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# Non-syndromic hearing loss: clinical and diagnostic challenges

<https://doi.org/10.1515/medgen-2020-2022>

Received December 20, 2019; accepted April 7, 2020

**Abstract:** Hereditary hearing loss is clinically and genetically heterogeneous. There are presently over 120 genes that have been associated with non-syndromic hearing loss and many more that are associated with syndromic forms. Despite an increasing number of genes that have been implemented into routine molecular genetic diagnostic testing, the diagnostic yield from European patient cohorts with hereditary hearing loss remains around the 50 % mark. This attests to the many gaps of knowledge the field is currently working toward resolving. It can be expected that many more genes await identification. However, it can also be expected, for example, that the mutational signatures of the known genes are still unclear, especially variants in non-coding or regulatory regions influencing gene expression. This review summarizes several challenges in the clinical and diagnostic setting for hereditary hearing loss with emphasis on syndromes that mimic non-syndromic forms of hearing loss in young children and other factors that heavily influence diagnostic rates. A molecular genetic diagnosis for patients with hearing loss opens several additional avenues, such as patient tailored selection of the best currently available treatment modalities, an understanding of the prognosis, and supporting family planning decisions. In the near future, a genetic diagnosis may enable patients to engage in preclinical trials for the development of therapeutics.

**Keywords:** clinical and genetic heterogeneity, hearing loss diagnostics, non-syndromic hearing loss, syndromic hearing loss

