The Genetics of Hearing Loss and the a Advent of Gene Therapy

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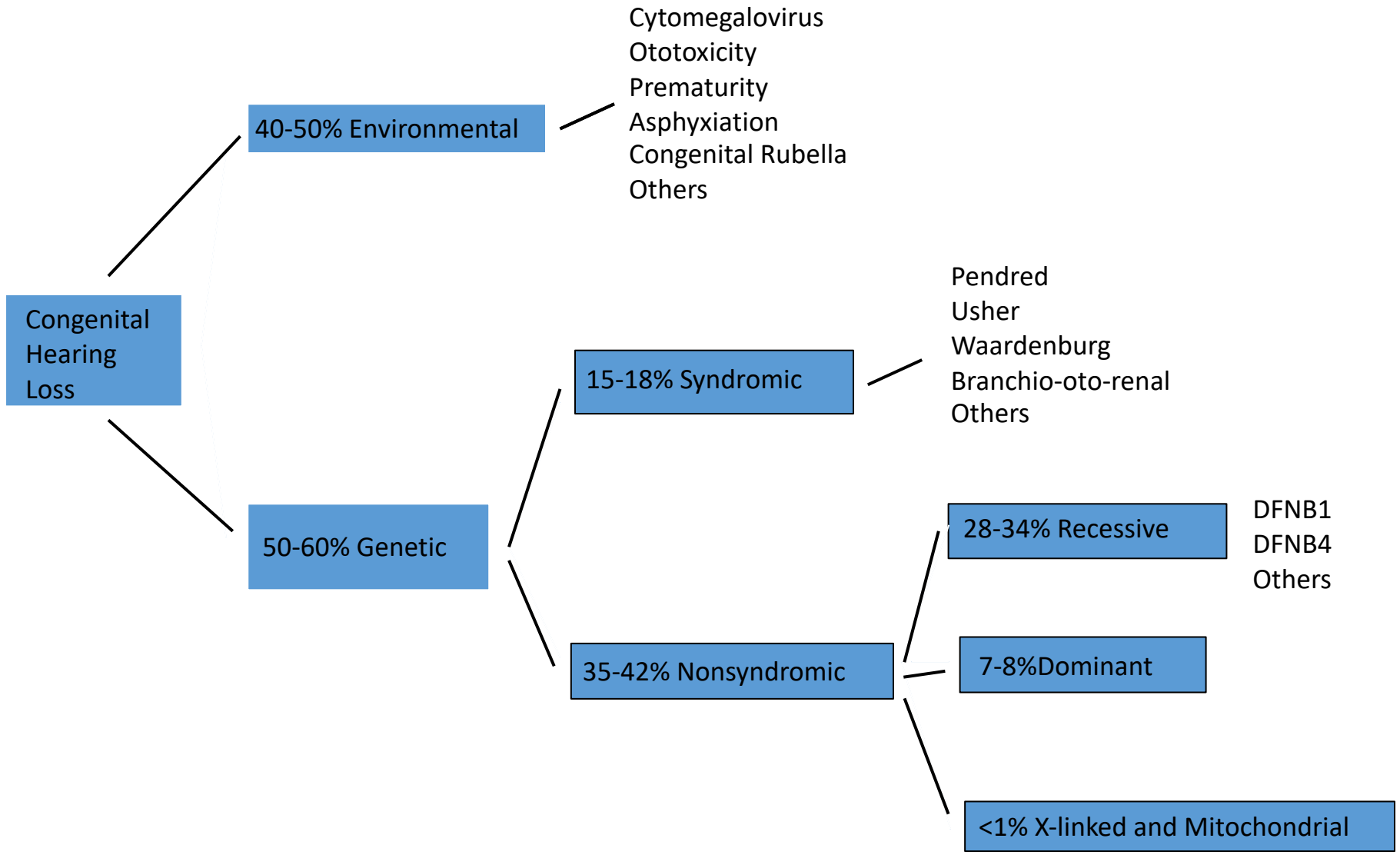
Disclosures

- Site Principal Investigator for the Regeneron corporation CHORD study for gene therapy in children but I have no financial relationship with the company.

Incidence of SNHL in Children

- Hearing loss is the most common congenital sensory impairment
- **Congenital**: 1-2/1000 live births with bilateral severe to profound SNHL
  - Another 1-2/1000 have milder or unilateral hearing loss
- **Later onset/Acquired**
  - 1-3/1000 develop hearing loss later on
  - 15% aged ≥18 years have some degree of HL (NIDCD, 2016)
- **Acquired** since birth: ototoxicity, meningitis, head trauma
- May be the hearing loss manifestation of a **prenatal** occurrence: CMV, anatomic abnormalities, genetics


Congenital Hearing Loss
40-50% Environmental
Cytomegalovirus
Ototoxicity
Prematurity
Asphyxiation
Congenital Rubella
Others
50-60% Genetic
15-18% Syndromic
Pendred
Usher
Waardenburg
Branchio-oto-renal
Others
35-42% Nonsyndromic
28-34% Recessive
DFNB1
DFNB4
Others
7-8%Dominant
<1% X-linked and Mitochondrial

Determining the Etiology of Hearing Loss

- History and Physical
- Labs
- Imaging
- Consults

History and Physical

- Beneficial ~5-10% of the time in directing further workup if an acquired, environmental factor or a syndrome is the cause of HL

Labs/Tests

- In general, these are not routinely done
  - If concerned about autoimmune issues
    - 68 kD Heat Shock Protein Western blot
    - Refer to Rheumatology
  - Renal
    - Urinalysis, BUN, creatinine if concerned about Alport's syndrome

Imaging

- For all patients with SNHL (unilateral or bilateral), obtain MRI of the IACs without contrast
- For patients with mixed and conductive loss, obtain CT of the IAC without contrast

Consults

- Cardiology: EKG to evaluate for prolonged QT interval (Jervell and Lange-Nielson
  - Only do if severe to profound bilateral loss

    and if genetic testing is not done.
- Ophthalmology: impact of a second sensory deficit
  - All patients should have this at some point
- Genetics: to determine inheritance pattern and evaluate for syndromic features
  - In all congenital patients with SNHL
  - Older patients with a dominant or recessive family history or if onset time is uncertain
  - In cases of likely acquired hearing loss after the age of 10 if the family desires

Syndromic Hearing loss

- Associated with malformations of the outer ear or medical problems associated with other organs
- 30% of Hereditary Hearing Loss is syndromic
  - >400 genetic syndromes include hearing loss
  - Autosomal dominant, autosomal recessive, X-linked
  - Not always obvious at birth
    - Ex. Jervell and Lange-Nielsen syndrome
    - Usher syndrome

Examples of Hearing Loss syndromes

- Waardenburg syndrome
- Brachiootorenal syndrome
- Stickler syndrome
- Neurofibromatosis 2
- Usher syndrome
- Pendred syndrome
- Jervell and Lang-Nielsen syndrome
- Riboflavin deficiency
- Refsum disease
- Alport Syndrome
- Mohr-Tranebjaerg syndrome
- Mitochondrial disease
  - MELAS
  - MERRF
  - NARP

