# Medical Genetics

Sheet: 15 – Genetics of Hearing Loss

Writer: Mothana Olimat

Scientific: Mothana Olimat

Final: Odai Bani Monia

Doctor: Bilal Azab

Hearing loss is a relatively common disease, and it affects around 17 in 1000 children and adolescents younger than 18 years old. The average incidence of hearing loss in neonates in the United States is 1.1 per 1000.

The prevalence of childhood and adolescent hearing loss was 3.1%, with higher rates in Hispanic Americans and in families with lower incomes showing that hearing loss is influenced by socioeconomic status.

Hearing loss could be due to a **genetic cause** or **factors other than that genetic mutations** such as prematurity, postnatal infections, ototoxic drugs, or maternal infection (CMV, rubella).

Prevalence of hearing loss increases with age and this reflects the impact of genetics and environment, it also reflects some interaction between environmental triggers an individual genetic predisposition.

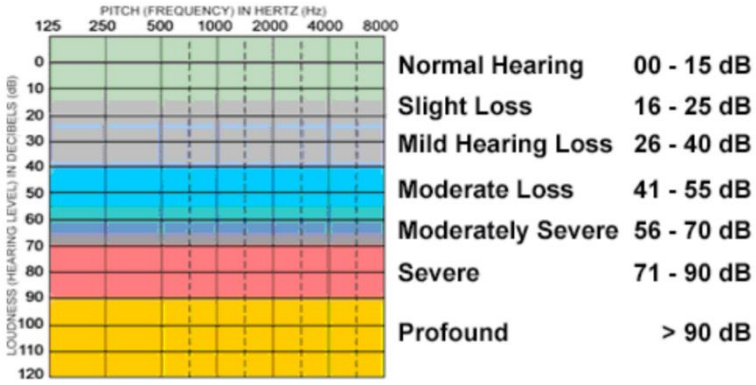
Hearing loss can be classified based on: **type**, **severity**, and **age of onset**

**1) Type**

- Conductive hearing loss: abnormalities of the external ear and/or the ossicles of the middle ear
- Sensorineural: malfunction of inner ear structures (cochlea)
- Mixed: conductive and sensorineural
- Central auditory dysfunction: results from damage or dysfunction at the level of the eighth cranial nerve, auditory brain stem, or cerebral cortex

**2) Severity**

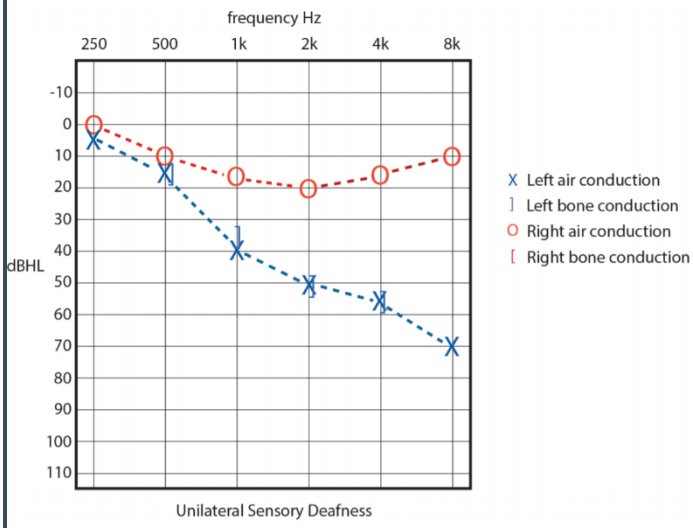
Hearing loss could be classified into a spectrum of severities based on the **minimum loudness** (decibels) required to hear the sound.