## Introduction

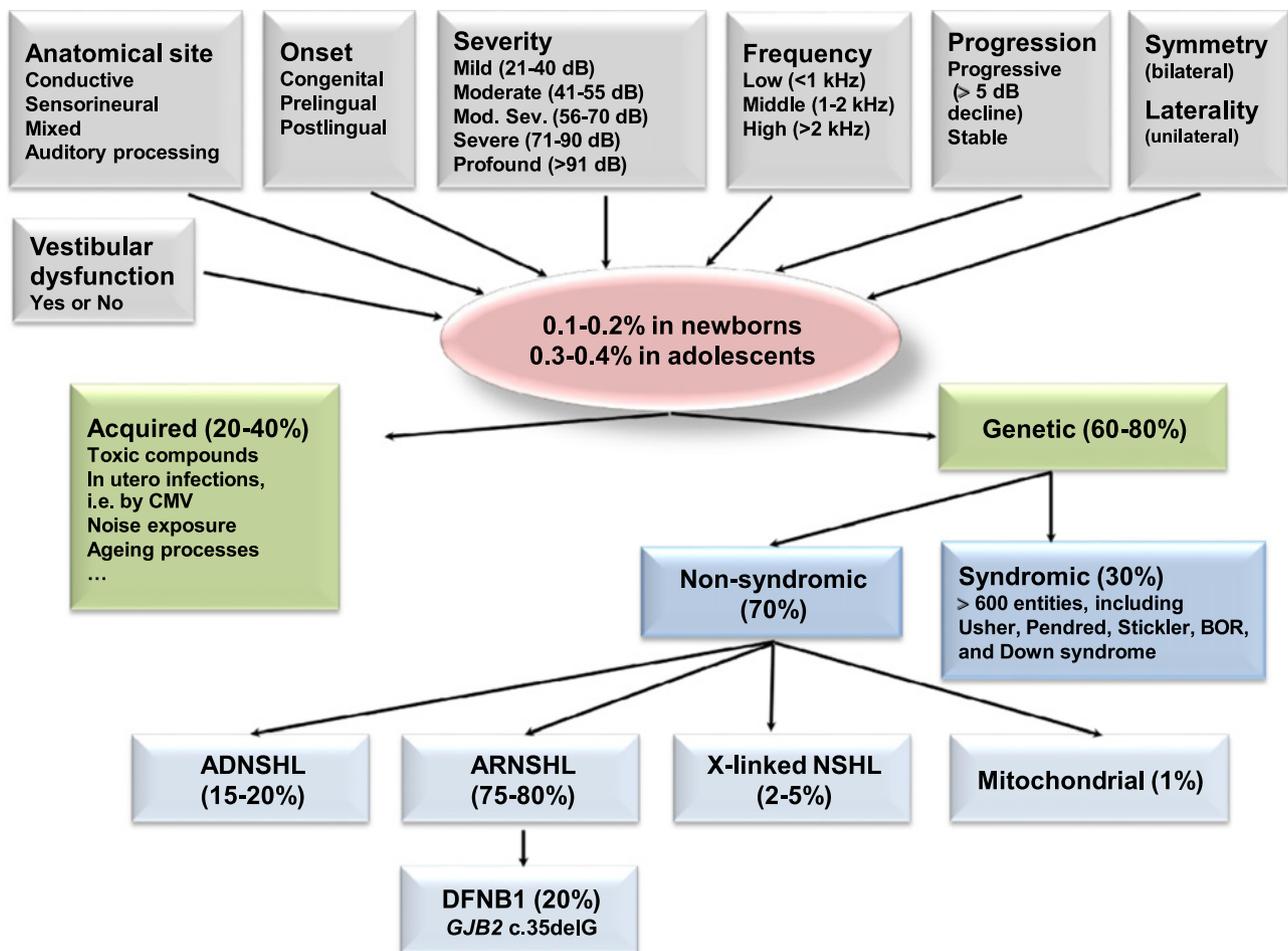
With an incidence of 1–2 in 1000 newborns, hearing impairment is one of the most common congenital disorders in humans [1, 2]. In populations with high rates of consanguinity, the incidence increases to up to 3–4 in 1000 [3]. Because hearing is important for language and cognitive development, untreated hearing loss (HL) leads to impaired cognitive functions and social competencies. To prevent this, many countries, including Germany, require newborn hearing testing by measuring otoacoustic emissions or auditory brainstem responses, which ideally is done during the first three days to one month of life [4–6]. Clinically, hearing impairment can be further classified by virtue of the affected anatomical site(s), age of onset, severity, affected frequencies, progression, symmetry/laterality, and presence/absence of vestibular dysfunction (Figure 1). Hearing impairment may be acquired, most importantly, by intrauterine (e. g., cytomegalovirus) infections, as well as toxic compounds (e. g., aminoglycoside antibiotics) and noise exposure. In developed countries, up to 80 % of congenital cases have a genetic basis [7, 8]. Hereditary forms of HL can be syndromic (30 %) with other organ system abnormalities or non-syndromic (70 %) with isolated HL. To date, approximately 600 syndromes have been associated with HL [9], including Usher, Pendred, Stickler, branchio-oto-renal, Down, and many others. The absence of additional symptoms in newborns or infants does not necessarily exclude syndromic forms, as HL may appear as the first manifestation in a long list of syndromes.

## Genetic heterogeneity of non-syndromic hearing loss (NSHL)

The auditory system is highly complex with many diverse and highly specialized cell types. Therefore, it is not unexpected that variants in a considerable number of genes interfere with normal hearing. To date, the Hereditary Hearing Loss Homepage (Van Camp G, Smith RJH, <https://hereditaryhearingloss.org>; last accessed November 2019) lists 46 autosomal dominant (DFNA), 76 autosomal recessive (DFNB), and 5 X-linked (DFNX) NSHL genes. There are

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**Figure 1:** Characteristics and clinical classifications used to describe hearing loss.

Abbreviations: ADNSHL = autosomal dominant non-syndromic hearing loss, ARNSHL = autosomal recessive non-syndromic hearing loss.

new genes discovered almost every month. Although exceptions exist, autosomal dominant NSHL is commonly described as progressive with a postlingual (>5 years of age) onset, whereas autosomal recessive NSHL is usually non-progressive, severe to profound with a congenital or prelingual (until 5 years of age) onset. Despite unparalleled genetic heterogeneity, recessive alleles in *GJB2* (DFNB1A) predominate (Figure 1). It is noteworthy that one particular pathogenic variant, c.35delG, accounts for up to 80% of recessive *GJB2* variants in European populations [10].

