

## **Molecular testing in non-syndromic hearing loss**

**Disease definition:** Normal hearing is defined as hearing thresholds between 0-20 dB across the 125-8000 Hz range. Hearing loss (HL) is defined as a loss of more than 20 dB. Most often the pure tone average (PTA) is calculated: average hearing sensitivity at 500, 1000 and 2000 Hz. It should closely match the speech reception threshold (SRT).

**Frequency:** HL is the most common sensory handicap. Prelingual HL affects at least 1 in 1000 newborns, whereas postlingual HL is much more common, and by the age of 80, 50% of octogenarians have at least mild HL.

**Main clinical symptoms:** Non-syndromic HL is not associated with additional signs and symptoms affecting other organ systems, but can be associated with vertigo, tinnitus, and anatomical anomalies of the middle and inner ear, most frequently enlarged vestibular aqueduct (EVA) and Mondini dysplasia.

**Inheritance:** At least 50% of prelingual HL is caused by monogenic mutation(s), whereas postlingual HL usually is multifactorial, although monogenic inheritance occurs in a minority of cases.

**Clinical diagnosis:** When prelingual hearing impairment is diagnosed, audiological assessment is indicated to evaluate the severity and type of hearing loss. In order to provide the best flow chart for molecular testing it is essential to:

- Define the type of hearing loss by audiological assessment (typically pure tone audiometry, alternatively ABR)
- Define the mode of inheritance by pedigree analysis
- Exclude syndromic hearing loss by :

1. Physical examination to exclude dysmorphic features
2. Eye fundus examination and electroretinogram (ERG) to exclude retinitis pigmentosa
3. Microscopic analysis of urine to exclude Alport syndrome
4. CT scan and/or MRI of the temporal region (including the cochlea) to exclude anatomical anomalies, like enlarged vestibular aqueduct, Mondini-like malformations, aplasia or hypoplasia of the cochlear nerve, etc. suggestive of Pendred syndrome or other forms of syndromic HL.

When postlingual hearing impairment is suspected, audiometric results should be plotted on age-related audiometric curves in order to exclude normal age-related HL (presbycusis).

**Clinical classification:** HL can be classified on clinical grounds as:

- Mild, moderate, severe, or profound
- Syndromic or non-syndromic
- Prelingual or postlingual
- Sensorineural, conductive or mixed
- Autosomal recessive, autosomal dominant, sex-linked, or mitochondrial inheritance

Hearing loss is considered mild if thresholds are between 20-40 dB, moderate if thresholds are between 40-60 dB, severe if thresholds are between 60-80 dB, and profound if thresholds are above 80 dB.

About 70% of prelingual and most postlingual HL is non-syndromic.

Monogenic prelingual HL usually is severe-to-profound, and non-progressive.

Postlingual inherited HL, in contrast, is usually progressive.

Nearly all monogenic forms of non-syndromic HL are sensorineural, with the sex-linked *POU3F4*-related HL a notable exception.

Eighty percent of prelingual monogenic HL is autosomal recessive, 15% is autosomal dominant, and 5% is sex-linked or mitochondrial. Postlingual monogenic HL usually is autosomal dominant.

**Molecular testing:** More than 100 loci and 46 nuclear genes have been implicated in non-syndromic HL. Loci are designated DFN (for DeaFNess) followed by a number indicating the chronological order of locus identification. **DFNA** numbers are given to autosomal dominant loci, **DFNB** numbers to autosomal recessive loci, **DFN(X)** numbers to X-linked loci, and **DFNY** to the single Y-linked locus. More than 10 mutations in mitochondrial DNA (mtDNA) have been reported to be associated with HL.

- **DFNA:** More than 21 genes have been shown to cause autosomal dominant HL, but their relative contribution is virtually unknown. The *KCNQ4* and *WFS1* genes are among the most prevalent genes involved. The phenotype caused by *WFS1* mutations is highly characteristic with upsloping audiometric pattern (low tone losses). *KCNQ4* mutations are common in Western Europe and lead to downsloping curves (high tone losses). Families in which HL and vertigo co-segregate may have mutations in the *COCH* gene. Also the *GJB2* gene should be analyzed in families with high tone losses. In families with autosomal dominant HL in the midfrequencies starting before the age of 10 years, the *TECTA* gene might be analyzed (Tables 1 and 2).
- **DFNB:** Although more than 24 genes have been shown to cause autosomal recessive HL, in developed countries more than 50% of neonates born with autosomal recessive severe-to-profound HL, have mutations in the *GJB2* gene. The frequency of various *GJB2* mutations is ethnically based, with the 35delG mutation being most frequent in persons of Southern European ancestry. Other genes commonly

implicated as a cause of autosomal recessive HL, include *GJB6*, *SLC26A4*, *CDH23*, *STRC*, and *OTOF*. *GJB6* encodes connexin 30 and should be analyzed in each patient heterozygous for a mutation in *GJB2*. *SLC26A4* should be analyzed if an inner ear anomaly (such as Mondini dysplasia or EVA) is present. The *OTOF* gene could be analyzed if auditory neuropathy is present, but the gene is quite large, and the test therefore expensive. A microarray with 200 mutations in *GJB2*, *GJB3*, *GJB6*, *SLC26A4*, *SLC26A5*, and mtDNA is available for diagnostic testing. Due to their large size, the *CDH23* and *STRC* genes are not really amenable to routine diagnostic testing.

In patients with severe-to-profound HL, where no mutation can be identified in the genes listed above, Usher syndrome should be excluded by an electroretinogram (in patients > 5 years) or molecular testing (in patients < 5 years). A microarray-based test of more than 400 mutations in all 8 Usher disease genes *MYO7A*, *CDH23*, *PCDH15*, *USH1C*, *USH1G*, *USH2A*, *GPR98*, and *USH3A* is available. This test detects 40-50% of mutations in Usher type 1, and 20-25% of type 2, the two types of Usher syndrome that are characterized by prelingual HL (Tables 1 and 2).