**3) Age of Onset**

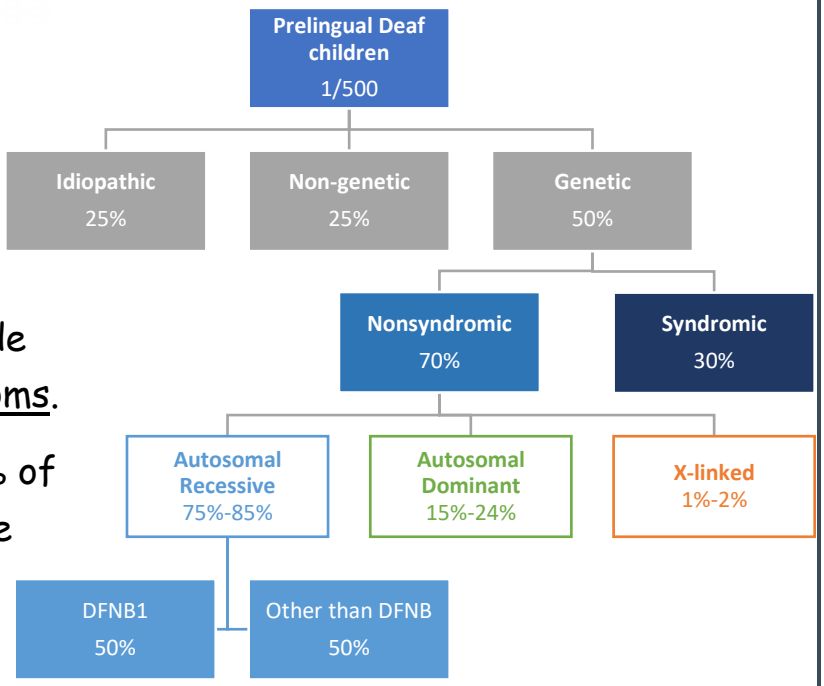
- Pre-lingual: congenital, or in early infancy (before speech develops)
- Post-lingual: occurs after the development of normal speech (more common)

**Hearing impairment may exist in only one ear (**unilateral**) or in both ears (**bilateral**).




This is an audiogram for a patient with hearing loss in one ear (the left). This assessment is achieved by exposing the tested patient to sounds of different frequencies and increasing the loudness of each until the patient can hear it. The more decibels required to hear the sound means a more severe difficulty in hearing.

This lecture we will focus on the genetic etiology of **prelingual** hearing loss.




## A) Syndromic hearing impairment

Over 400 genetic syndromes include hearing loss as one of their symptoms.

Syndromic hearing loss causes 30% of prelingual deafness, but its relative contribution to all deafness in general is much smaller.

Syndromes which are accompanied by hearing loss are divided based on their pattern of inheritance into: **autosomal dominant**, **autosomal recessive**, **X-linked**, and **mitochondrial**, and here are some examples of such syndromes:

| Autosomal dominant | Autosomal recessive | X-linked |
|---|---|---|
| Waardenburg syndrome (WS)<br>Branchiootorenal syndrome (BOR)<br>Stickler syndrome<br>Neurofibromatosis 2 (NF2) | Usher syndrome<br>Pendred syndrome<br>Jervell and Lange-Nielsen syndrome<br>Biotinidase deficiency<br>Refsum disease | Alport syndrome<br>Mohr-Tranebjaerg syndrome (deafness-dystonia-optic atrophy syndrome) |

**Such syndromes may be caused by a mutation in one identified gene (**Treacher Collins syndrome** - TCOF1) or by a mutation in one of many genes that can cause the syndrome (**Usher syndrome**) and we will discuss these **two examples**.

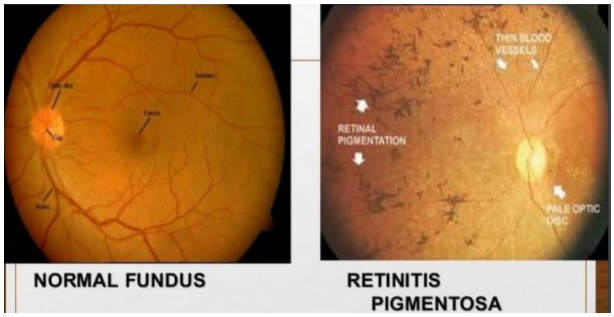
# Usher Syndrome

Usher syndrome is the most common condition that affects both hearing and vision.

