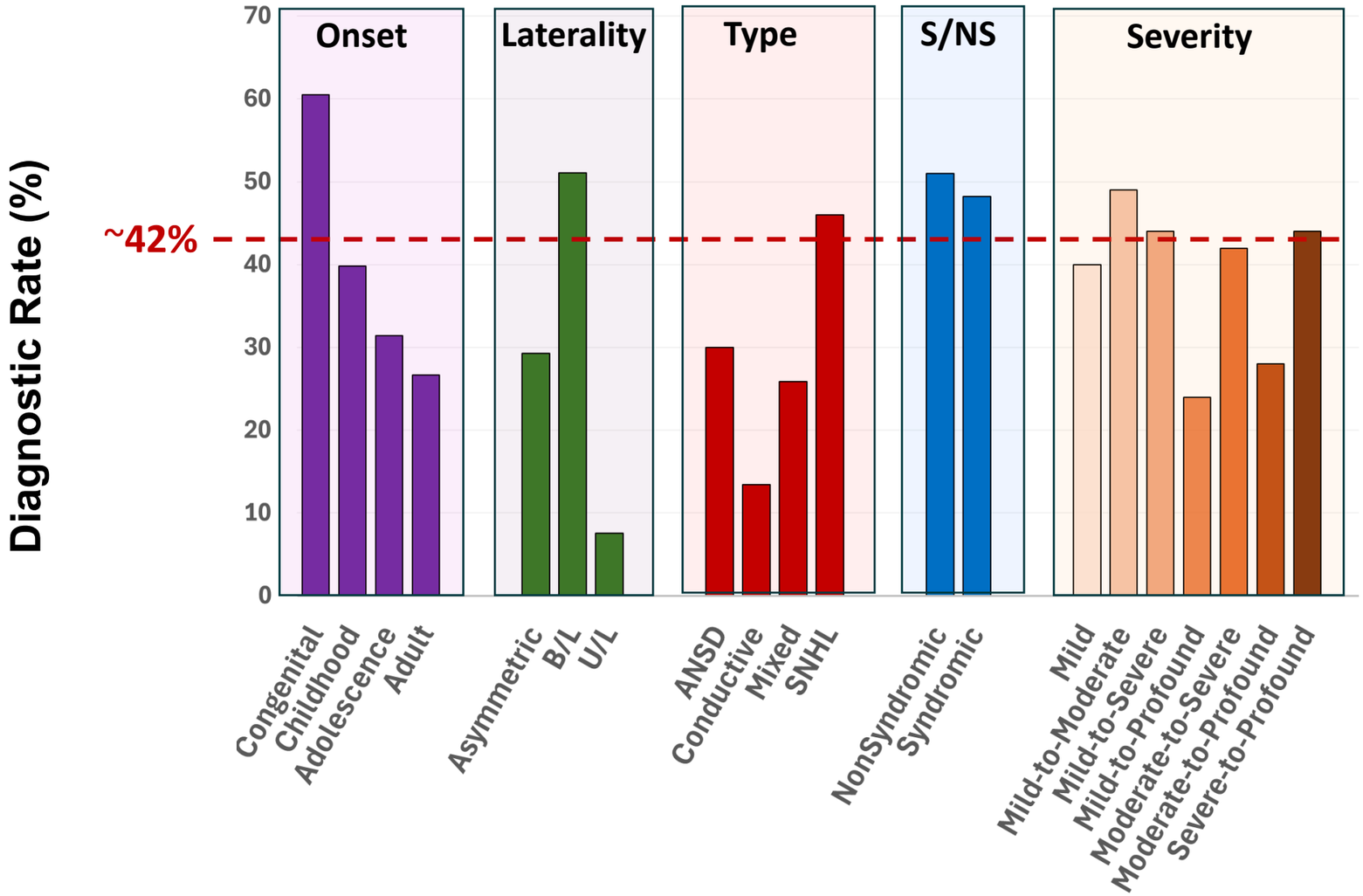
VUS detected
Clinical significance unclear due to limited evidence

NEGATIVE

No P/LP variants found to account for patient's condition

Genetic diagnosis is dependent on patient-specific characteristics

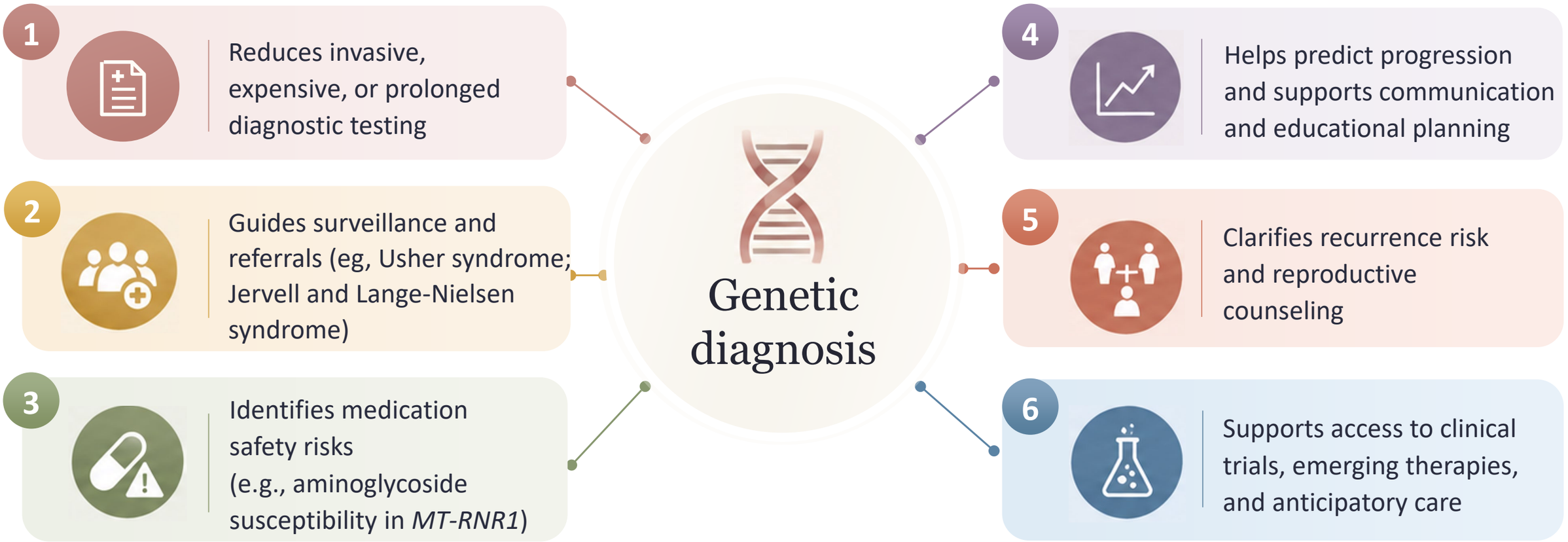
Data from > 8,000 patients



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Clinical Benefits of Genetic Testing

Why a genetic diagnosis matters in hearing loss care



Additional Value

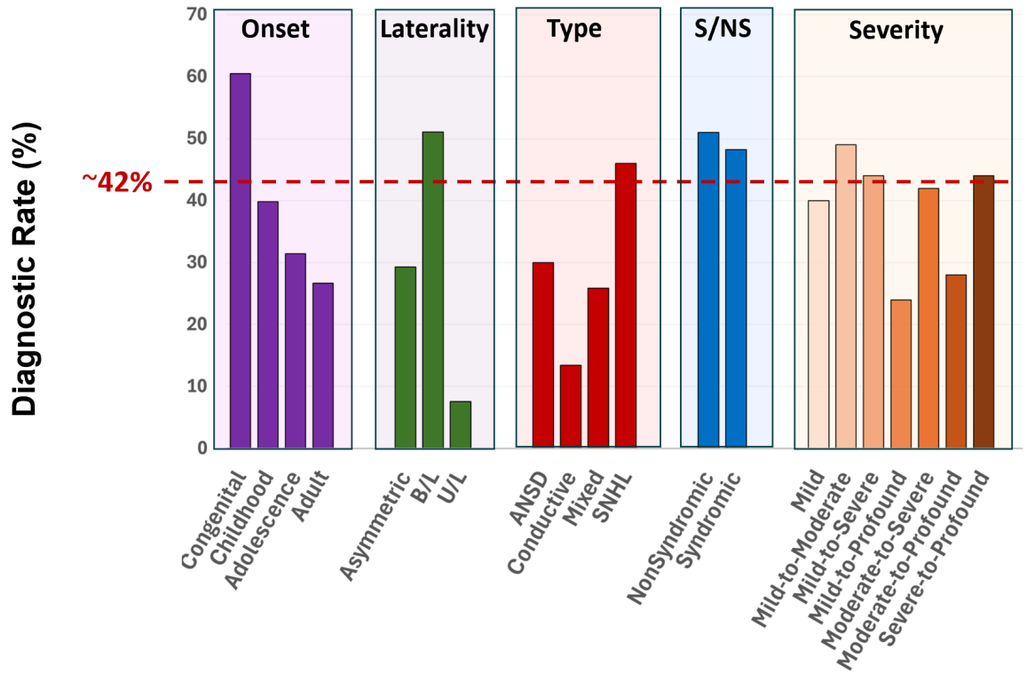
A genetic diagnosis can also provide families with clarity, closure, and more realistic expectations for the future.

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Limitations of Genetic Testing

Testing limitations

- The genetic cause remains unsolved in ~60% of cases
- Diagnostic yield is lower in certain types of hearing loss
- Results may be inconclusive or ambiguous
- A negative result does not exclude a genetic cause



Other Considerations

Practical counseling considerations

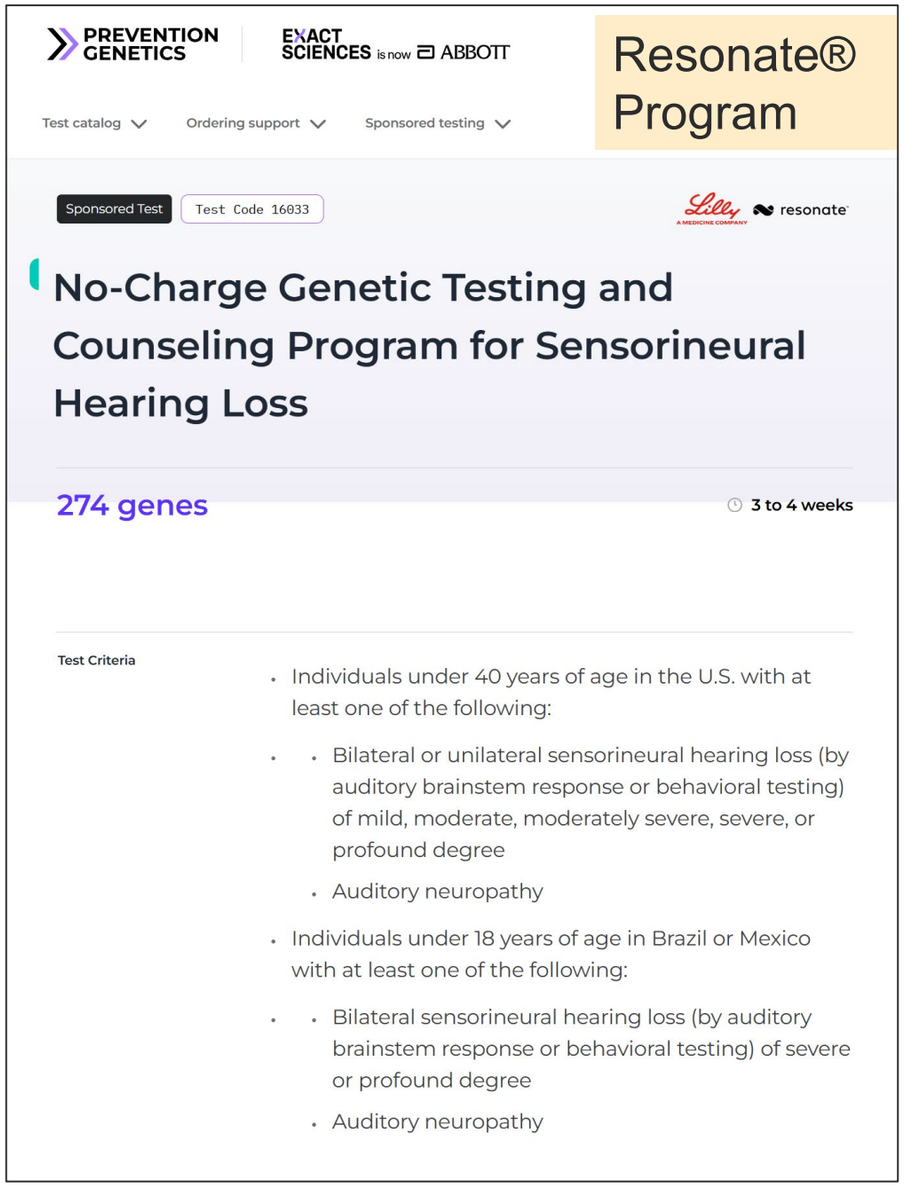
- The process and results may be emotionally challenging for some families
- Access to testing may be limited by geography or local resources
- Insurance coverage and out-of-pocket costs vary
- Families should make informed decisions after understanding both benefits and limitations

Clinical message: Even when results are negative or inconclusive, they still help narrow the differential diagnosis and guide follow-up planning.