Nonsyndromic Hearing Loss

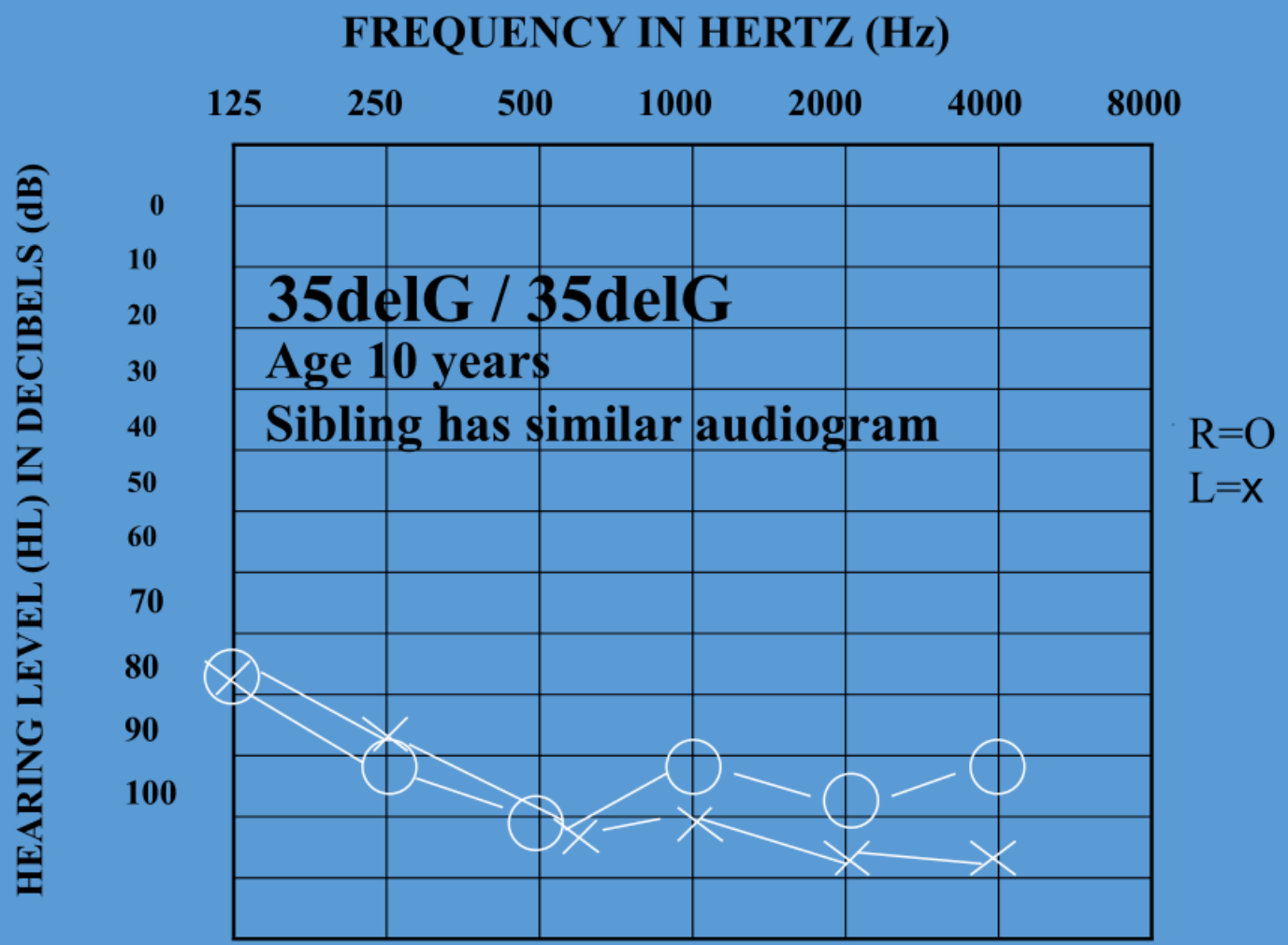
- Not associated with other abnormalities
- Accounts for more than 70% of hereditary hearing loss
- Many loci can cause nonsyndromic HL:
  - ~25 autosomal dominant (DFNA)
  - ~40 autosomal recessive (DFNB); 75-80% of prelingual HL is recessive
  - Several X-linked recessive (DFNX)
  - Several mitochondrial loci

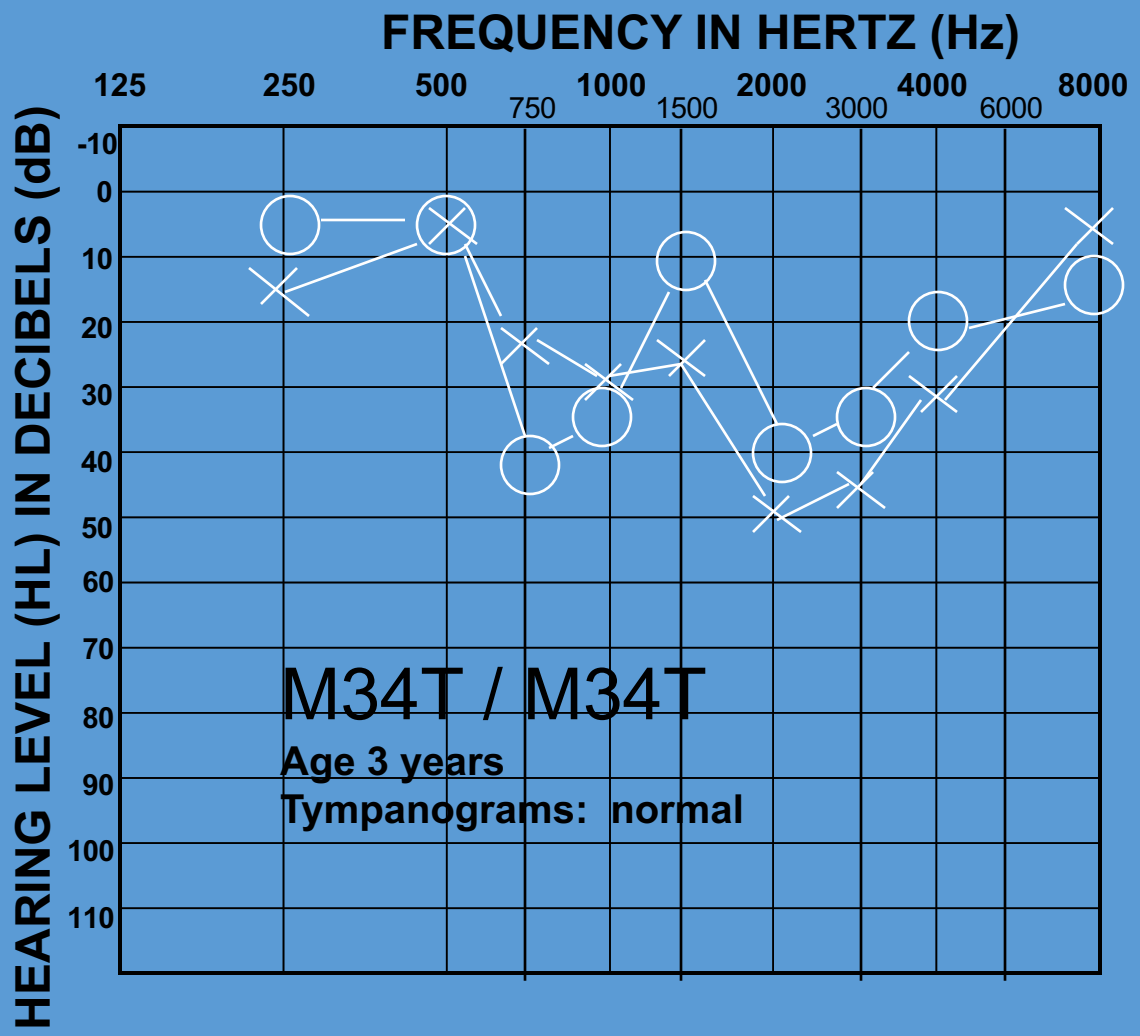
Hundreds of genes identified

- motor (*MYO7A, 15*)
- structural (*TECTA, COL11A2, STRC*)
- ion/mechanic channels
  - *SLC26A4, GJB2, KCQN4, TMC1*
- transcription factors (*POU4F3/3F4, SIX1*)
- extracellular (*ESPN*, *OTOA, OTOG*)
- neural (*OTOF, PJVK)*
- membrane/transporter
  - *TMPRSS33, SLC52A2/3, CLDN*

Prevalence of *GJB2*

- Up to 50% in some populations
- EHC population-18% patients positive
- BCH population-17% positive
- Significantly higher yield in bilateral severe-to-profound SNHL (37.8%) vs. mild-moderate (13.9%) (p=0.004)
- Mutation type correlated with SNHL severity
  - severe SNHL: biallelic nonsense
  - mild SNHL: at least one missense
  - p=0.0006
  - Truncating vs. non-truncating mutations


FREQUENCY IN HERTZ (Hz)
125
250
500
1000
2000
4000
8000
HEARING LEVEL (HL) IN DECIBELS (dB)
0
10
20
30
40
50
60
70
80
90
100
35delG / 35delG
Age 10 years
Sibling has similar audiogram
R=O
L=x