It impairs vision by causing **retinitis pigmentosa (RP)** which is characterized by:

- Nyctalopia (night-blindness) as the disease begins in deterioration of **rods** photoreceptors.
- Tunnel vision as the disease deteriorates **cones** lose their function which leads to loss of peripheral vision (side vision) which could further progress into complete blindness.
- Severe balance problems

Fundus imaging shows a characteristic **retinal pigmentation** called bone spicules, **pale optic disc**, and **thin blood vessels**.




The prevalence of Usher syndrome in the general US population has been conservatively estimated at 4.4:100,000 and the carrier frequency may be as high as 1:70, while the prevalence in persons of Scandinavian descent has been estimated at around 3.6:100,000.

Usher syndrome has been estimated to be responsible for 3%-6% of all childhood deafness and approximately 50% of all deaf-blindness.

Usher syndrome is divided into **three** types based on the severity and age of onset of hearing, visual, and vestibular symptoms. Types I and II account for 90% to 95% of all cases of children who have Usher syndrome in the US.

| | Hearing loss | Vestibular system | Retinitis Pigmentosa |
|---|---|---|---|
| **Type I** | Congenital onset, profound symptoms (worst) | Congenital balance problems | Pre-puberty onset |
| **Type II** | Congenital onset, mild to severe sloping symptoms | Normal | Teens to 20s onset |
| **Type III** | later onset, progressive symptoms | Progressive balance problems | Variable onset |

Each type of Usher syndrome is divided into **subtypes** based on the gene that causes it and its location (locus) on the chromosome. Notice that some genes are unknown which means they haven't been discovered yet but the location of the gene when altered is known to cause the syndrome. The relative incidence of each subtype is also noted, showing the most common causative genes.

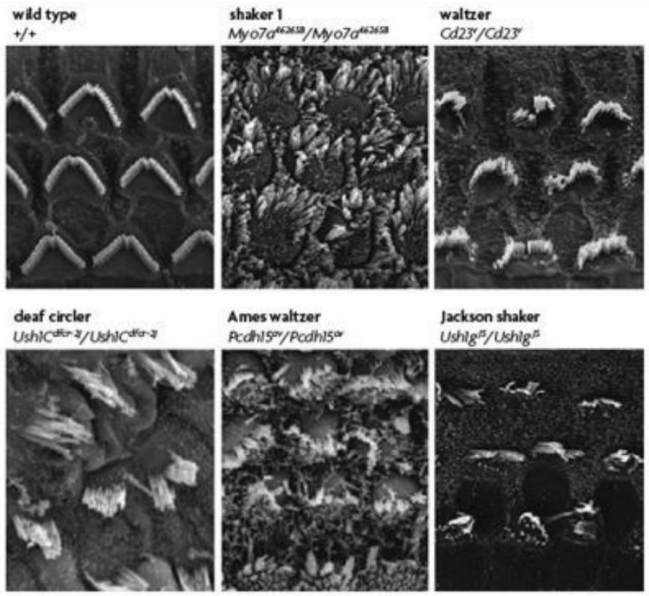
| Usher Type | Locus | Gene | Relative Incidence |
|---|---|---|---|
| USH1A | 14q32 | unknown | 2% |
| USH1B | 11q13.5 | *MYO7A* | 60% |
| USH1C | 11p15.1 | *USH1C* | 5% |
| USH1D | 10q | *CDH23* | 10% |
| USH1E | 21q | unknown | Rare |
| USH1F | 10q21.1 | *PCDH15* | Rare |
| USH1G | 17q24-25 | *SANS* | Rare |
| USH2A | 1q41 | *USH2A (+51)* | 80% |
| USH2B | 3p23-24.2 | unknown | Rare |
| USH2C | 5q14.3-q21.3 | *VLGR1* | 15% |
| USH3 | 3q21-q25 | *USH3* | 100% |

**We aren't required to know which gene causes which subtype.

There are different genetic **testing methods** for a disease entity, so if the clinician suspects a genetic disease and wants to run genetic testing to diagnose the individual, they should be aware of the appropriate testing method for the suspected disease. For example, you can't use whole exon sequencing for a disease where known causative mutations are in non-coding regions.