- **DFN(X):** The *POU3F4* gene has been implicated in about 50% of all X-linked non-syndromic HL. Anatomical anomalies of the inner ear are frequently present and include dilatation of the internal auditory meatus, deficient bone between the internal auditory meatus and the cochlea resulting in an abnormally wide fistulous communication, anomalies of the oval window and stapedial footplate, and partial hypoplasia of the cochlea called pseudo-Mondini dysplasia (Tables 1 and 2).
- **DFNY:** A single family with Y-linked HL has been described, but the disease causing gene has not yet been identified.

- **MtDNA:** Ten mutations in the *MTRNR1* gene (A1555G, 961delT/insC, T961G, T1095C, C1494T, A827G, T1005C, T1291C, T1243C, and A1116G) encoding 12S rRNA have been associated with HL. Several mutations in the *MTTS1* gene (A7445G, T7445C, 7472insC, T7510C, and T7511C) encoding the tRNA for Ser(UCN), have been associated with both syndromic (with palmoplantar keratoderma) and non-syndromic HL (Tables 1 and 2).
- **Testing in sporadic cases:** Mutations in the *GJB2* gene are thought to account for 10-30% of sporadic cases. In single cases or small families in which the mode of inheritance cannot be unambiguously determined, molecular genetic testing should be offered if the HL is prelingual; in such cases *GJB2*, *GJB6*, the mutation microarray, and the 10 *MTRNR1* mutations should be tested. If CT or MR imaging disclose temporal bone anomalies like Mondini dysplasia or EVA, *SLC26A4* should be analyzed. The 10 *MTRNR1* mutations could also be tested when the HL is postlingual (Tables 1 and 2).

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## Databases

[Http://webh01.ua.ac.be/hhh/](http://webh01.ua.ac.be/hhh/)

<http://life2.tau.ac.il/GeneDis/Tables/Deafness/deafness.html>

<http://hearing.harvard.edu/>

<http://www.boystownhospital.org/Hearing/info/genetics/index.asp>

Table 1. Different types of non-syndromic hearing loss with the proportion of the respective gene, its size, price indication, and test advise

Type	Specific Feature	Gene	Protein	Contribution	Number of Exons (AA)	Price Indication (Euro)*
<b>Autosomal dominant (DFNA)</b>		GJB2	Connexin 26	5 %	2 exons (226 AA)	200
	Menière	COCH	Cochlin	5-10 %	11 exons (550 AA)	950
	Low frequency losses	WFS1	Wolframin	5 %	8 exons (890 AA)	300
	High frequency losses	KCNQ4	K channel	5 %	14 exons (695 AA)	1000
	Mid frequency losses	TECTA	Alpha tectorin	< 3 %	23 exons ( 2155 AA)	1350
		16 other genes	Various	< 3 %	Various	Various
<b>Autosomal recessive (DFNB)</b>		GJB2	Connexin 26	50 %	2 exons (226 AA)	200
		GJB6	Connexin 30	5 %	3 exons (261 AA)	200
	Mondini, EVA, hypothyroidism	SLC26A4	Pendrin	5 %	21 exons (781 AA)	800
		CDH23	Cadherin-related 23	5 %	69 exons (3354 AA)	> 3000
		STRC	Stereocilin	5 %	29 exons (1775 AA)	> 2000
	Auditory neuropathy	OTOF	Otoferlin	3 %	48 exons (1997 AA)	1250
		18 other genes	Various	< 3 %	Various	Various
		GJB2, GJB3, GJB6, SLC26A4, SLC26A5, Mt DNA	Various	> 50 %	Mutation microarray	600
		MYO7A, CDH23, PCDH15, USH1C, USH1G, USH2A, MASS1, USH3A	Various		Mutation microarray	600

Table 1. Different types of non-syndromic hearing loss with the proportion of the respective gene, its size, price indication, and test advise (Continued)

<b>X-linked (DFNX)</b>	Stapes fixation/ Peri-lymphatic gusher	POU3F4	POU-domain transcription factor	50 %	1 exon (361 AA)	300
<b>Y-linked (DFNY)</b>		Not Identified	Not Identified			
<b>Mitochondrial</b>		MTRNR1	12S rRNA	< 50 %	954 bp	600
	Palmoplantar keratoderma	MTTS1	tRNA for Ser(UCN)		72 bp	300
<b>Sporadic</b>		GJB2	Connexin 26		2 exons (226 AA)	200
		GJB6	Connexin 30		3 exons (261 AA)	200
	Mondini, EVA, hypothyroidism	SLC26A4	Pendrin		21 exons (781 AA)	800
		GJB2, GJB3, GJB6, SLC26A4, SLC26A5, Mt DNA	Various		Mutation microarray	600
		MTRNR1	12S rRNA		954 bp	600

\*Prices were calculated according to market prices in US and Western Europe, taking into account the number of exons of each gene

Table 2. Guidelines for molecular testing in pre-and postlingual deafness

### Prelingual HL

- In general : GJB2  
GJB6  
Deafness microarray
- If Mondini / EVA anomaly, or TSH increase SLC26A
- If temporal bone anomaly or X-linked POU3F4
- If auditory neuropathy OTOF
- If matrilineal inheritance 10 MTRNR1 mutations
- If ectodermal dysplasia GJB6

### Postlingual HL

- If loss of high tones and AD inheritance KCNQ4
- If loss of low tones and AD inheritance WFS1
- If loss of mid tones and AD inheritanceTECTA1
- If mitochondrial inheritance 10 MTRNR1 mutations
- If vertigo COCH