Free Genetic Testing Programs

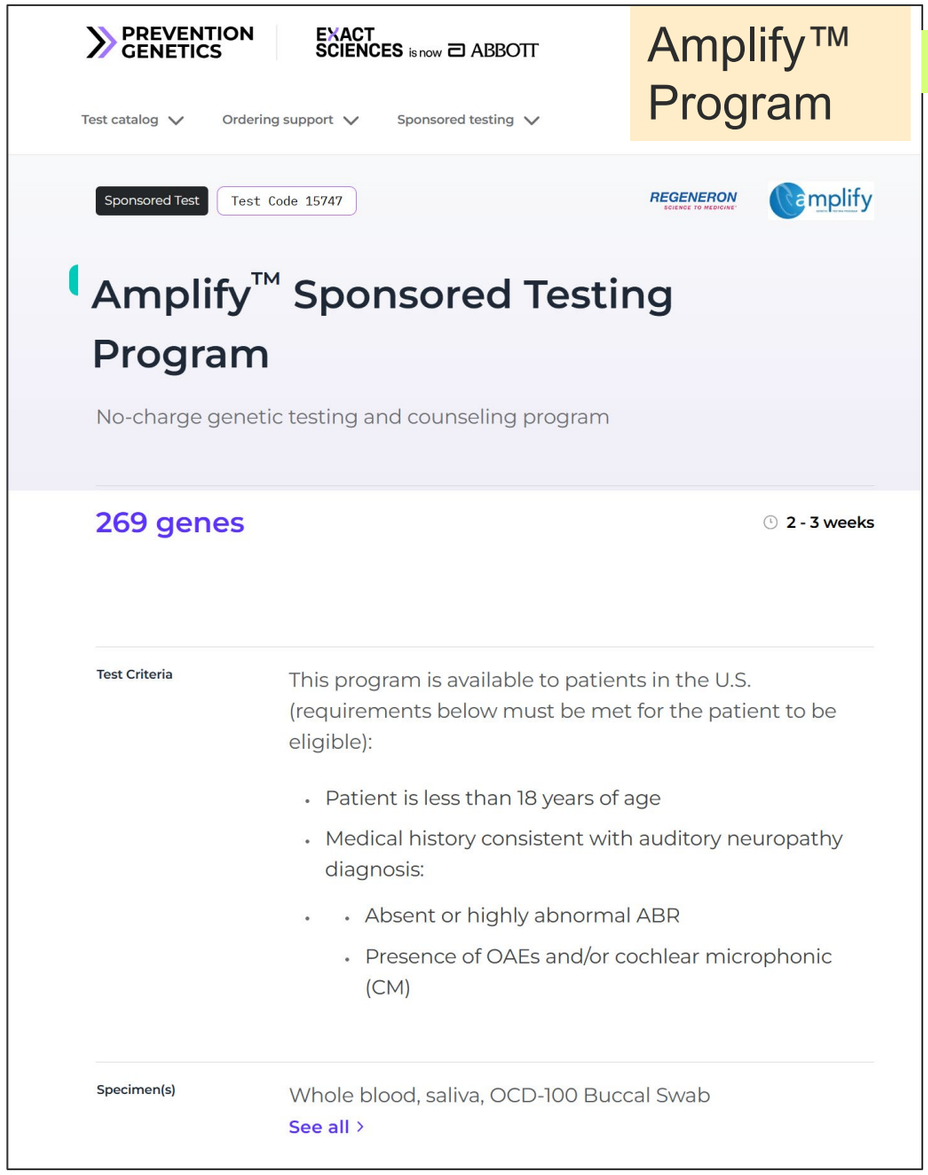
Eli Lilly / Akouos

Resonate® Program



Regeneron

Amplify™ Program

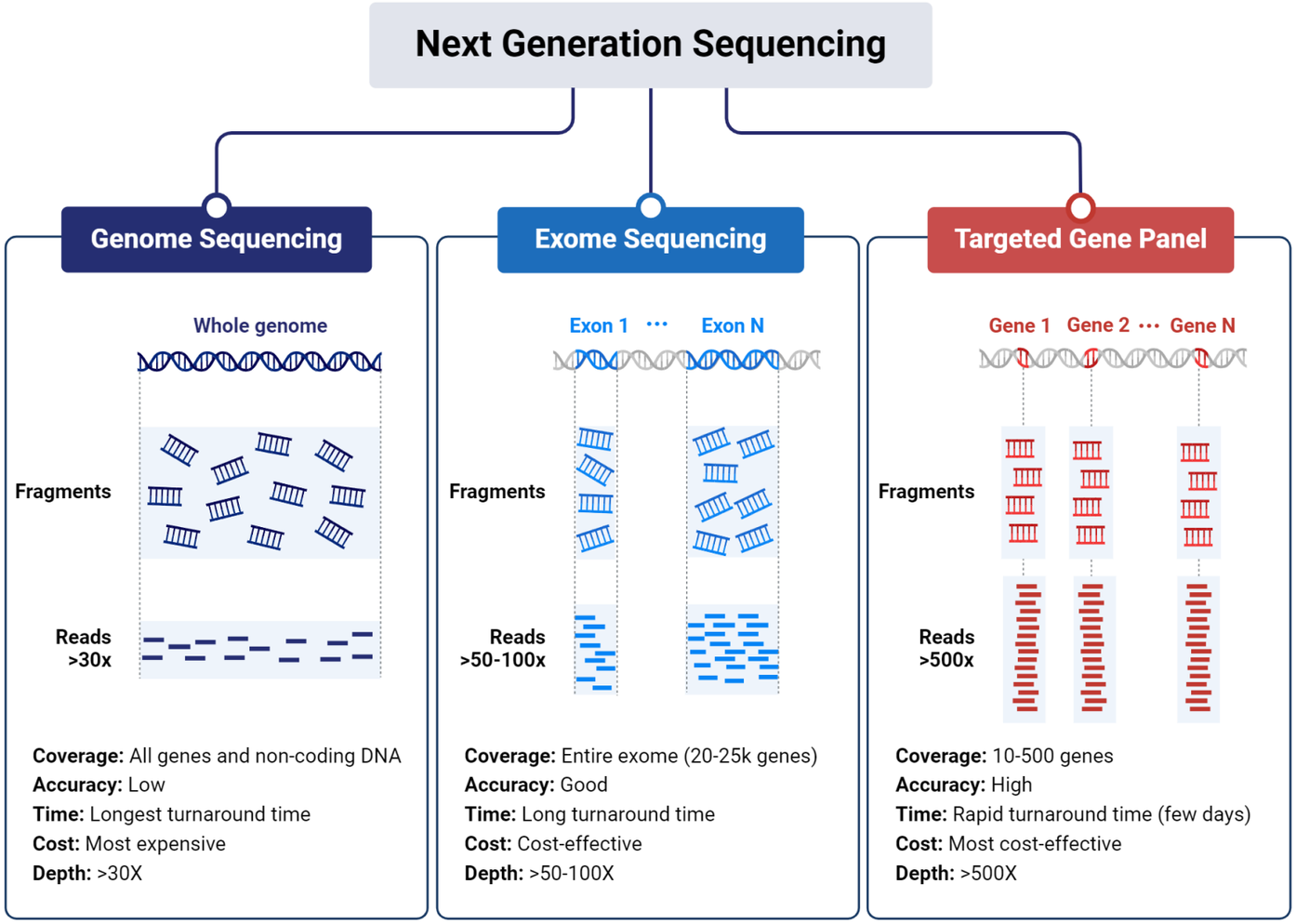


Genetic Testing

1. Assessment
2. Counseling
3. **Testing**
4. Interpretation & Results
5. Counseling

Test selection

- Hearing-loss gene panel: efficient first-line approach.
- Exome/genome sequencing: useful when the phenotype is syndromic, broad, atypical, or when prior testing is negative.



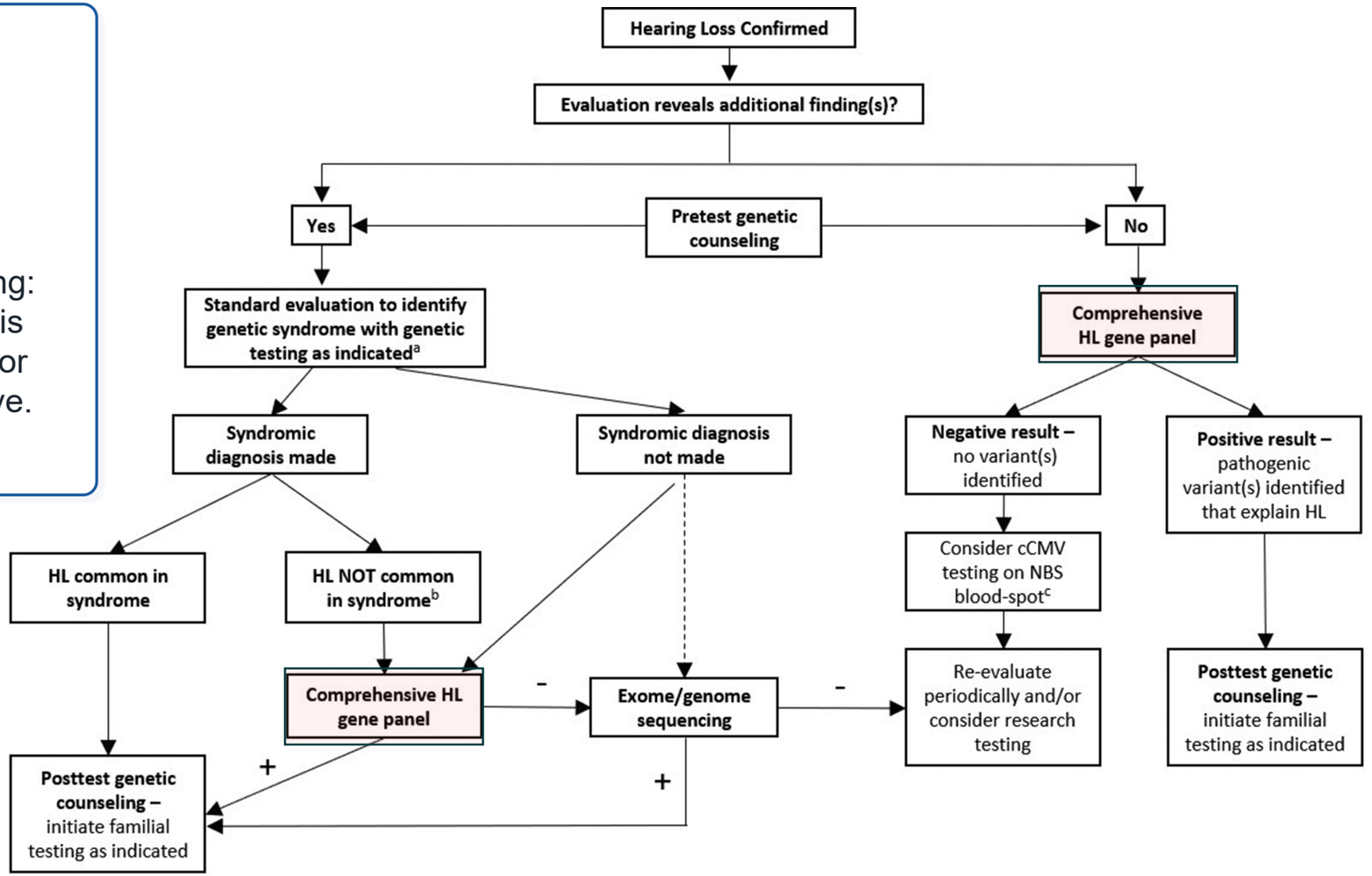
Template adapted from: *Dr. Roshini Abraham*
Clinical Immunologist at Nationwide Children's Hospital