| Percent of All USH1 [1] | Gene Symbol (Locus Name) | Test Method | Mutations Detected | Mutation Detection Frequency by Gene and Test Method [2] |
|---|---|---|---|---|
| 39%-55% | *MYO7A* (USH1B) | Sequence analysis | Sequence variants [3] | ~90% [1, 4] |
| | | Targeted mutation analysis | Panel of targeted known sequence variants [5] | See footnote 5 |
| | | Deletion / duplication analysis [6] | Exonic or whole-gene deletions | Unknown |

**Usher syndrome is a devastating disase where patients suffer from hearing and vison loss and tend to rely on touch for communication.




←This figure shows the phenotype happening in the cochlea to the inner hair cells of mice, notice how it differs as the causative mutation changes.

## Treatment

Hearing aids: Young children can benefit from early fitting of hearing aids and speech training to normalize language. Cochlear implantation may be necessary.

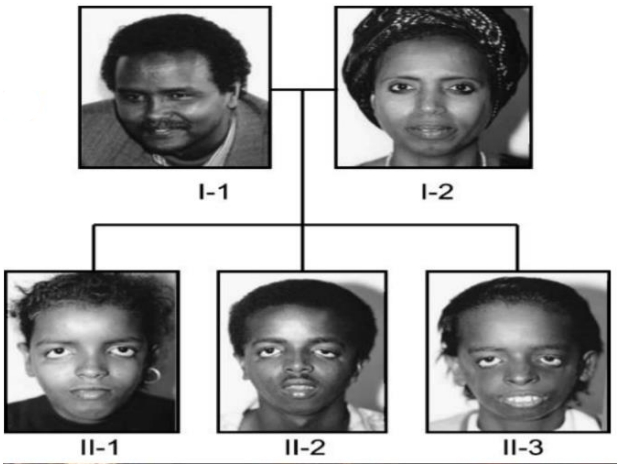
# Treacher Collins Syndrome

It's an **autosomal dominant** disorder of craniofacial development (pharyngeal arches 1, 2 and 3). It has a prevalence of 0.2–1/10,000, and more than 60% of cases **do not** appear to have a previous family history. Characterized by:

- External ear abnormalities (77%): including absent, small, and malformed ears (**microtia**) or rotated ears.
- External auditory canals atresia
- Impairment of the middle ear ossicles: which are formed embryologically from the **pharyngeal arches**, and this causes **conductive** hearing loss in 40% to 50% of patients.
- Facial anomalies: including lower eyelid abnormalities as **coloboma** (notching) of the lower eyelid, sparse & partially or totally absent cilia (lashes).
- Mandible hypoplasia & Zygomatic complex hypoplasia

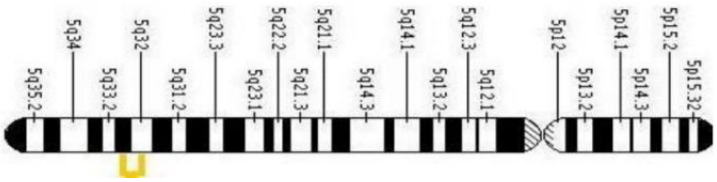
## Variability & Penetrance

TCS has **variable severity** and **incomplete penetrance**. This means that not all individuals carrying the dominant mutation will suffer from the syndrome, also the severity of the symptoms differs even between siblings. Notice in the example aside a child is showing the phenotype for TCS but its unclear phenotypically from which parent did he inherit the mutation.




So, an affected individual with both parents not showing the symptoms could be due to a **de novo mutation** in the germline (mutation doesn't exist in parents) or because of the **variable severity** of the syndrome or because of the **incomplete penetrance** of the syndrome

**40% of individuals diagnosed with TCS have an affected parent, 60% of probands with TCS have the disorder as the result of a de novo gene mutation.