FREQUENCY IN HERTZ (Hz)
125
250
500
750
1000
1500
2000
3000
4000
6000
8000
HEARING LEVEL (HL) IN DECIBELS (dB)
-10
0
10
20
30
40
50
60
70
80
90
100
110
M34T / M34T
Age 3 years
Tympanograms: normal

Other Findings in *GJB2* Hearing Loss

- Most studies show normal inner ear structures
  - A few show mild abnormalities
  - One study shows significant abnormalities
  - Differences may be due to varying populations, one vs. two mutations
- Progression rates vary
  - <10-50% (Lee et al, 2008; Kenna et al, 2010; Chan et al, 2010)
- Some patients have other medical findings (autism, urologic) but unclear whether related to *GJB2* status or reflects tertiary care institutional population

GJB6 (Connexin 30)

- Contiguous with GJB2 on 13q2 in the DFNB1 locus
- At least three recognized pathogenic deletions
- Heterozygous deletion of a 150-300 kb region (includes a common promotor region)
  - Lerer et al, 2001-Ashkenazi
  - Del Castillo et al, 2002-Spain
  - Second deletion described in 2005 del Castillo et al
- In association with *GJB2* heterozygotes causes severe-profound SNHL (recessive)
- Common in Spain and Israel, less so in US

Digenic inheritance

- *GJB2/GJB6*
- Possibly TMPRss3/GJB2
- Newly described deletion in *GJB2* in Russian patients of Ingush ethnicity, del(GJB2-D13S175)
  - Founder mutation going back 3000 years

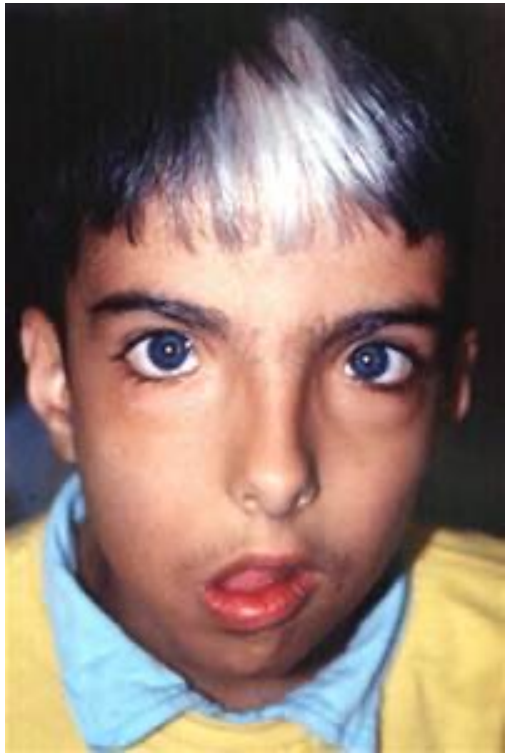
Other common genes for SNHL

- Stereocilin (STRC)
  - Mild mid-frequency saucer-shaped hearing loss
- Usher genes
  - MYO7A, CDH23, USH1C, PCDH15, WHRN
- SLC26A4
  - Pendred Syndrome and nonsyndromic EVA
- Otoferlin
  - Non-syndromic and Auditory Neuropathy
- MYO15A, non-syndromic
- TMC1

Branchio-oto-renal syndreome

- AD, ~100% penetrance, 1/40,000 infants (2% of profoundly deaf children)
- Otologic findings
  - Inner, middle, and outer ear anomalies
  - CHL, SNHL, or Mixed HL, may be severe or progressive
- Branchial anomalies
  - Pits, sinuses, clefts
- Renal anomalies
  - Agenesis or dysplasia
- EYE1 gene- transcription factor

Waardenburg Syndrome



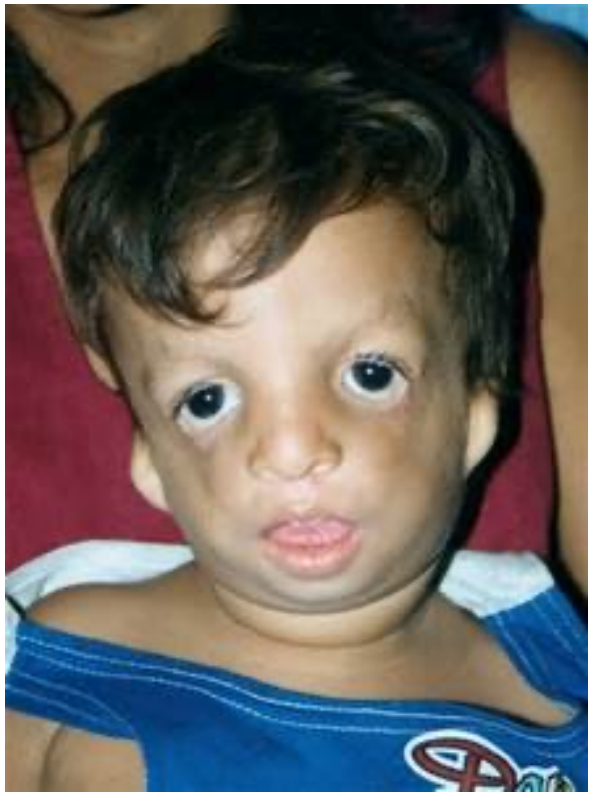
Waardenburg Syndrome

- A common form of hereditary congenital deafness (1:4,000 births)
- Subtypes
  - 1. SNHL (33-66%), heterochromia irides, pigment anomalies, dystopia canthorum
  - 2. As above w/o dystopia canthorum (SNHL 57-85%)
  - 3. Klein-Waardenburg's – #1 + microcephaly, mental retardation, limb and skeltal abnormalities
  - 4. Shah-Waardenburg's - #2 + Hirschsprungs (AR)

Waardenburg Syndrome

- Mutation of *PAX3* gene (types 1 & 3)
- Dystopia Canthorum
  - Shortened and fused medial eyelids with small medial sclera, lateral displacement of inferior puncta, and hypertelorism

Treacher Collins Syndrome



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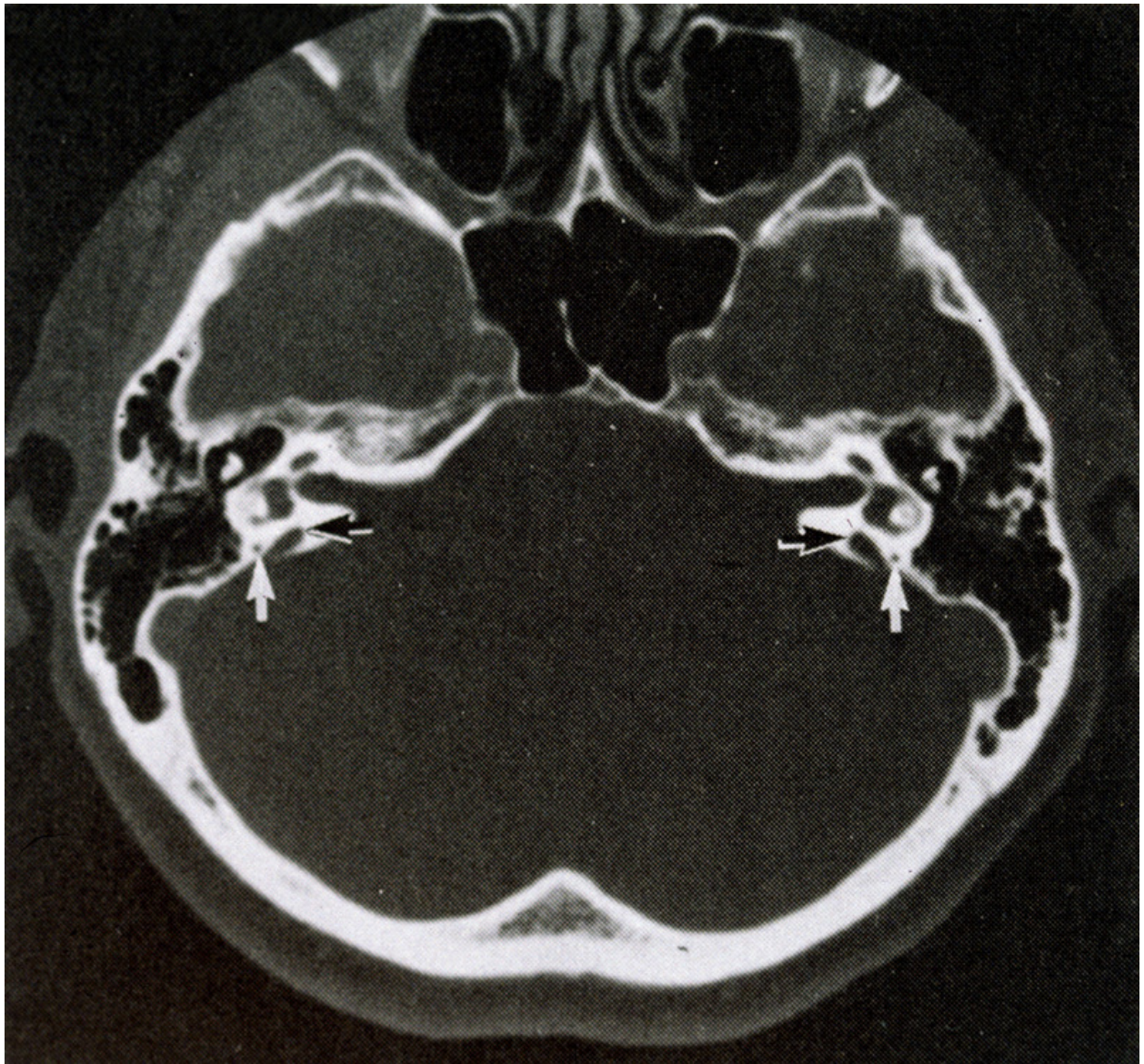
Treacher Collins Syndrome