Genetic Testing

1. Assessment
2. Counseling
3. **Testing**
4. Interpretation & Results
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Test selection

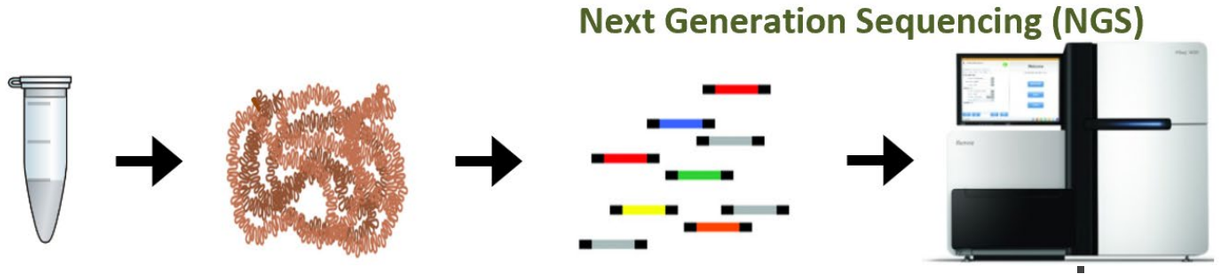
- Hearing-loss gene panel: efficient first-line approach.
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ACMG clinical practice

Li et al., 2022 (PMID: 35802133)

Genetic Testing Workflow



Bioinformatics

- **Raw sequence data**
- **Read mapping** BWA
- **Mark Duplicates** Picard
- **Indel Realignment** GATK
- **Variant Calling** GATK
- **Variant Annotation** Custom pipeline

Sequencing QC

Variant Filtering

Total Variants
Over 500

-Depth of Coverage ≥ 10
- ≥ 30% of Reads
- Q/D ≥ 5

Hi Quality Variants
Over 400

-Non-synonymous
-Splice-site variants
-Insertion/deletions
-Synonymous

NS/SS/Indel
~100

- MAF ≤ 2% in gnomAD

Rare Variants
~10

Variant Classification



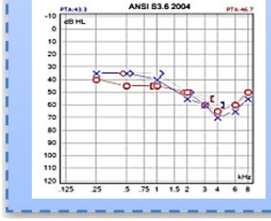
Multidisciplinary Team Meeting

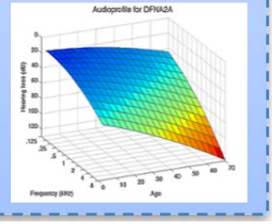
Deafness Variation Database (**DVD**)

Known mutation

Novel Variant

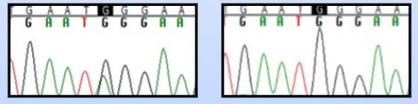
Audiometric profile **AudioGene**



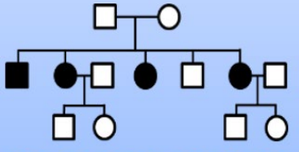


Variant Confirmation

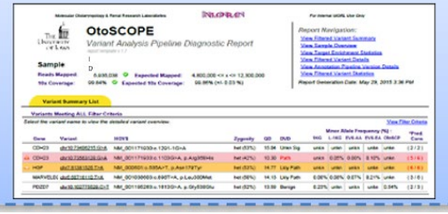
Sanger Sequencing



Segregation Analysis

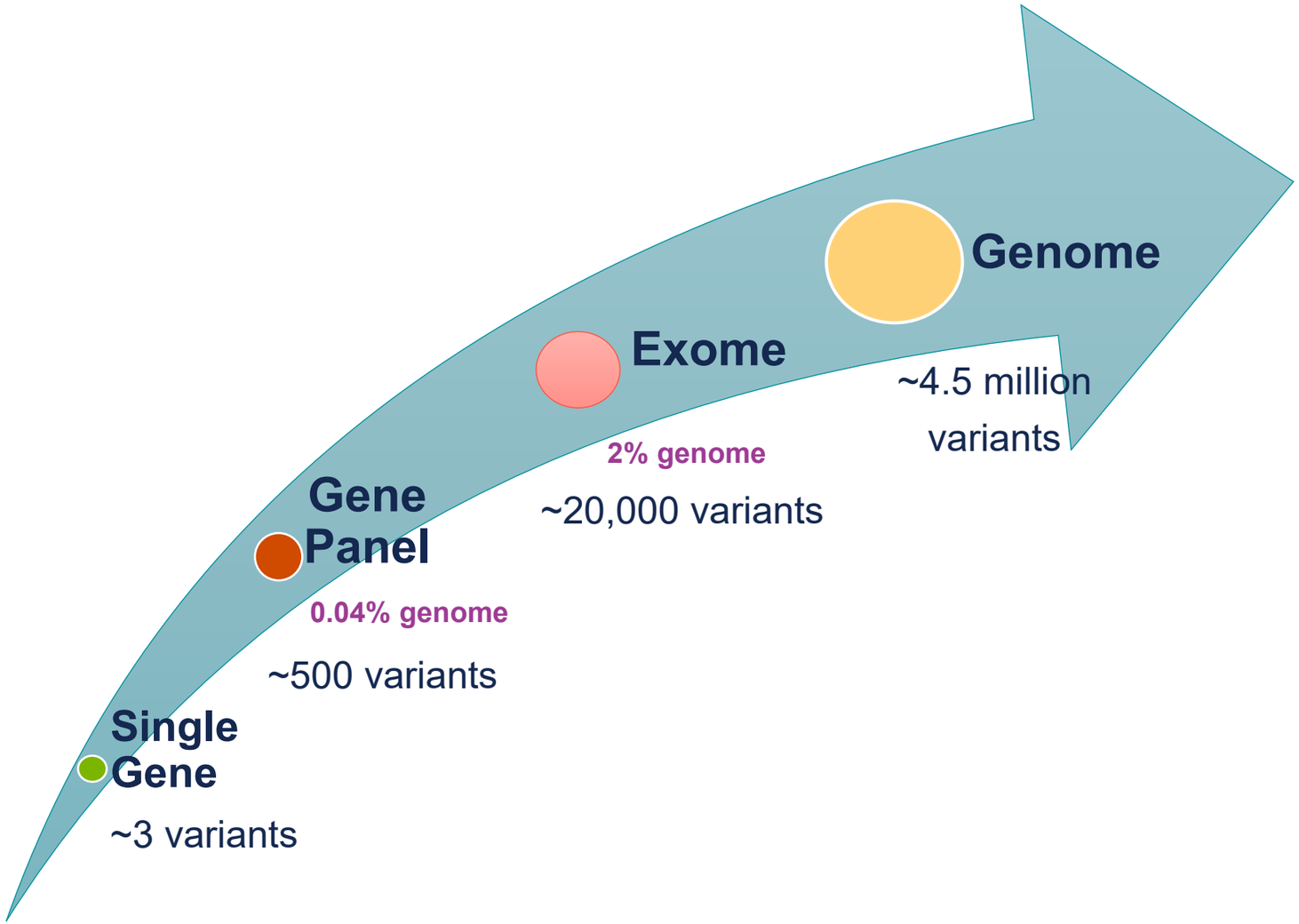


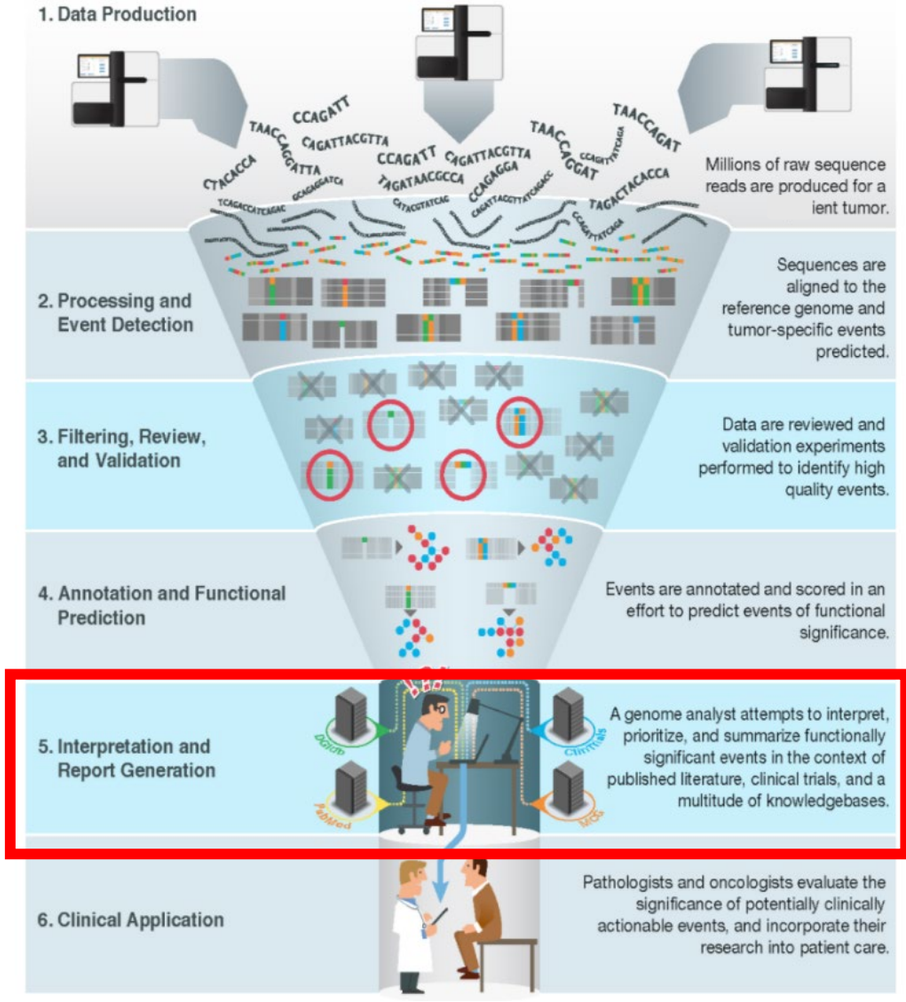
Report Generation



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The Challenge of Genetic Variant Interpretation





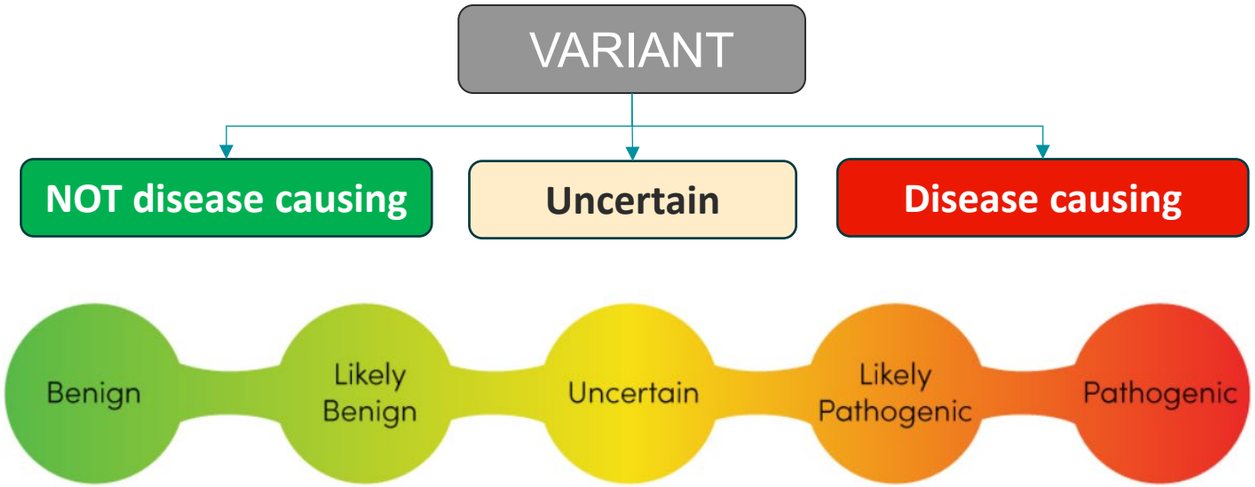
Good et al, Genome Biology 2014

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Genetic Testing: Variant Classification

© American College of Medical Genetics and Genomics
ACMG STANDARDS AND GUIDELINES | Genetics inMedicine

Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology



Genetic variants are classified into ***5*** categories to indicate the likelihood that a particular variant is associated with the disease

Post-test Counseling: Translate Results Into Action

1. Assessment
2. Counseling
3. Testing
4. Interpretation & Results
5. **Counseling**

✓ Positive result

- Confirms or strongly supports a genetic diagnosis
- May guide prognosis, specialist referral, and family testing
- Can inform management and surveillance for associated conditions

X Negative result

- Does not rule out a genetic cause
- Current tests may miss some variant types or unknown genes
- Reanalysis or broader testing may be useful later

? Inconclusive

- Evidence is not yet sufficient to confirm disease causality
- Should not drive irreversible medical decisions
- Segregation studies, new data, or reclassification may clarify the result

POSITIVE

Identified P/LP variant linked to the patient's condition, and can guide treatment and management decisions



NEGATIVE

No P/LP variants found to account for patient's condition



INCONCLUSIVE

VUS detected; clinical significance unclear due to limited evidence



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? Inconclusive

- Evidence is not yet sufficient to confirm disease causality
- Should not drive irreversible medical decisions
- Segregation studies, new data, or reclassification may clarify the result

Post-test counseling should translate the report into action

Use plain language, document residual uncertainty, evaluate siblings promptly, coordinate referrals, and align genetic information with amplification, cochlear implant evaluation, communication planning, and speech-language/habilitation goals.