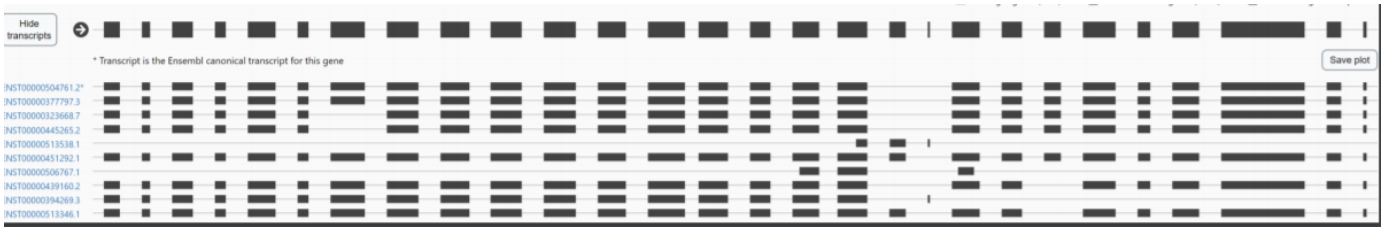



Three genes are known to be involved in TCS:

**TCOF1 | POLR1C | POLR1D**

Pathogenic variants in TCOF1 are responsible for about 80–85% of TCS cases with typical facial features, whereas POLR1C and POLR1D are involved in less than 10% (POLR involvement has recently been discovered)

TCOF1 is <u>located</u> on **chromosome 5** on the **q arm** at region 32 to 33(5q32-5q33) and <u>affects</u> the craniofacial complex development that arise from the neural crest in the first and the second branchial arches.

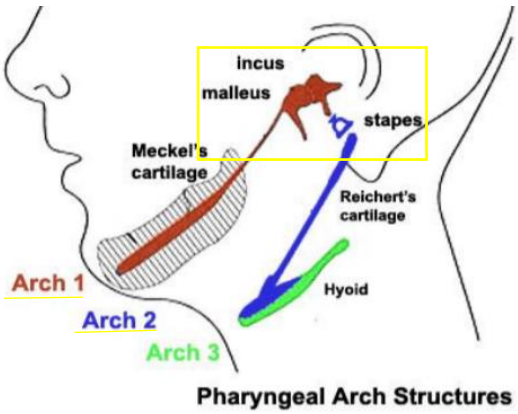


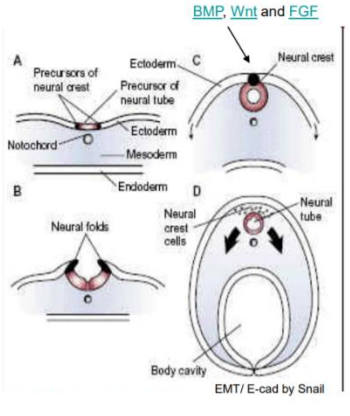

^This figure shows the **different mRNAs** that are transcribed from TCOF1 gene, so the gene codes for multiple proteins and this is achieved by **alternative splicing**.

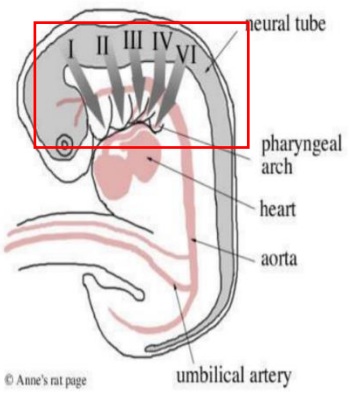
**Variants affecting TCOF1 could happen in coding regions or the boundaries between introns & exons, and you could check the gene's sequence using Sanger Sequencing.

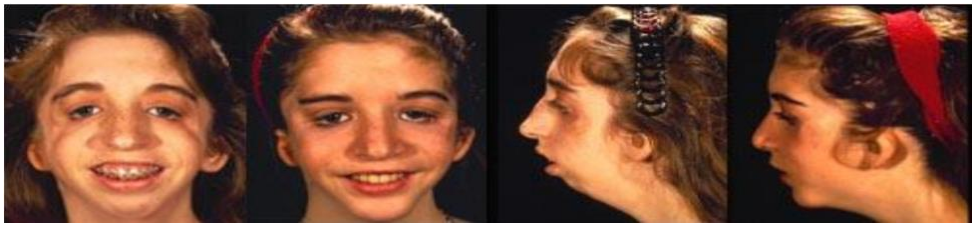
## <u>Pathology of TCS</u>

Pathogenic TCOF1 variants can reduce the number of neural crest cells (NCCs) and affect their migration to pharyngeal arches, which are needed for craniofacial embryological development and this causes facial abnormalities and middle ear hearing loss. Notice that the ossicles are derived from pharyngeal arches.