- AD
- Craniofacial abnormalities
  - Lower lid colobomas, downward slanting palpebral fissures, hypoplastic mandible, malformations of external ear, cleft palate, hearing loss
- Hearing loss is conductive (ossicular fixation)
- TCOF gene, protein—T*reacle*

Pendred Syndrome

- Congenital deafness and thyroid goiter
- May account for 5-10% hereditary deafness
- Gene *SLC26A4* (or Pendrin gene—*PDS*), codes for pendrin protein (chloride/iodide transporter)
- Perchlorate discharge test
  - Perchlorate blocks Na/I symporter, will displace more iodine than normal from thyroid gland
  - Not very sensitive, genetic testing preferred
- Associated w/ EVA or Mondini dysplasia

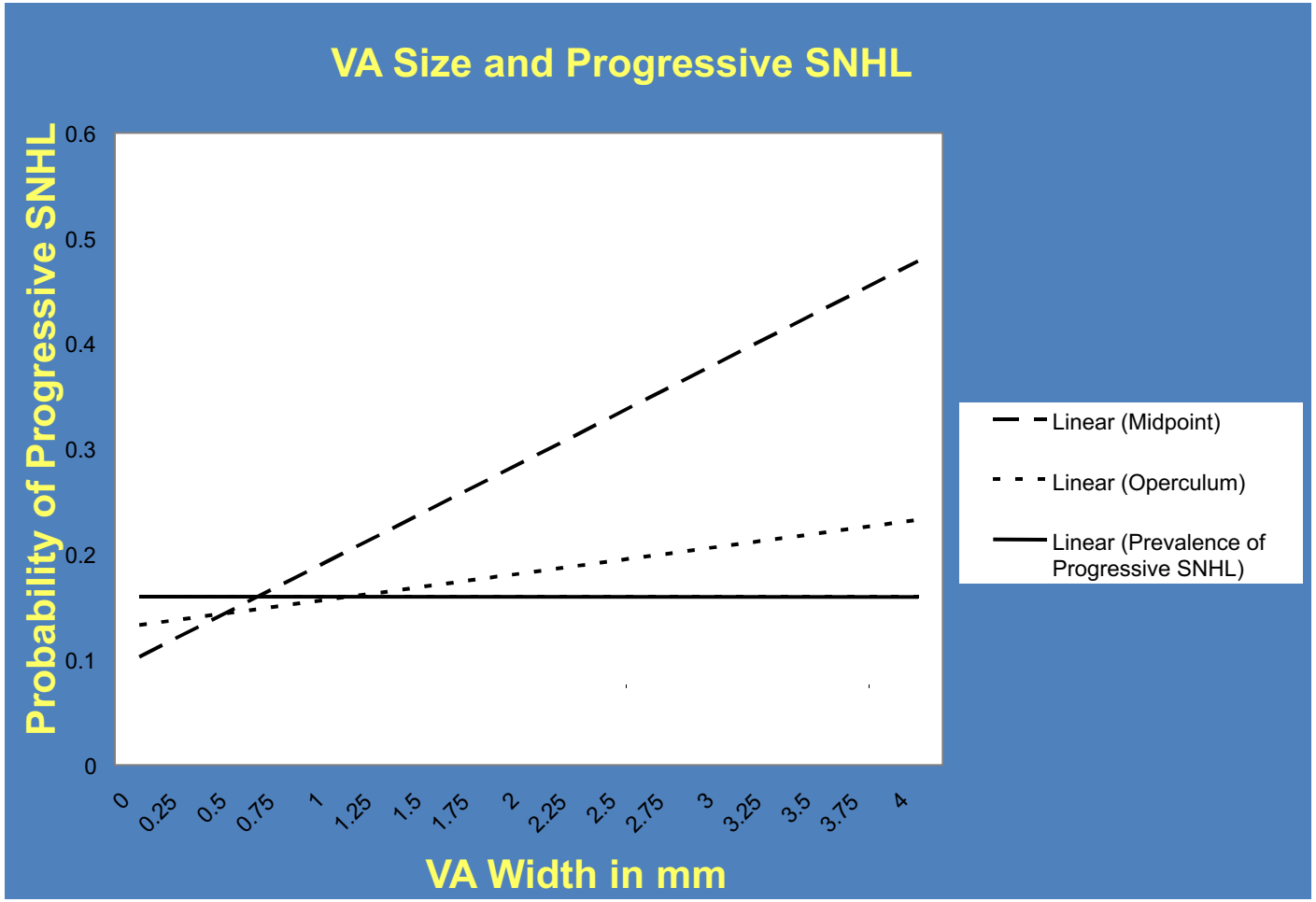


Phenotype

- Characterized by variable onset
  - Infancy (*SLC26A4*) to childhood
- Fluctuation-common
- Progressive SNHL
  - 12-65% (~30%)
- Audiogram
  - SNHL
  - Variable configuration
  - Mixed component at lower frequencies

Distinct EVA Phenotypes

- Syndromic
  - Pendred syndrome (PS)
  - Waardenburg syndrome
    - Madden et al (2003)
  - Branchio-oto-renal syndrome
    - Chen et al (1995)
- Nonsyndromic
  - DFNB4
    - Found to be syndromic (PS)
  - EVA syndrome