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Same VUS, Different Clinical Context, Different Outcomes

Genetic finding

A child has a **VUS** in a gene associated with **later onset progressive autosomal dominant hearing loss**.

Case A: fit is weak

- Congenital profound SNHL
- Stabel HL
- Parents unaffected
- Audiogram NOT matching prior reports

Variant is unlikely to be causing HL in child

Case B: fit is stronger

- Later-onset mild high-frequency SNHL
- Worsening thresholds over time
- Multiple affected relatives across generations
- Audiogram consistent with prior reports

Variant becomes more suspicious; family testing may help

Bottom line: The same VUS can be interpreted very differently depending on phenotype, inheritance, and family history.

Azaiez H

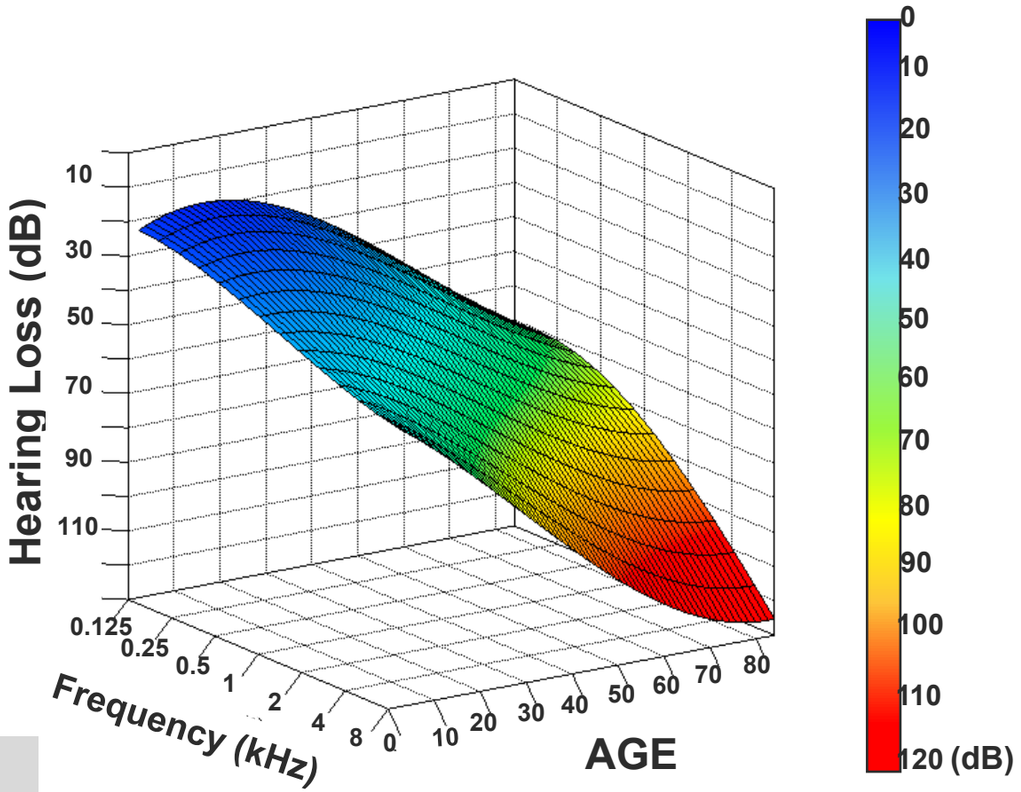
Azaiez H

Precision Audiology



Need: To Check concordance between audiograms and genetic variants

- AudioGene is a machine learning algorithm that uses audiometric data to predict genetic cause
- Audioprofile surface: The planar surface shows age-specific average thresholds
- Allows prognostication over time
- Essential for clinical trials (natural history data)



AudioGene v4 uses 3246 audiograms collected from 1445 persons with ADNSHL (34 DFNA loci)

https://audiogene.eng.uiowa.edu/

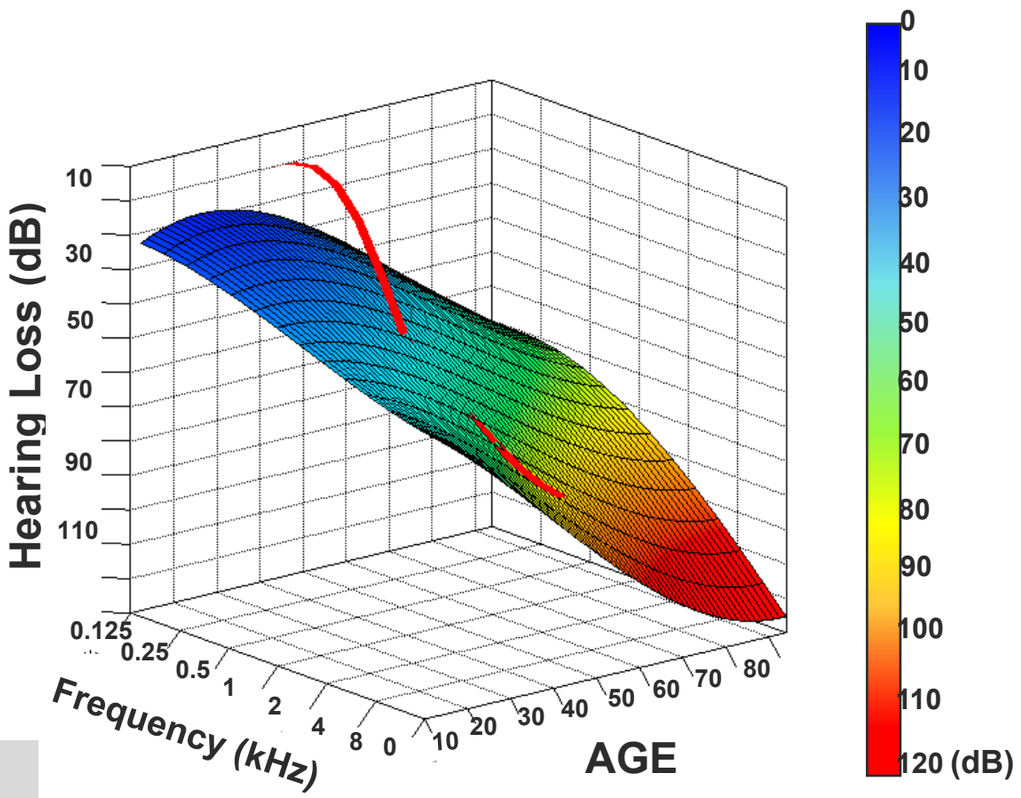
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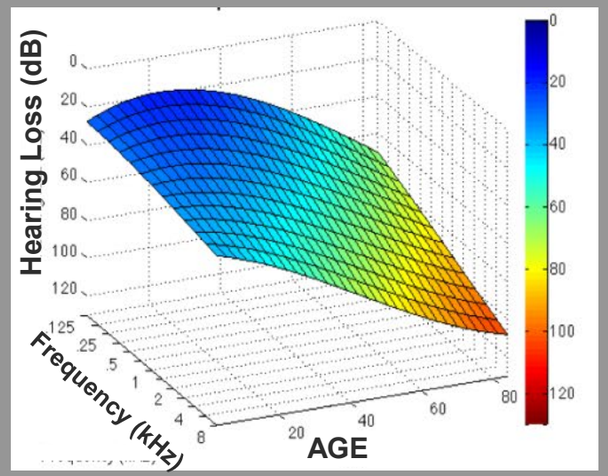
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Precision Audiology

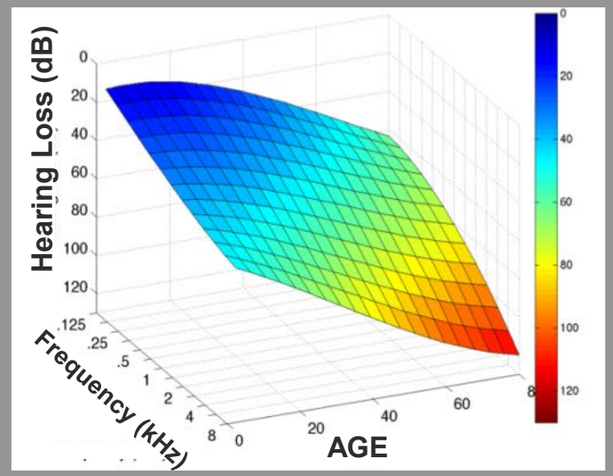


Mutation-specific phenotypic differences associated with a given gene

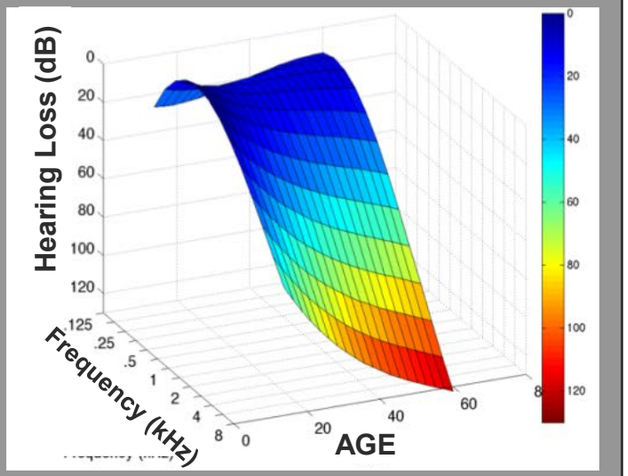
KCNQ4



Missense Mutations



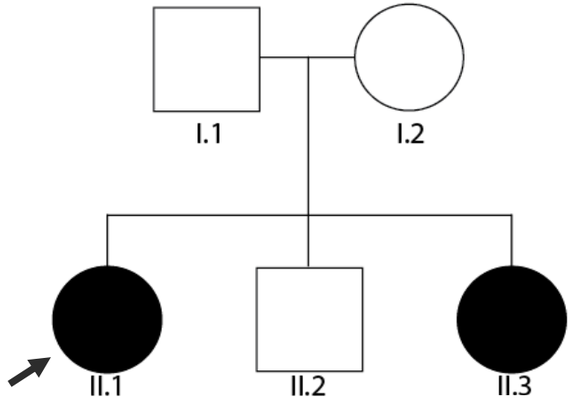
Truncating Mutations

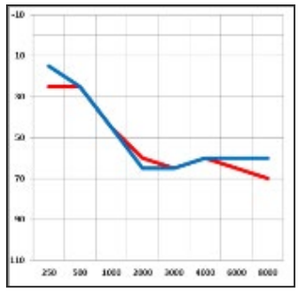


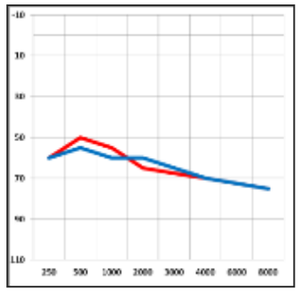
Taylor et al. (2015)- PMID: 23280582

Early Detection, Prevention and Management

Case 1







- Congenital mild-to-severe HL
- Family history: sister with HL
- Vision: astigmatism
- Medical exam: normal

Genetic Testing Results:

Pathogenic Variant 1: ***USH2A***: p.Gly268Ter

Pathogenic Variant 2: ***USH2A***: p.Met3868AsnfsTer4

Diagnosis: Usher syndrome Type 2

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Molecular Otolaryngology and Renal Research Laboratories (MORL)

Accurate Clinical Diagnosis

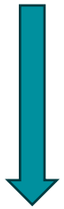
Case 2:

Clinical Presentation:

- 15 yo
- bilateral HL + renal dysplasia
- Family History
- Clinical Diagnosis: **BranchioOtoRenal (BOR) syndrome**

Genetic Testing Results:

Pathogenic Variant: **GATA3: p.Tyr64Ter**



Diagnosis:

- Change the diagnosis from BOR to **hypoparathyroidism with sensorineural deafness and renal dysplasia (HDRS)**.