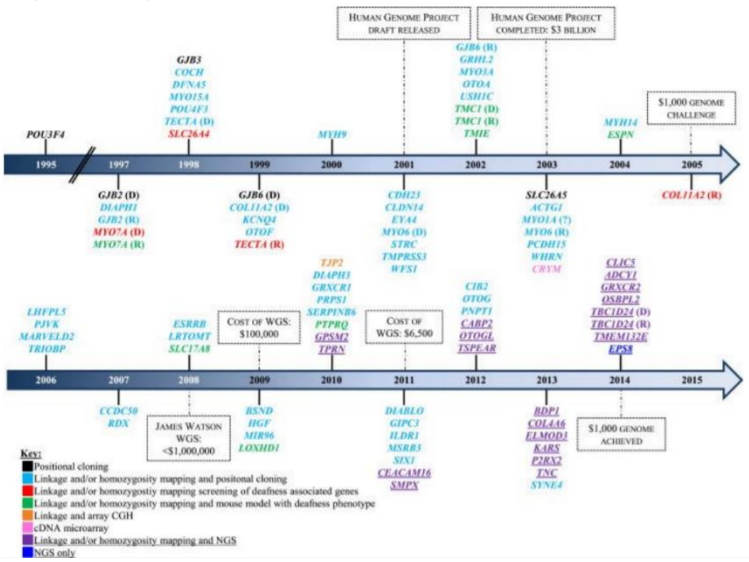



## B) Nonsyndromic hearing impairment (NSHL)

70% of the genetic causes of hearing loss are nonsyndromic which is mainly autosomal recessive. Around 100 genes can cause nonsyndromic hearing loss, and those genes have been discovered using different techniques which are expressed by the color key below.




-Positional cloning (old method)
-Linkage analysis (popular now)
-Next generation sequencing (NGS): very powerful as it could sequence the entire genome or used to sequence all the exons for all genes.

**Nonsyndromic hearing impairment** could be inherited in multiple manners:

1) Autosomal Recessive [DFNB1 -> DFNB84]

Most autosomal recessive loci cause **prelingual** bilateral severe to profound hearing loss. An exception is DFNB8, in which the hearing impairment is **postlingual** and rapidly progressive.

50% of DFNBs are DFNB1 so DFNB1 is **the most common subtype** of AR impairment.

2) Autosomal Dominant [DFNA1 -> DFNA51]

Most autosomal dominant loci cause **postlingual** hearing impairment. Some exceptions are DFNA3, DFNA8, DFNA12, and DFNA19 which cause **prelingual** impairment. There's no identifiable single gene responsible for most of the cases (unlike AR)

3) X-linked [DFNX1, DFNX2, DFNX3]

X-linked nonsyndromic hearing loss can be either **pre- or postlingual**, DFNX3 has **mixed** hearing loss.

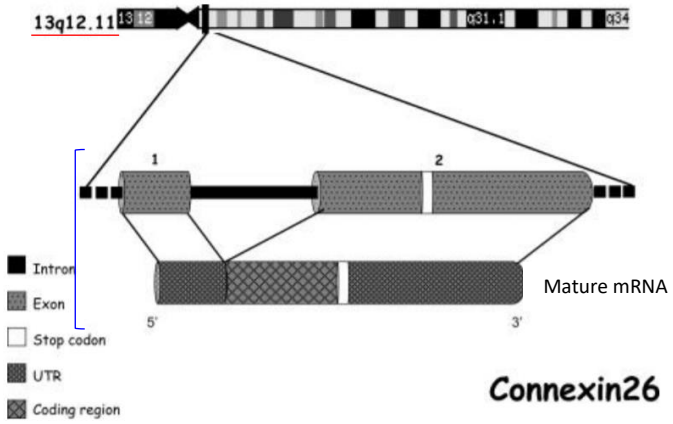
4) Mitochondrial [MT-RNR1, MT-TS1]

Now we will further discuss the subtype DFNB1 which revolves around the genes **GJB2** & **GJB6** that are familiar to us by now.