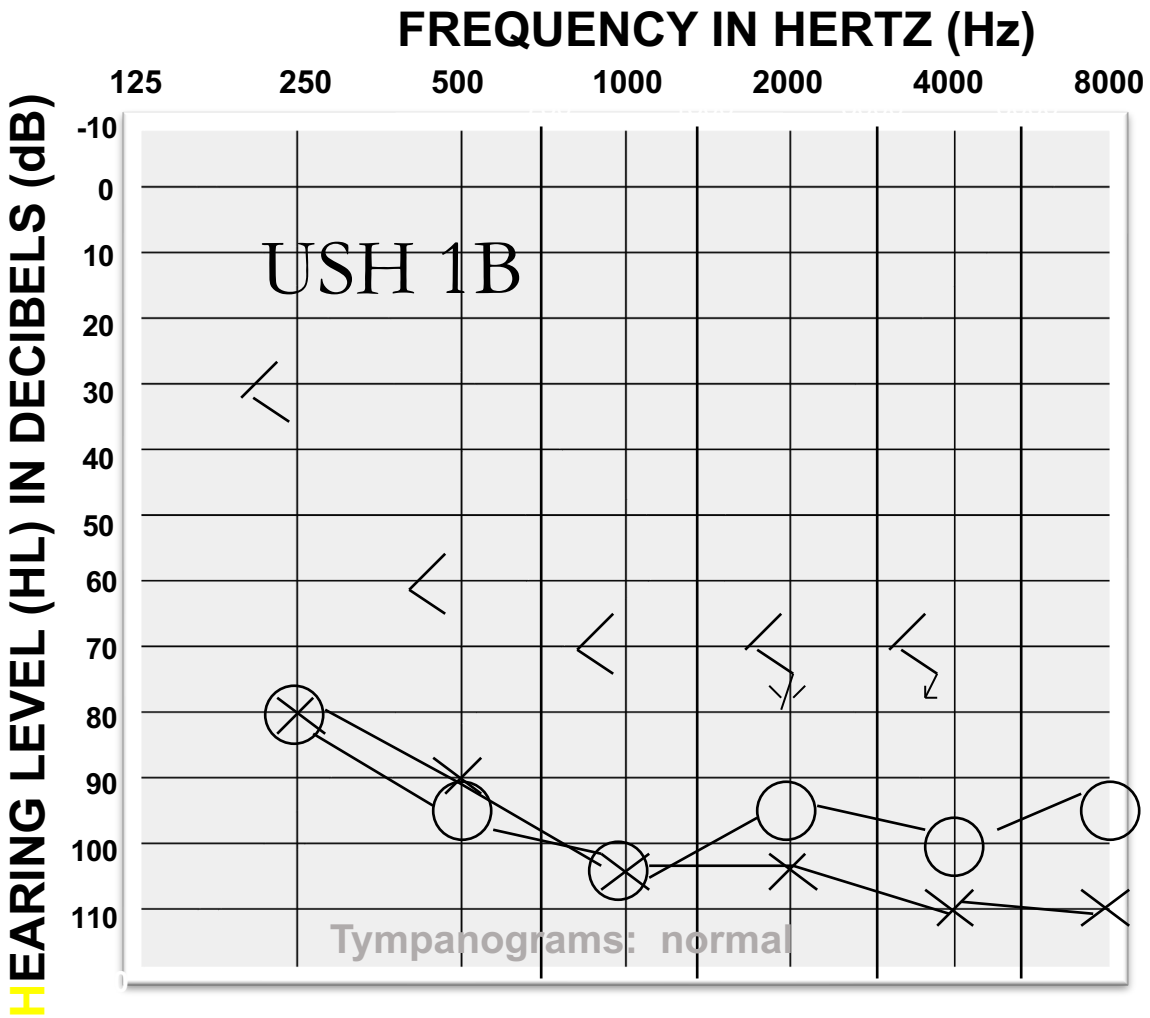

VA Size and Progressive SNHL
Probability of Progressive SNHL
0.6
0.5
0.4
0.3
0.2
0.1
0
0
0.25
0.5
0.75
1
1.25
1.5
1.75
2
2.25
2.5
2.75
3
3.25
3.5
3.75
4
VA Width in mm
Linear (Midpoint)
Linear (Operculum)
Linear (Prevalence of Progressive SNHL)

Jervell and Lange-Nielsen Syndrome

- Congenital Deafness, Prolonged QT, syncopal attacks
- Genes code for K channel in heart and inner ear (*KCNQ1 and KCNE1*)
- Hearing loss is congenital, bilateral, severe to profound
- Prolonged QT can lead to ventricular arrhythmias, syncopal episodes, death in childhood (tx w/ beta-blockers)

Usher Syndrome

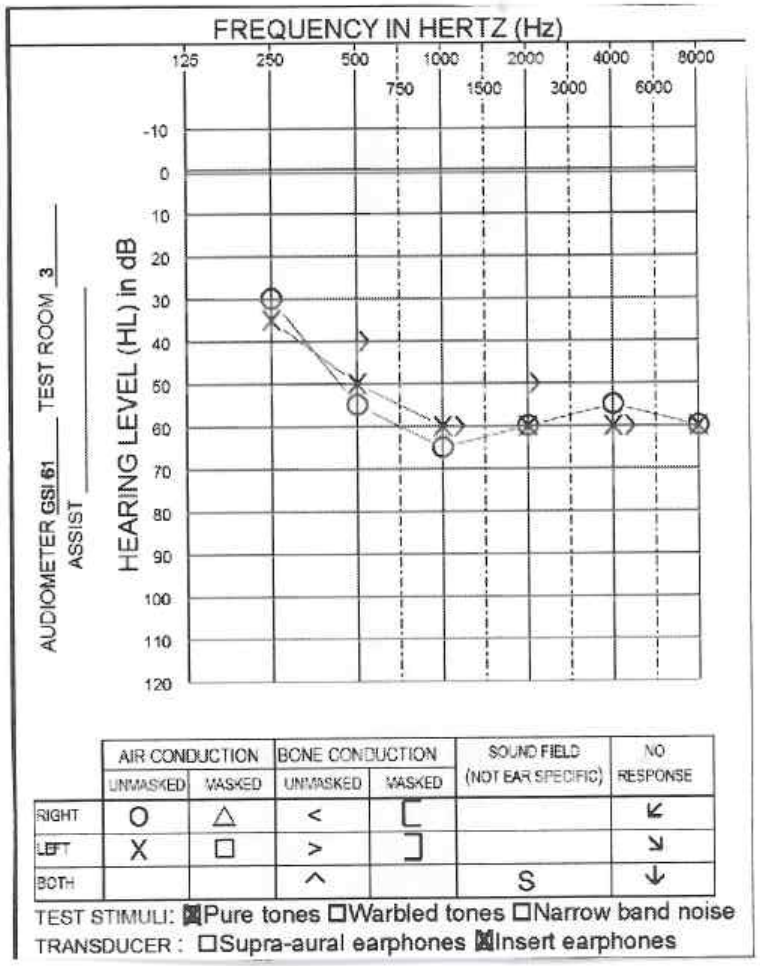
- 3-6% of congenitally deaf, 50% of blind/deaf
- Type I—severe-profound HL, absent vestibular fxn, pre-pubertal RP
- Type II—moderate-sever HL, normal vestibular fxn, post-pubertal RP
- Type III—progressive HL, variable vestibular fxn and RP