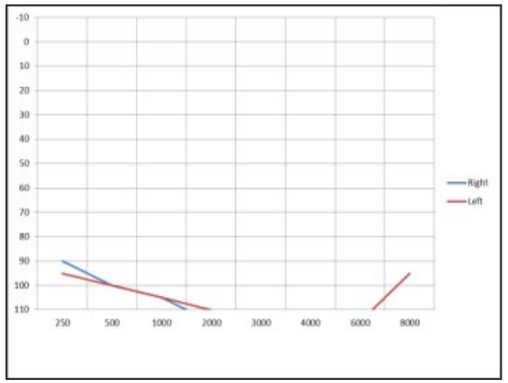
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Accurate Clinical Diagnosis

Case 3:

Clinical presentation:

- 43 yo
- bilateral profound HL
- Unknown Family History
- Clinical Diagnosis: **Usher Syndrome**



Genetic Testing Results:

2 Pathogenic variants in ***SLC26A4***

+

1 pathogenic variant in ***OPA1***

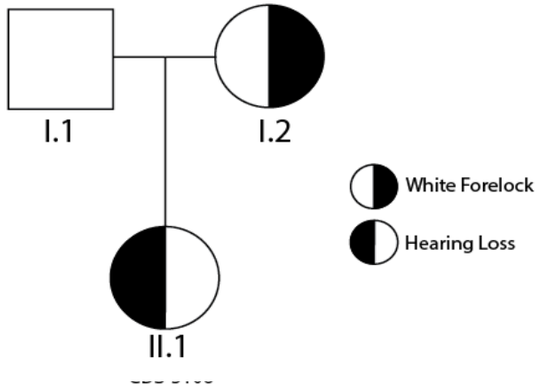
Diagnosis:

- *SLC26A4* for the profound HL
- *OPA1* for optic atrophy
- Changes diagnosis from Usher Syndrome to hearing loss and optic atrophy

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Importance of Comprehensive Testing

Case 4

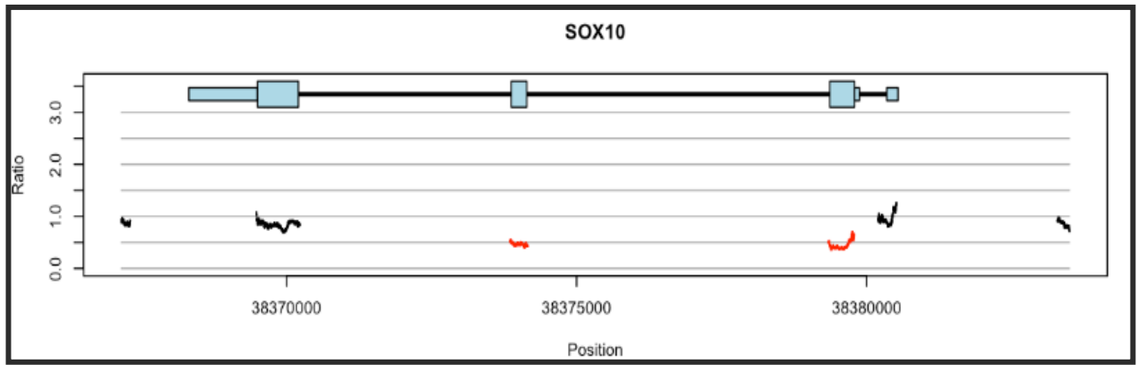


Phenotype:

- 5 yo with congenital severe-to-profound SNHL.
- Family History: extended family with HL and a mother with white forelock.

Previous genetic screening: negative

❖ Waardenburg Panel (negative)



Results:

❖Two-exon frameshift deletion of *SOX10*

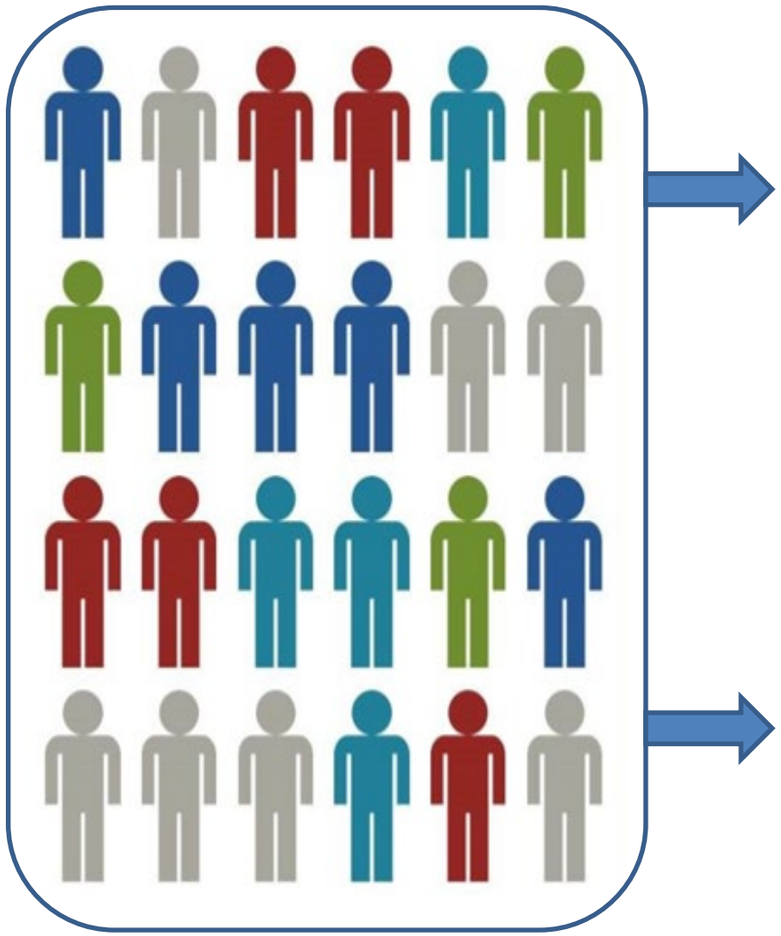
❖Diagnosis: Waardenburg Syndrome

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Precision Medicine for Hearing Loss

Tailored Care: Precision Medicine

Hearing Loss

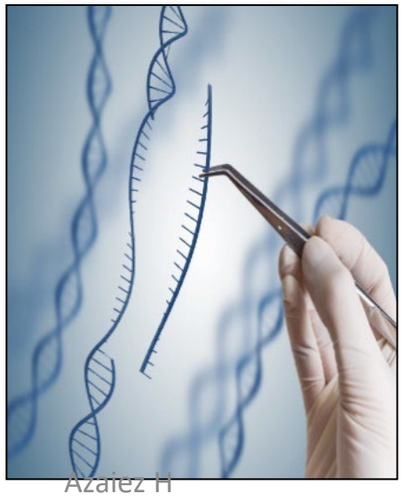


Treatment

Hearing Aid
Cochlear Implant



Molecular Intervention
Gene Therapy



One-size-fits-all Medicine

"Aims to understand how a person's genetics, environment, and lifestyle can help determine the best approach to prevent or treat disease."

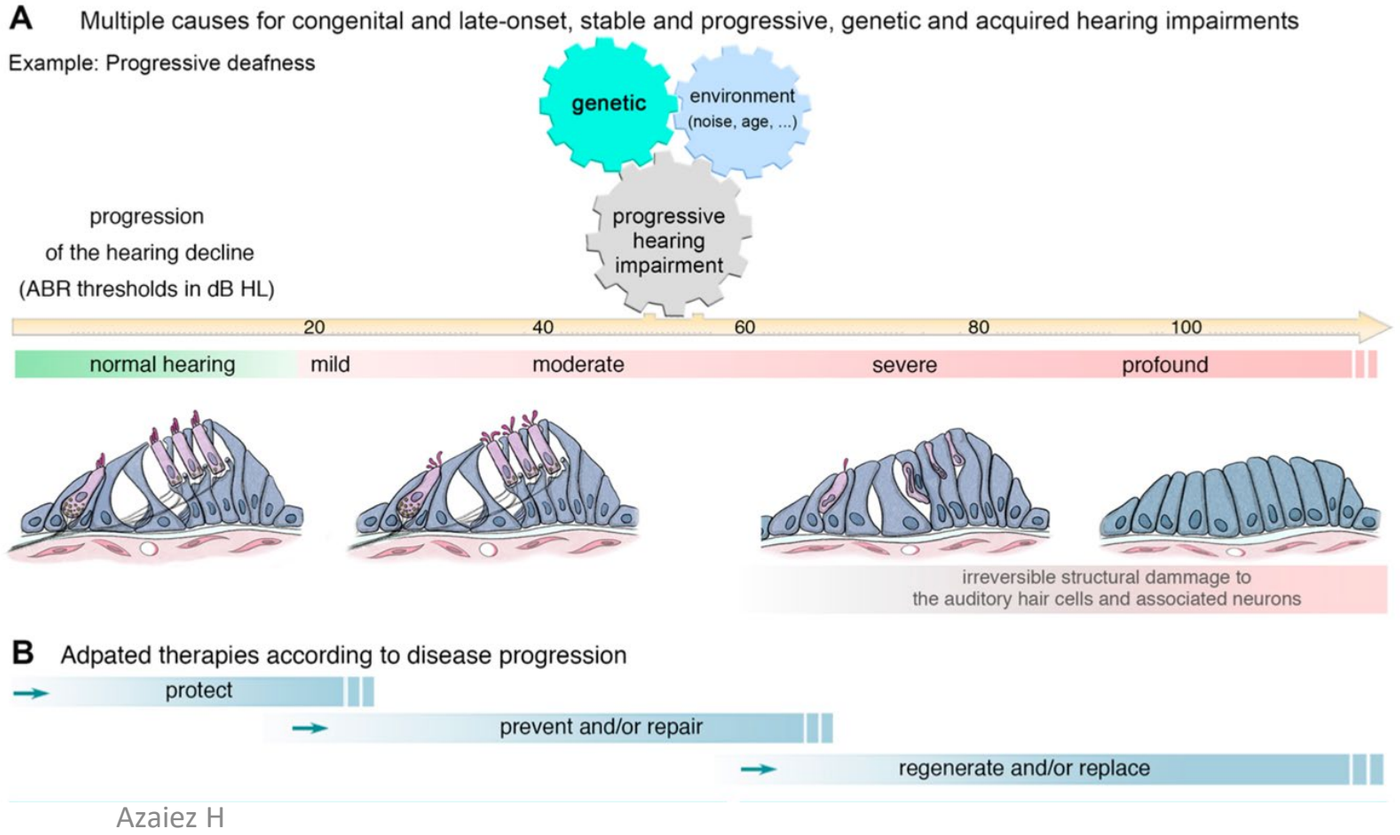
Precision Medicine

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Gene Therapy

- Gene therapy involves introducing, removing, or modifying genetic material within a person's cells to *treat* or *prevent* disease.

- For hearing loss, the goal of gene therapy is to ***preserve*** or ***restore*** auditory function.