**GJB2** encodes gap junction beta 2 protein, known as **connexin26** which forms gap junctions between cells that transport potassium ions to maintain its correct level. Other research suggests that connexin 26 is required for the maturation of certain cells in the cochlea.
**GJB2 variants cause DFNB1 which is inherited in an autosomal recessive manner.

**GJB6** encodes gap junction beta 6 protein, known as **connexin30** and it participates in causing DFNB1.

GJB2 & GJB6 are located on the **q arm** of **chromosome 13**, regions 11 and 12 (same locus for both).




Zooming at GJB2, it consists of two exons and one intron on the genetic level. On the mRNA level, the entire exon 1 is the **5'UTR**. **3'UTR** is part of exon 2 and is preceded by a stop codon

**Exon 2 can have many variants, with some being more common than others

Clinically, DFNB1 is characterized by congenital, non-progressive, mild to profound sensorineural hearing impairment with no other associated medical findings.

98% of individuals with DFNB1 have **two** identifiable GJB2 mutations. They are either **homozygotes** for the mutation or **compound heterozygotes** with two different mutations on the same gene.

2% of individuals with DFNB1 have **one** identifiable GJB2 mutation and **one** of two **large deletions** that include a portion of GJB6. (**Double heterozygotes**)

Testing for GJB2 variants

**Sequence analysis** of the entire coding region detects both mutations in 98% of persons with DFNB1. Mutation screening for DFNB1 is not complete (not 100%) unless screening for the **splice site mutation** (exon 1 of GJB2) and screening for **large deletions** in GJB6 is included.

**Targeted mutation analysis** (looking for only one or several specific mutations) is generally not recommended because this type of analysis has an ethnic bias:

c.35delG mutation is most common in populations of northern European ancestry
c.167delT mutation is most common in the Ashkenazi Jewish population
c.235delC mutation is most common in the Japanese and Chinese populations

So, targeting those mutations which are common in other populations could **miss** detecting a mutation that isn't as common in other populations.

**Don't memorize the most common mutations mentioned above

Double heterozygotes are responsible for 2% of the etiology of DFNB1 subtype as mentioned above. Which means that we have **one** variant in GJB2 gene so its heterozygous, and another **one** variant (deletion) in GJB6 gene so its also heterozygous and that makes the **Double heterozygous**

For individuals suspected of having DFNB1: The first step in diagnosis is sequence analysis of GJB2 **exon 2**. If two deafness-causing mutations are identified, the diagnosis of DFNB1 is established. If one deafness-causing mutation is identified, deletion analysis for GJB6 deletions is done.

Mutations in GJB2 are mostly in exon 2, but when exon 1 is mutated it's a splice site mutation

Moving on to AD subtype DFNA3, it manifests clinically as **pre- or postlingual**, mild to profound, progressive sensorineural hearing impairment with no related systemic findings and with a family history of NSHL consistent with **autosomal dominant inheritance.**

Variants that cause DFNA3 are interestingly in genes **GJB2 (90%) & GJB6 (10%)** which are the same genes that were involved in the AR DFNB1.

**Sequence analysis** of both genes identifies:

- 10 substitution mutations in GJB2, any of which will cause DFNA3
- A couple of mutation in GJB6 that will also cause DFNA3

Take home message: depending on the **type of variant**, one variant in the GJB2 gene, for example, will cause AR mode of inheritance for the NSHL and a different mutation on the same GJB2 gene will lead to AD mode of inheritance. (same gene, different mutation)

For treatment of NSHL patients: appropriate hearing aids, enrolment into educational programs for the deaf, and considering cochlear implantation in case of profound HL.

Best of luck