1 year old male with USH1B and absent VOR

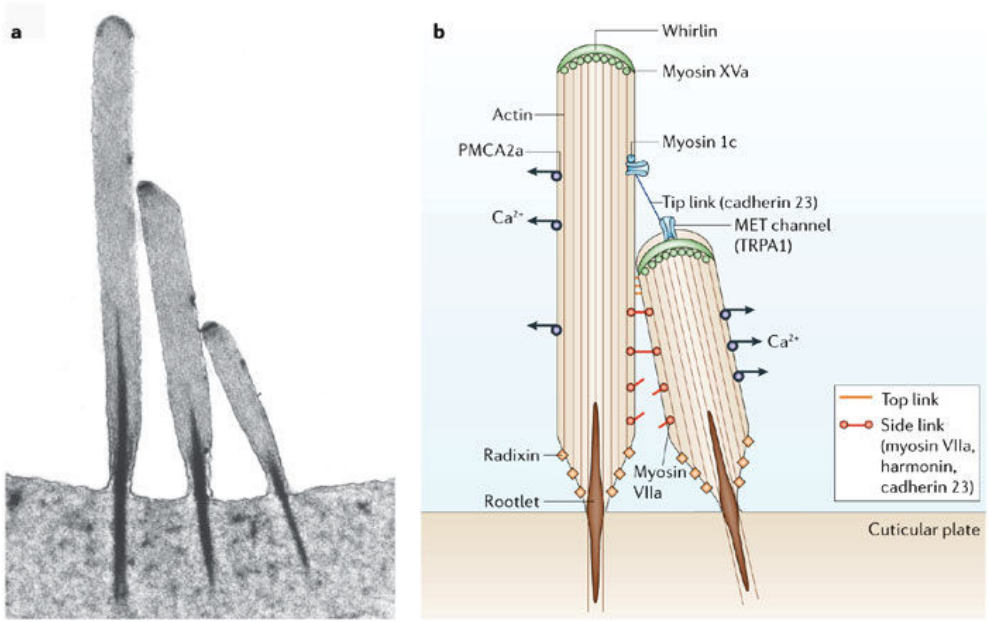
USH2A

10 year old male with USH2A and normal visual acuity



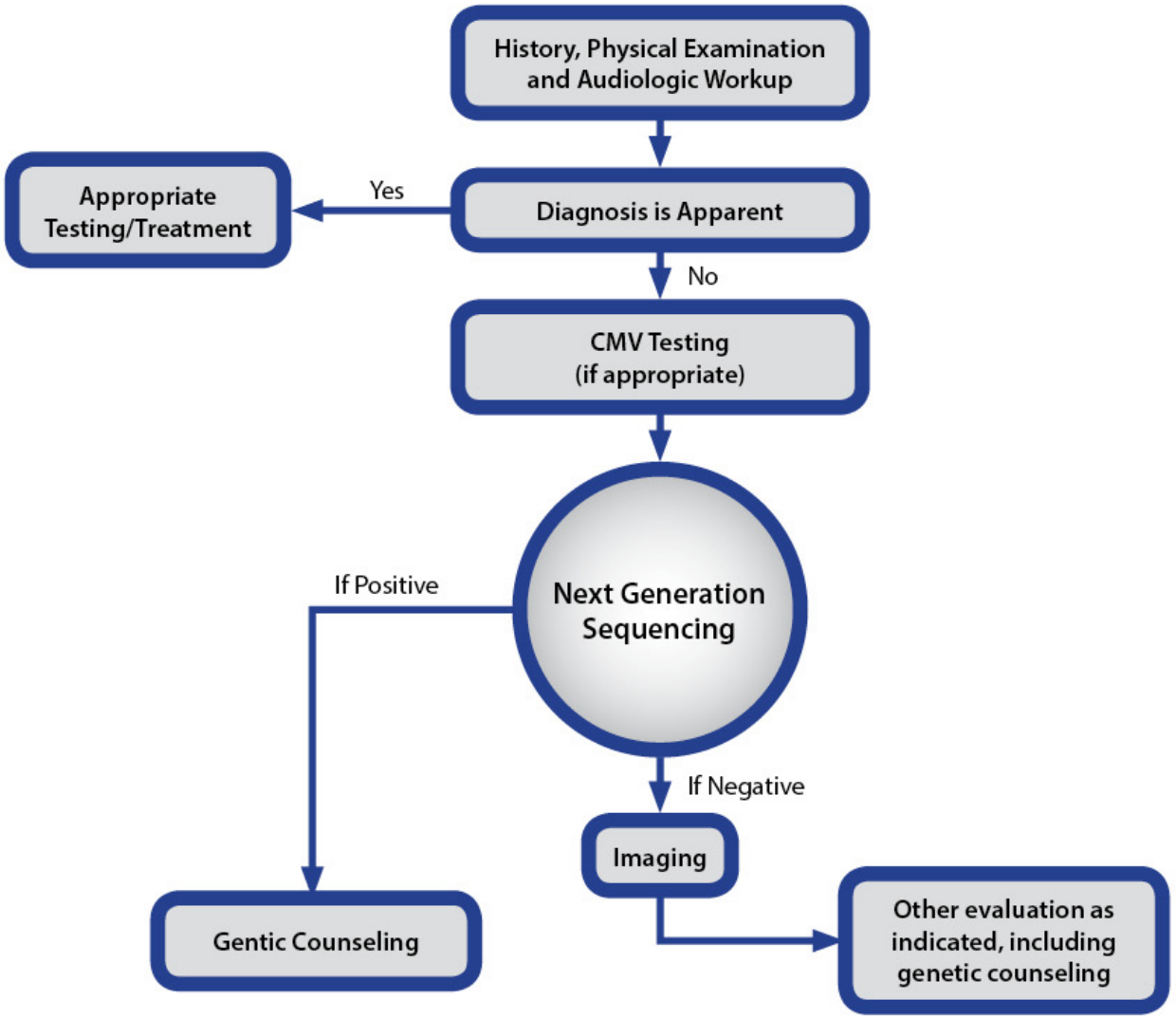
Usher Syndrome

- Structural and motor proteins
- Related to motion of tip links in the inner ear causing ion influx and depolarization
- *MYO7A*- type 1 and *USH2*- type 2



Alport

- Type IV collagen (basement membrane)
- Hematuric nephritis (all males progress to ESRD), hearing loss, ocular changes
- Predominantly x-linked (can be AD or AR)
- Hearing loss is HF SNHL by late childhood


History, Physical Examination and Audiologic Workup
Diagnosis is Apparent
Yes
Appropriate Testing/Treatment
No
CMV Testing (if appropriate)
Next Generation Sequencing
If Positive
Gentic Counseling
If Negative
Imaging
Other evaluation as indicated, including genetic counseling

Treatment for SNHL

- Hearing aids
- FM systems
- Cochlear implants
- Molecular medicine
  - Gene therapy
  - Hair cell regeneration

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Otoferlin as a target for gene therapy

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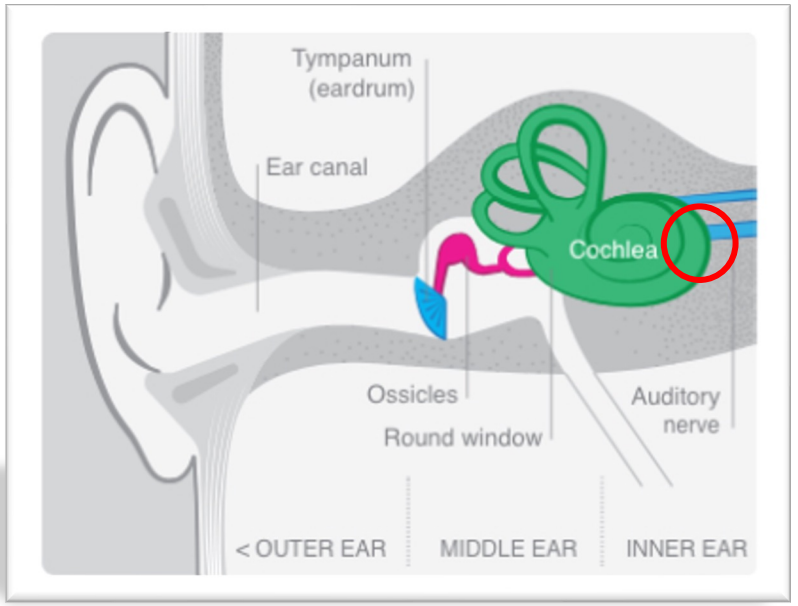
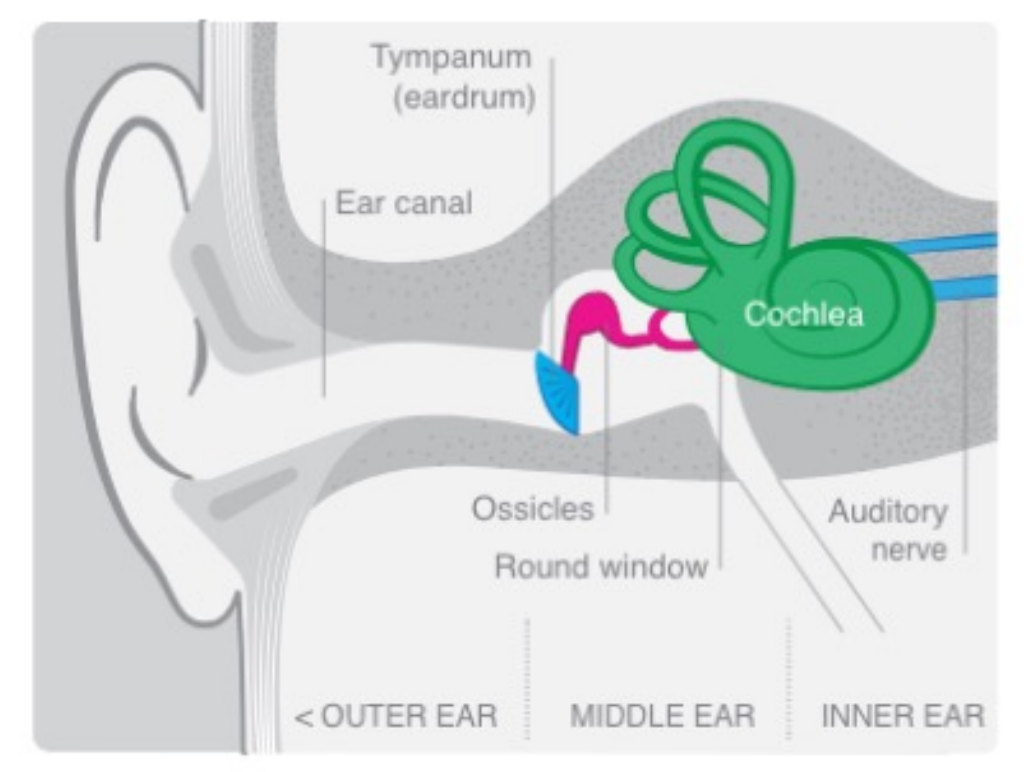
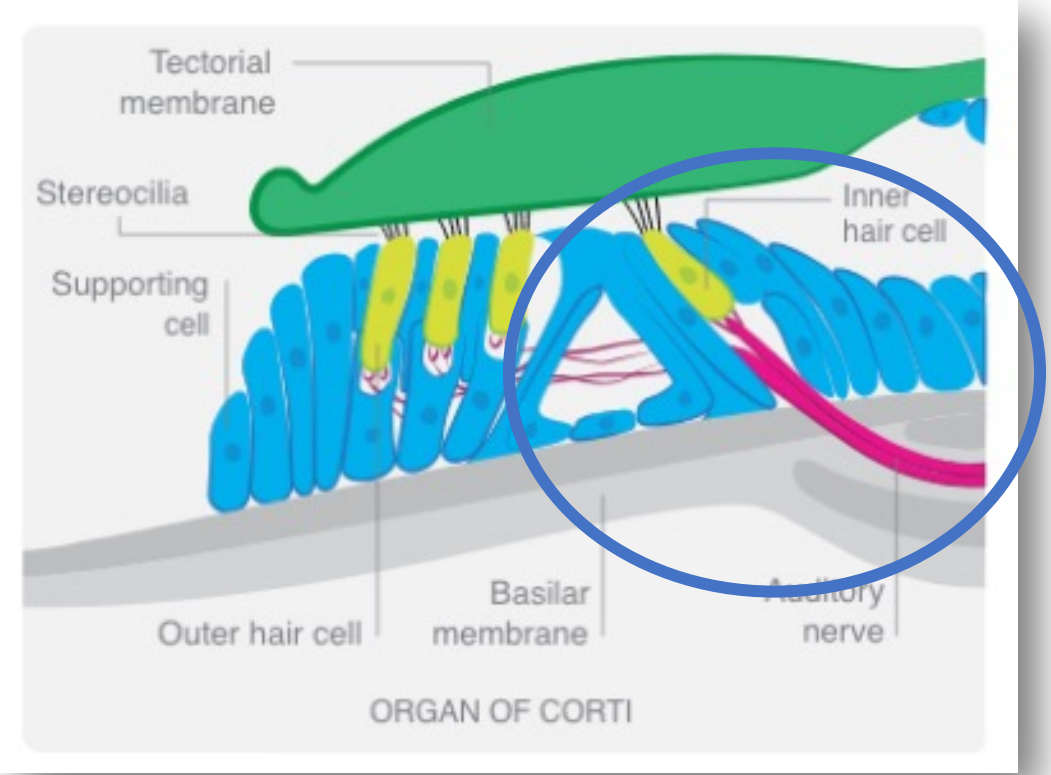
Hearing Loss