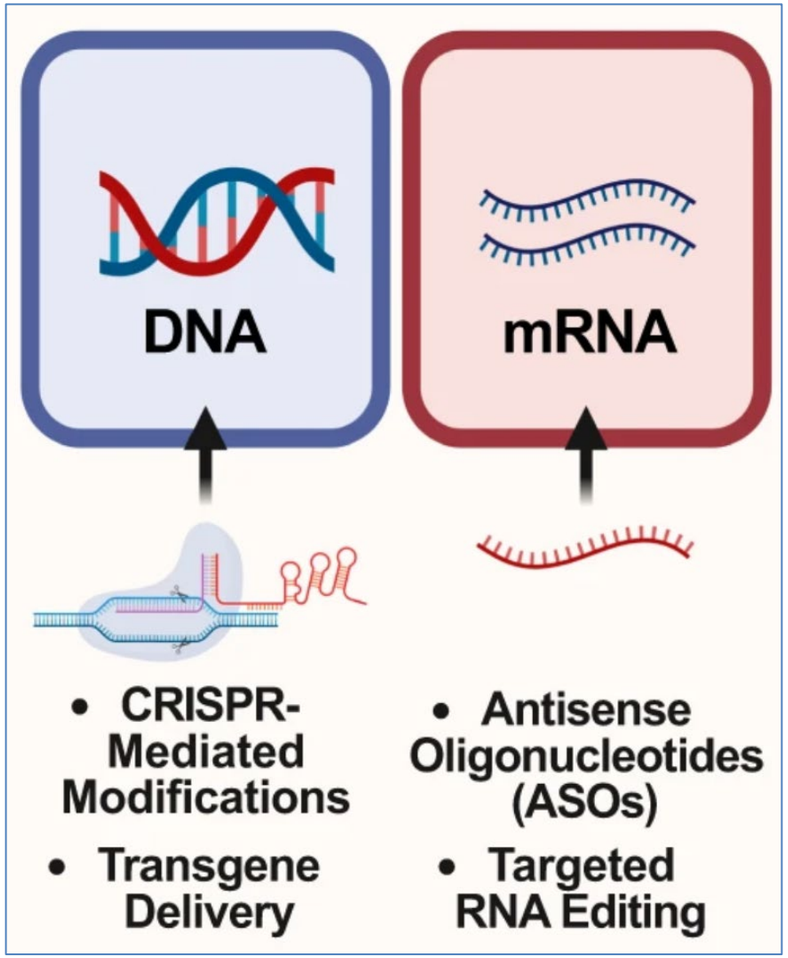


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Delmaghani and El-Amraoui 2020

Gene Therapy Modalities

- ***Gene replacement*** **:**
  - Introducing a functional copy of the gene.
  - AR conditions where the loss of gene function is the mechanism.
- ***Gene silencing:***
  - Suppressing the expression of a mutated gene through mechanisms such as *RNA interference*.
  - Typically for dominant-negative/GOF variants that produce a harmful protein.
- ***Gene editing*** **:**
  - Correcting mutations at the genomic level.
  - Offers the potential for permanent correction.



Gene Therapy for Hearing Loss!!



Drugs.com

FDA-Approved Drug

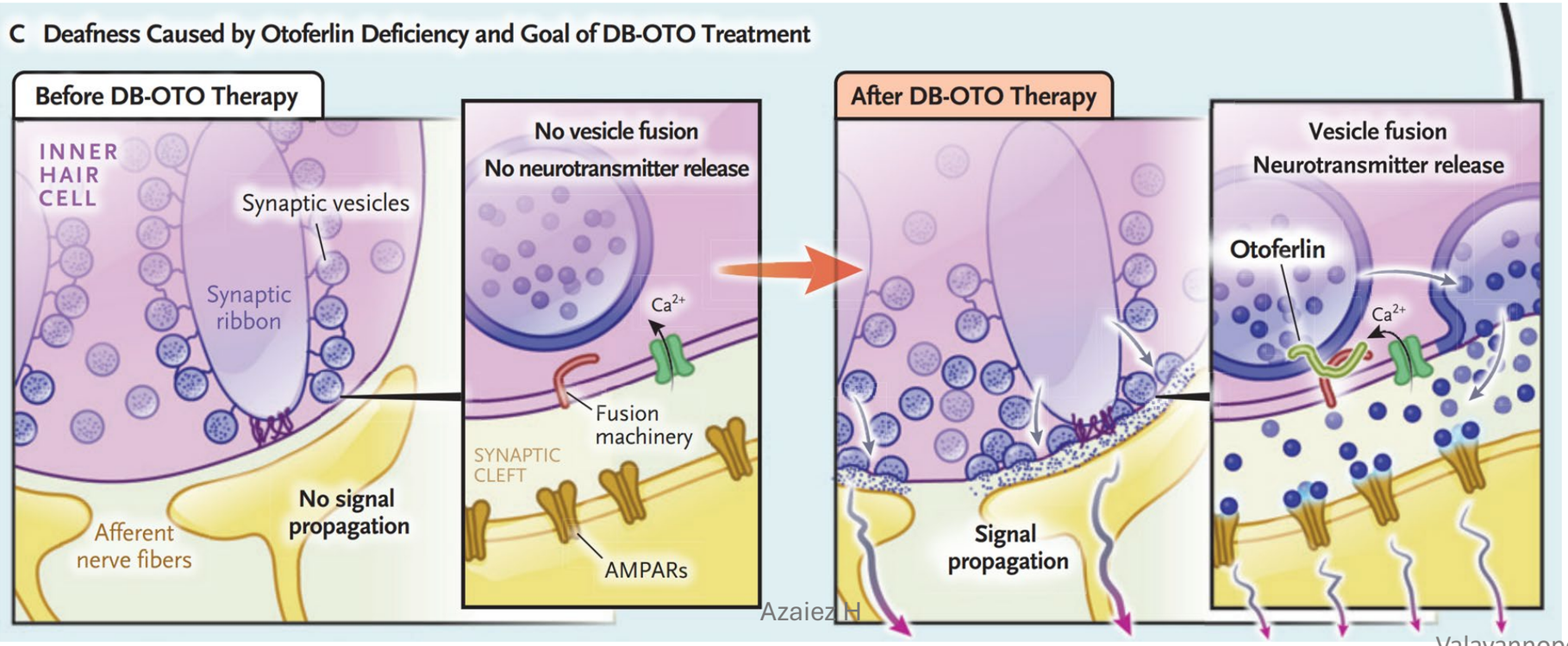
2026

OTOF -Related ANSD

- *OTOF* gene encodes Otoferlin protein
- Otoferlin is expressed in inner hair cells

Otoferlin is a major calcium sensor protein involved in synaptic exocytosis in Inner Hair cells

- Normal hearing relies on a temporally precise and sustained glutamate release



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Valayannopoulos et al, NEJM - Oct 2025

The NEW ENGLAND JOURNAL of MEDICINE

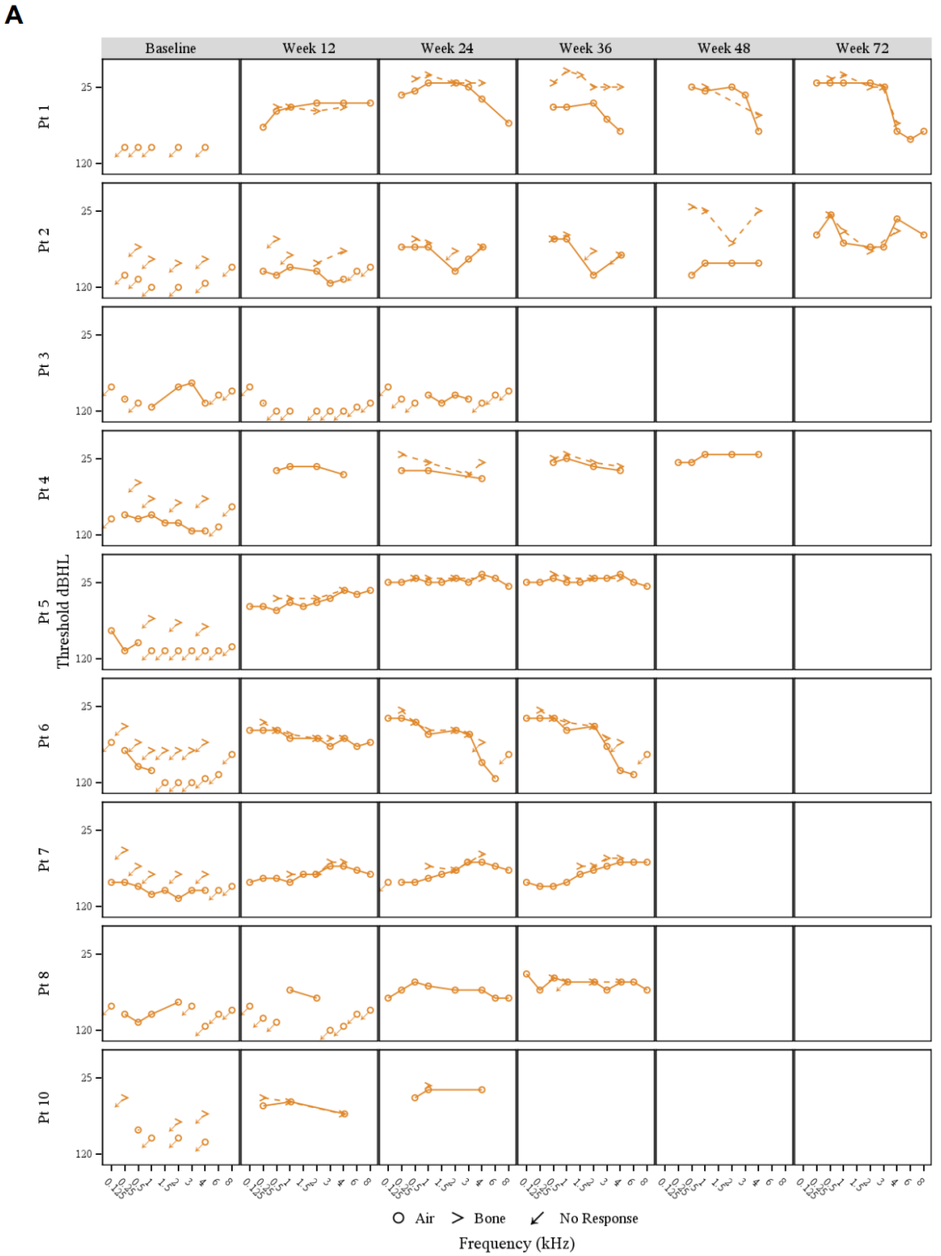
ORIGINAL ARTICLE

DB-OTO Gene Therapy for Inherited Deafness

REGENERON
SCIENCE TO MEDICINE®

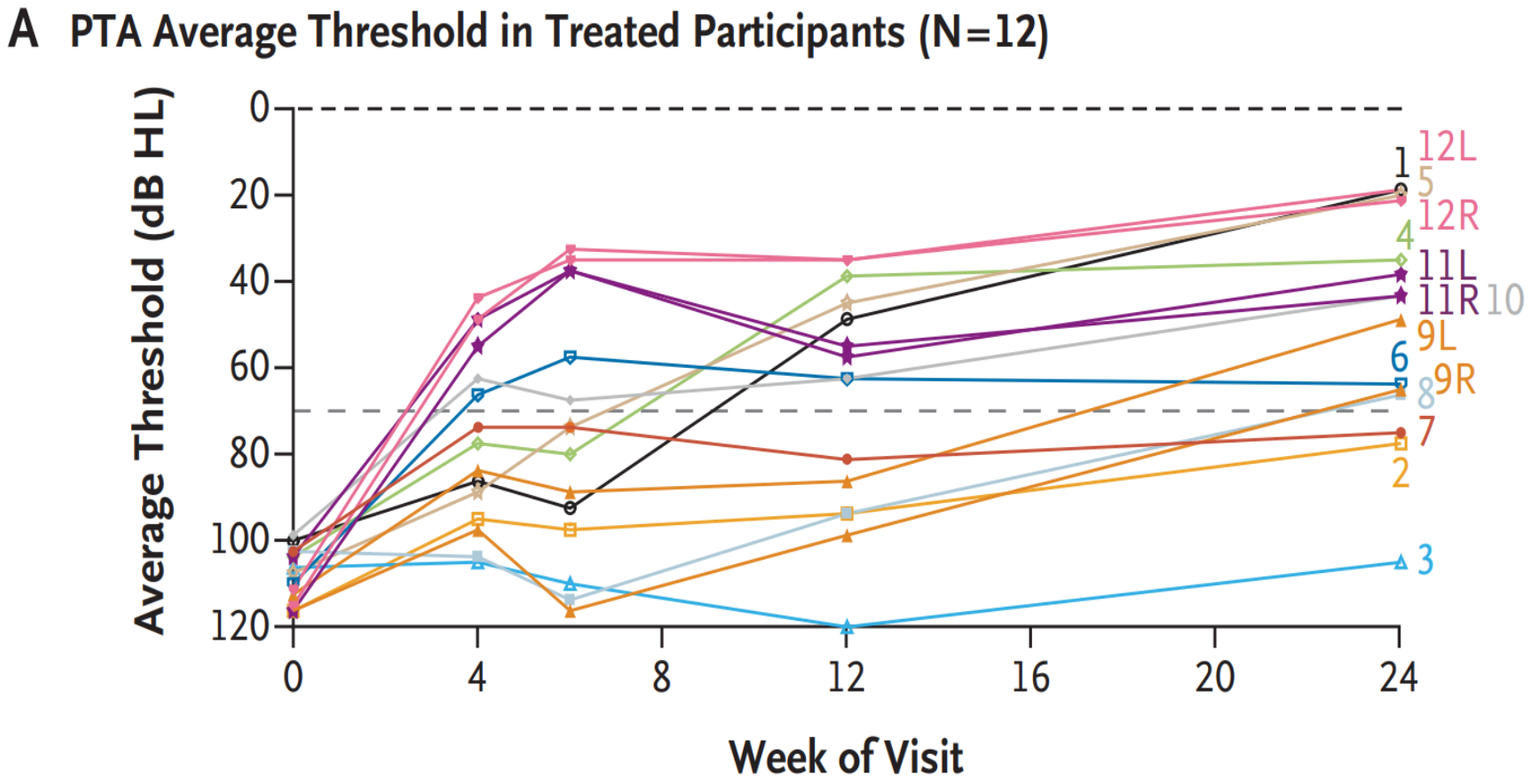
ABR improvement in most cases

- **12 participants** between 10 months and 16 yo
- Primary efficacy endpoint: PTA ≤70 dB HL at Week 24



Valayannopoulos et al, NEJM - Oct 2025

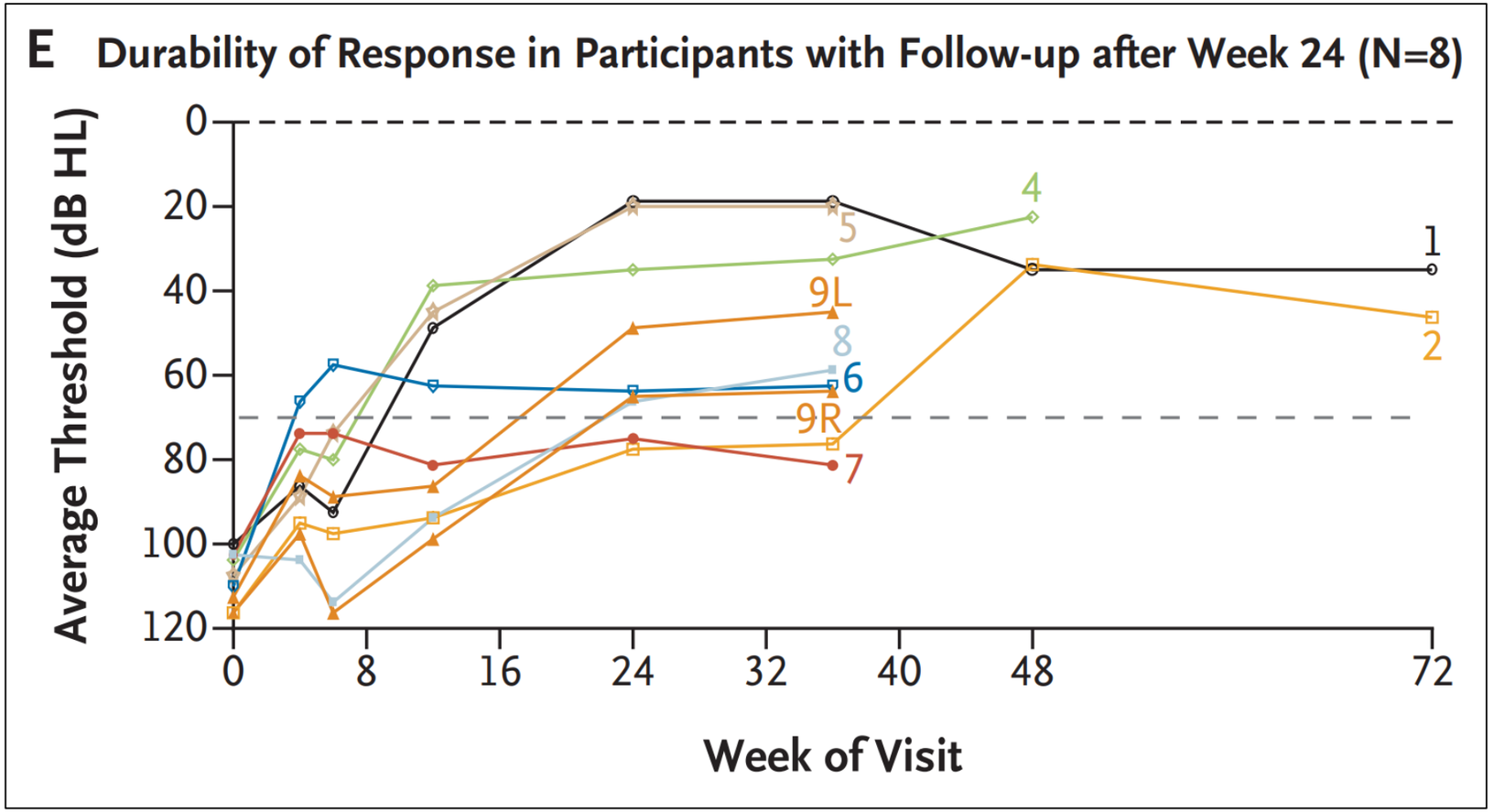
Overall Efficacy



- Primary efficacy end point was met in 9 of 12 participants (75%)
- 3 of 12 participants reached average normal hearing sensitivity levels (≤25 dB HL).