- Conductive
- Sensorineural
  - Auditory Neuropathy Spectrum Disorder (ANSD)



Relevant Anatomy for Auditory Neuropathy Spectrum Disorder (ANSD)





ANSD

- Present cochlear function coupled with abnormal auditory nerve activation
  - Diagnoses: Normal otoacoustic emissions (OAEs) or cochlear microphonics and an absent or abnormal auditory brainstem response (ABR) (Starr et al. 1996)
  - Can be mistaken for profound SNHL
- Decreased “function” hearing
  - Listening vs hearing
- Outer hair cell function “initially” preserved
- Hearing thresholds and SRT can be variable

Auditory Neuropathy Spectrum Disorder (ANSD) Etiologies

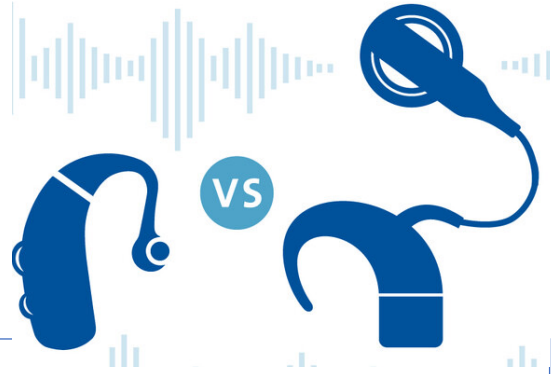
Genetic causes (syndromic/non-syndromic)	• Nonsyndromic: ***Otoferlin***, *CAPB2, SLC17A8, DIAPH3, SLC*52A2/3 • Syndromic: Charcot-Marie-Tooth (CMT), Friedreich's ataxia, *OPA1, CACNA1D* • X-linked: *AIFM1*
Environmental & acquired factors	• Prematurity • Hyperbilirubinemia (jaundice) • Perinatal hypoxia (lack of oxygen)
Other	• Cochlear nerve deficiency (CND) • Autoimmune

- Shearer AE, Hansen MR. Auditory synaptopathy, auditory neuropathy, and cochlear implantation. Laryngoscope Investig Otolaryngol. 2019 Jul 1;4(4):429-440

*OTOF (*Otoferlin*)*

- Function related to synaptic vesicle exostosis
  - Accounting for inner hair cell dysfunction
- Large gene on chromosome 2q23
- Phenotype
  - Congenital severe to profound “functional” hearing
  - Auditory dys-synchrony

Audiologic Management Strategies for ANSD



Hearing aids

- Infants and young children with mild to severe tone detection thresholds
  - *fitted with amplification as soon as ear-specific pure-tone and speech detection thresholds are determined*

Cochlear implants

- Once limited benefit from amplification is confirmed

Other communication strategies

- FM systems
- Auditory verbal therapy (AVT)
- Cued speech or sign language

-Hayes, D., & Sininger, Y.S. (2008). Guidelines for Identification and Management of Infants and Young Children with Auditory Neuropathy Spectrum Disorder.
-Picture: Dukehealth.org

Regeneron DB-OTO-001 (The CHORD Study)

A Phase 1/2, Open-Label, Multicenter Trial With a Single Ascending Dose Cohort With Unilateral Intracochlear Injection Followed by a Bilateral Injection Expansion Cohort to Evaluate the Safety, Tolerability, and Efficacy of DB-OTO in Children and Infants With Biallelic *hOTOF* Mutations

CHORD Study Training:
Study Protocol V2.0 US-4_28Feb2024
Audiometric Study Manual V5.0_11Nov2024
Prevention Genetics Lab Manual of Procedures V3.0_02Aug2024

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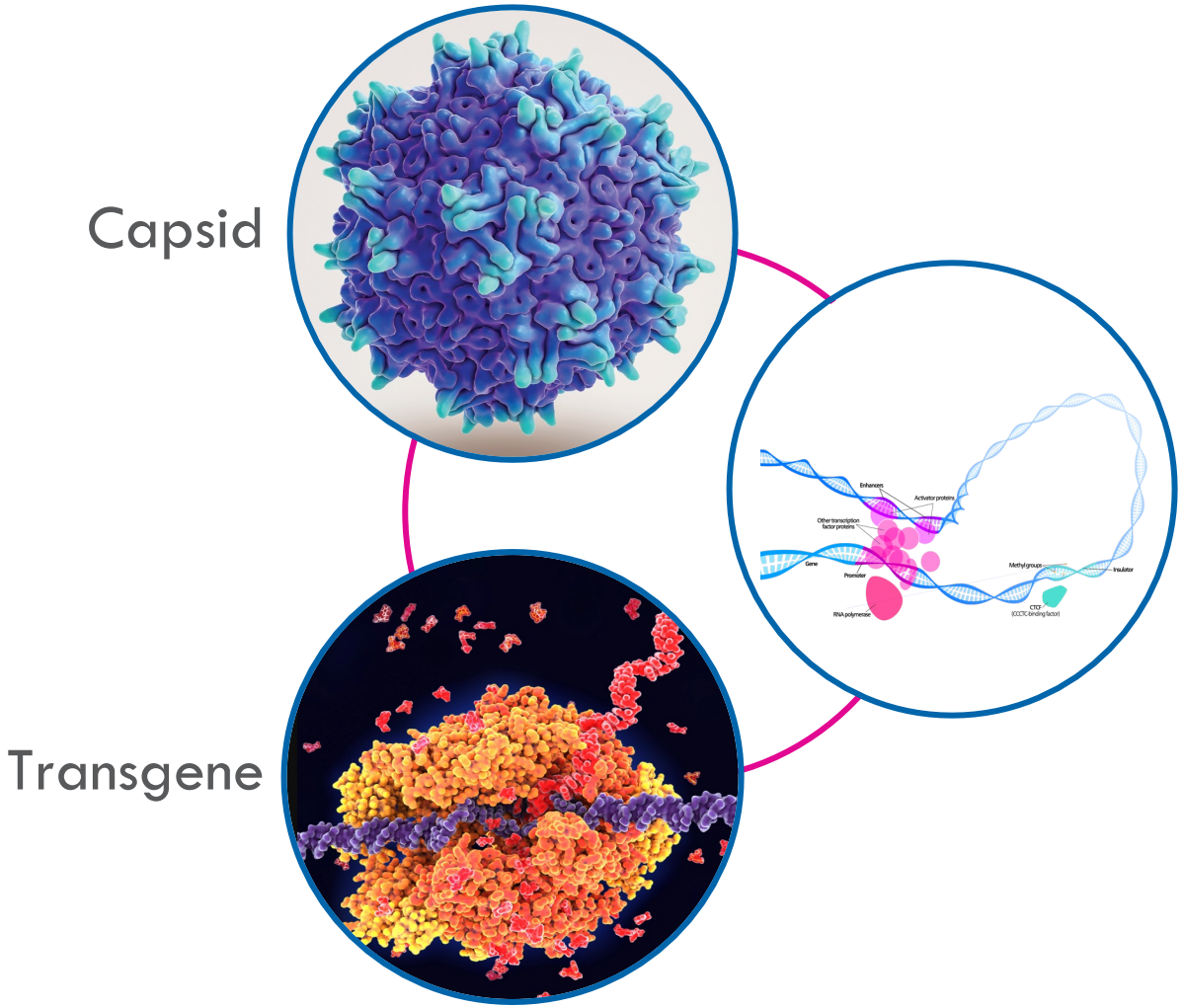
CHORD Clinical Trial™

Adeno Associated Virus (AAV) Gene Therapy



- Gene therapy inserts a normal copy of the gene
- The inner ear is an ideal organ since local injection can minimize any systemic effects and heighten local drug delivery
- Gene therapy is a different technology than CRISPR or attempts at hair cell regeneration
- Gene therapy requires normal organ of Corti and cochlear nerve anatomy

Endpoints

Primary Endpoint

- Incidence and severity of treatment-emergent systemic and local adverse events (AEs)

Secondary Endpoint

- Auditory brainstem response (ABR) – change in intensity threshold (decibel normalized Hearing Level [dB nHL]) across frequency domains
- Behavioral audiometry with pure-tone audiometry – change in intensity thresholds (decibel normalized Hearing Level [dB nHL]) in treated ear across frequency domains
- Speech awareness threshold (SAT)– change in threshold in treated ear

Study also includes a number of speech perception and quality of life exploratory endpoints

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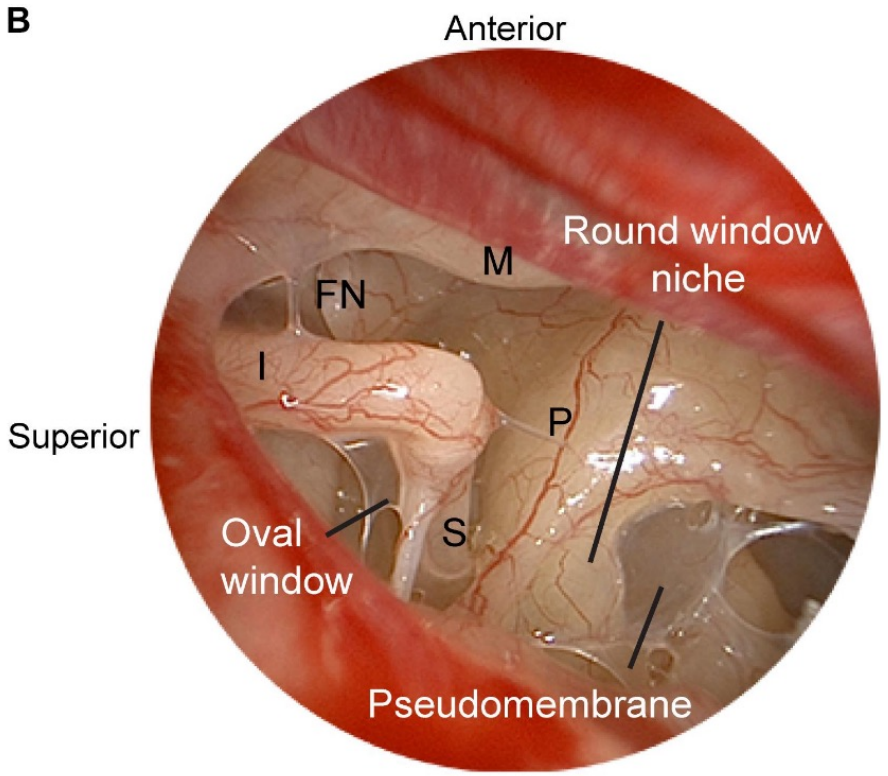
Operative protocol

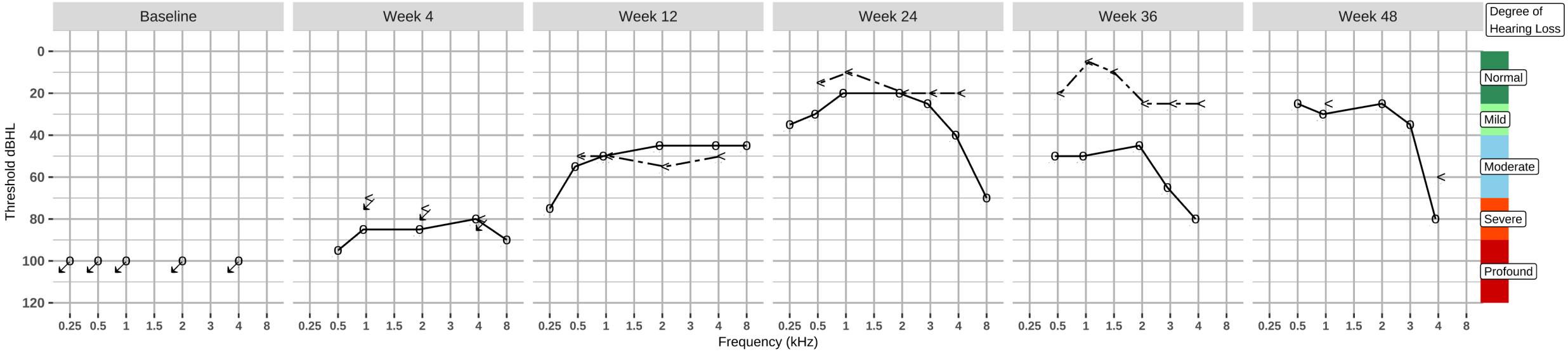
- Standard mastoidectomy similar to cochlear implants
- A fluid egress hole will be made in the horizontal canal to allow for fluid/pressure egress
- Inject 250 ul of gene therapy product via needle catheter through the round window via a micropump.
- Post operative treatment with steroids for several weeks



Preliminary efficacy: improvement close to normal hearing range in the DB-OTO–treated ear through Week 48

Patient 1

- Pure tone audiogram thresholds for the DB-OTO–treated ear reaching normal hearing levels at key speech frequencies by week 24
- No improvement was seen in the untreated ear with the cochlear implant turned off



HL, hearing level.

- Data from our first 12 patients has been accepted for publication in the New England Journal of Medicine
  - Not a traditional journal for ENT or audiology
  - Wide reaching implication of this research
- Data from our next 12 patients (24 ears) is being analyzed

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