Valayannopoulos et al, NEJM - Oct 2025

Durability out to 72 weeks



Valayannopoulos et al, NEJM - Oct 2025

Article

April 2026

Multicentre gene therapy for *OTOF*-related deafness followed up to 2.5 years

https://doi.org/10.1038/s41586-026-10393-y

Received: 14 September 2025

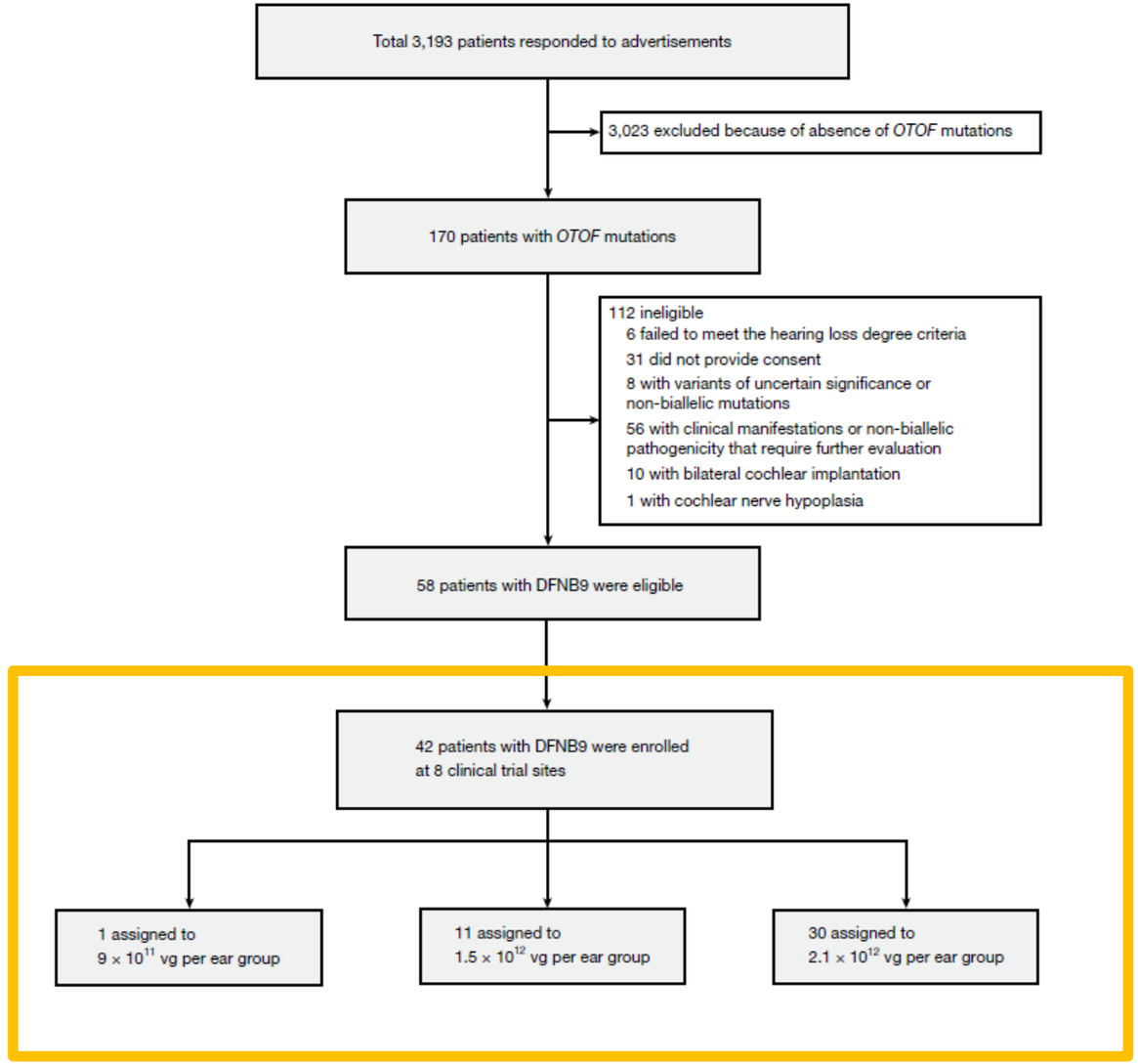
Accepted: 4 March 2026

Published online: 22 April 2026

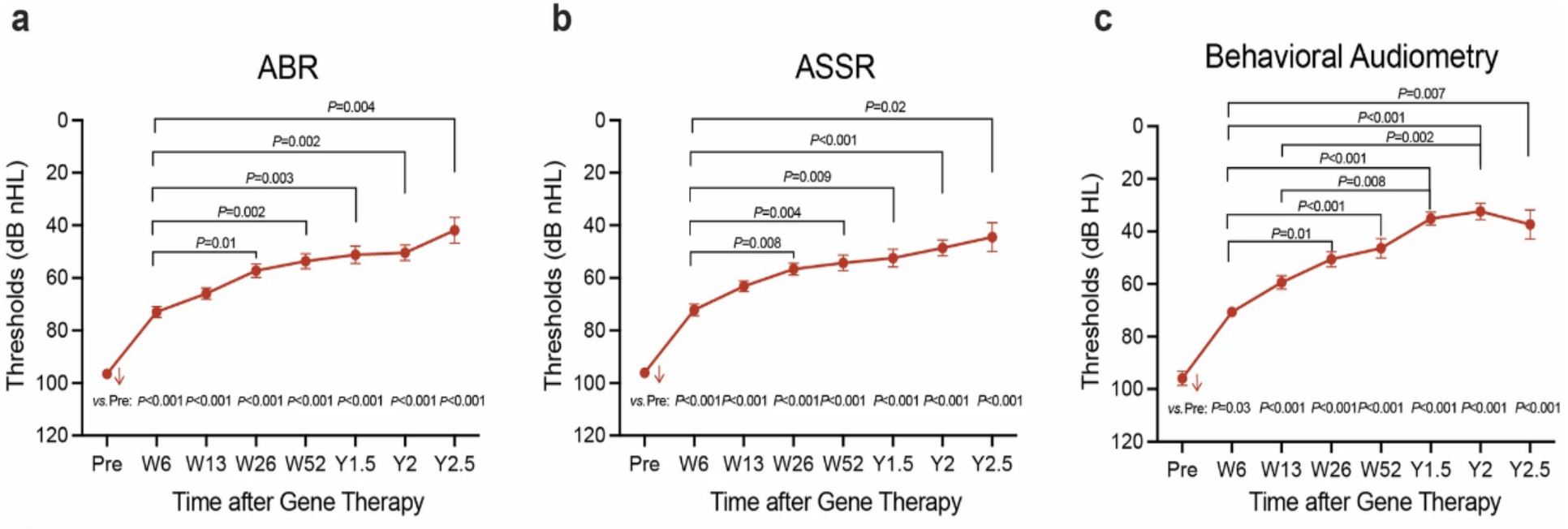
Check for updates

Luoying Jiang[1,2,3,4,5,18], Xiaoting Cheng[1,2,4,18], Jun Lv[1,2,4,18], Yuxin Chen[1,2,4,18], Xiaoyun Chen[1,2,3,4,5,18], Rongqun Zhai[6,18], Liqin Zhang[7,18], Lei Han[1,2,3,4,5], Yiling Zhang[8], Juhong Zhang[9], Di Deng[10], Zhicheng Huang[11], Qi Cao[1,4], Xin Zhang[1,2,3,4,5], Daqi Wang[1,2,4], Yizhe Wang[1,2,3,4,5,12], Liheng Chen[1,2,4], Sha Yu[1,2,4], Luo Guo[1,2,4], Bowen Zhang[1,2,4], Hui Wang[1,2,4], Yi Zhou[13], Liling Dai[1,2,3,4,5,14], Wei Wang[1,2,3,4,5], Longlong Zhang[1,2,4], Yanbo Yin[1,4], Guiqing Cheng[1,2,4], Ziyi Zhou[8], Wuqing Wang[1,4], Bing Chen[1,4], Wei Lu[6], Hongqun Jiang[15], Zhiqiang Gao[7], Dazhi Shi[11]✉, Yuanping Xiong[15]✉, Yu Zhao[10]✉, Wei Yuan[9]✉, Qin Wang[8]✉, Guodong Feng[7]✉, Huawei Li[1,2,3,4,5]✉, Zheng-Yi Chen[16,17]✉ & Yilai Shu[1,2,3,4,5]✉

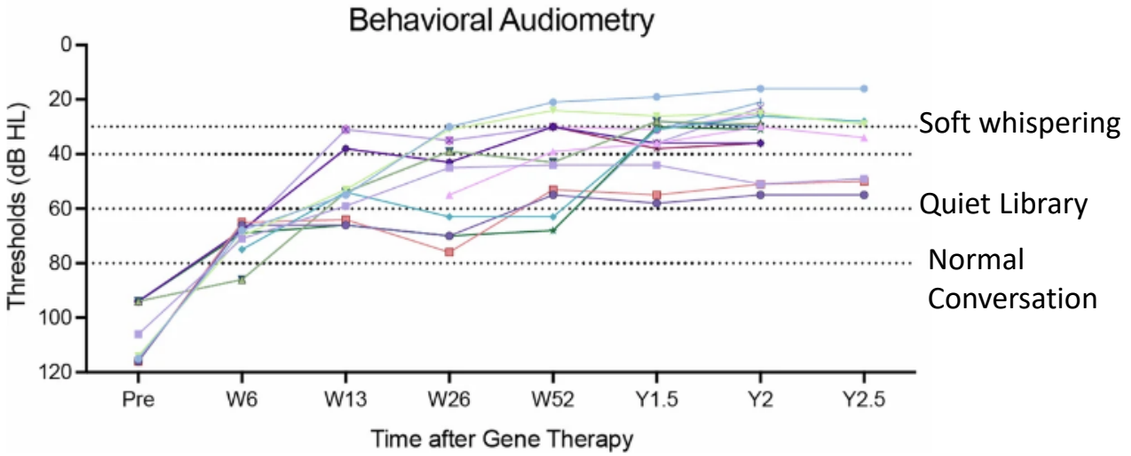
- Multicenter OTOF Gene Therapy Trial
- n = 42 patients
- Age: 0.8 – 32 years
- Follow-up: up to 2.5 years



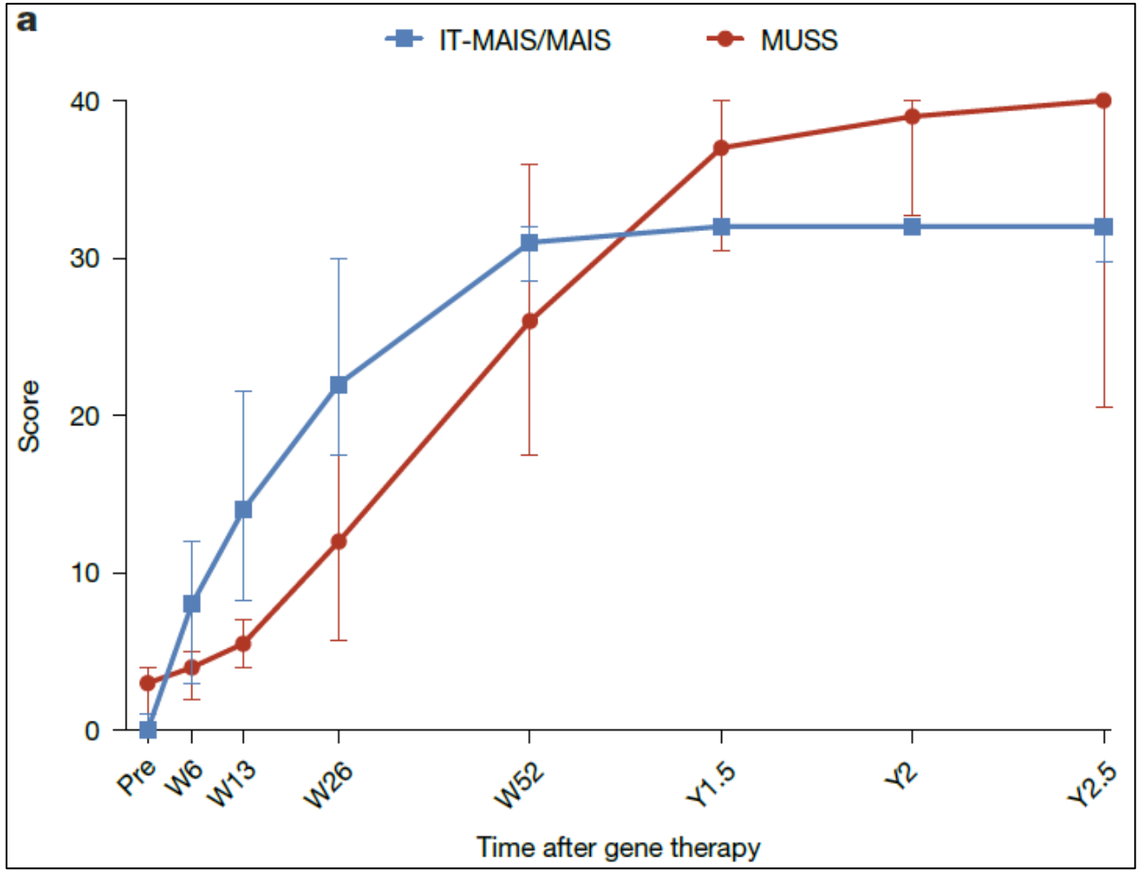
Improvement of hearing thresholds over time

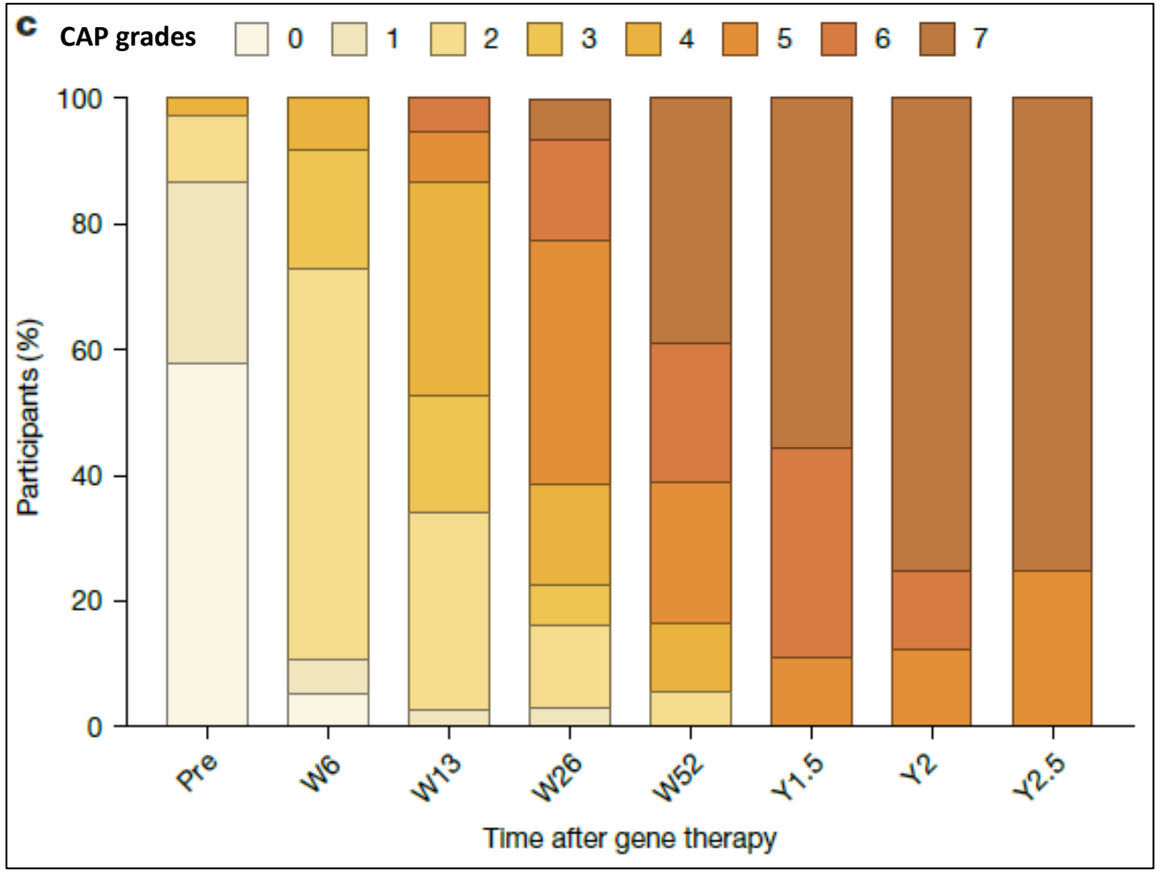


- **Significant improvement by 6–13 weeks**
- **Continued gains through 1–2.5 years**
- **Thresholds approach functional hearing range (~30–50 dB)**
- **Consistent across ABR, ASSR, and behavioral measures**



Speech Perception Improves Over Time





- **Continued improvement beyond 1 year reflects learning + neural adaptation**
- **Functional outcomes lag hearing recovery but continue to improve**

Beyond OTOF: which genes are next?

One therapeutic platform will not work equally well across cochlear cell types and mechanisms

Gene	Biology / target	Current status	Critical translational issue
GJB2	Connexin 26 • cochlear supporting cells	**Phase 1/2**	Cell-specific targeting; developmental defects
SLC26A4	Pendrin • endolymphatic sac/lateral wall	**Preclinical**	Therapeutic window; EVA/progression
STRC	Stereocilin • outer hair cell bundle	**Preclinical**	Oversized cDNA; early timing; OHC targeting
TMC1	Mechanotransduction • sensory hair cells	**Preclinical**	Dominant vs recessive mechanisms
MYO7A	Myosin VIIA • sensory bundles / vestibular	**Preclinical**	Early structural abnormalities; limited late rescue

More genes are under preclinical study (for example, USH1C, MYO15A, CDH23, PCDH15, KCNQ4, TMPRSS3). None has OTOF-level clinical evidence.

Azaiez H

3 Key Takeaways

Genetic testing is foundational

It supports diagnosis, prognosis, recurrence-risk counseling, surveillance, and eligibility assessment for current or future gene-based therapies.

Gene therapy is promising but constrained

It remains gene-specific, time-sensitive, eligibility-dependent, and rapidly evolving; availability and benefit cannot be assumed for every child.

Balanced counseling supports timely decisions

Families need clear explanations of benefits, limitations, uncertainty, and next steps without delaying proven interventions that support auditory access and language development.

Audiologists and SLPs are central to phenotype documentation, family education, referral, and longitudinal support

Azaiez H

Thank you