Different clinical outcomes can be evoked by variants in the same gene. For example, biallelic truncating variants in *GJB2* are generally associated with profound, compound heterozygous truncating and non-truncating variants with moderate, and biallelic non-truncating variants with mild hearing impairment [11]. Rare dominant variants in *GJB2* can also cause NSHL (DFNA3A), as well as several skin disorders (keratitis-ichthyosis-deafness syn-

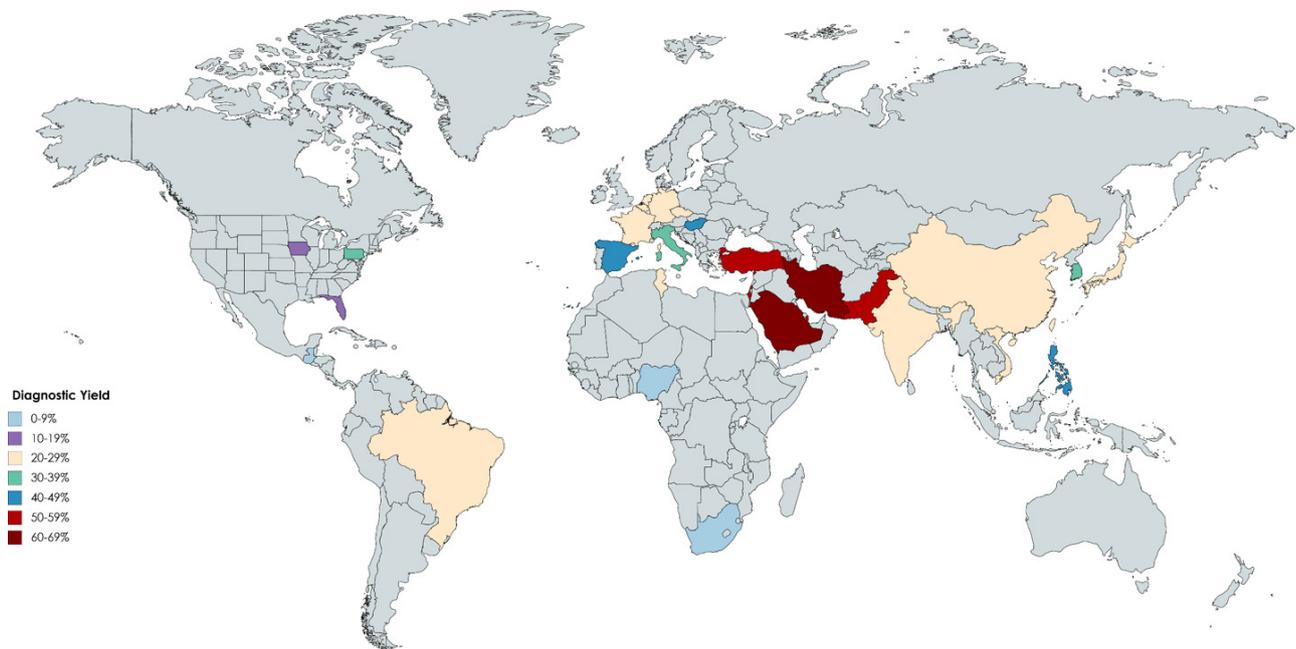
drome, palmoplantar keratoma with deafness, hystrix-like ichthyosis with deafness, and Vohwinkel syndrome).

Next generation sequencing (NGS) has greatly transformed the molecular diagnostics of heterogeneous Mendelian disorders, allowing rapid screening of large gene panels or the entire exome. In a diagnostic setting, we currently use exome sequencing and apply targeted gene analysis of 129 genes (Table 1), 13 of which have been associated with both autosomal dominant and autosomal recessive inheritance. Forty-nine genes have been associated with both NSHL and other genetic entities (Online Mendelian Inheritance in Man; <https://www.ncbi.nlm.nih.gov/omim/>). Consistent with other studies [13–15], our solve rate in *GJB2*-mutation negative Caucasian patients, the majority of them being sporadic cases, is 20%–30% (using different panels over the years), whereas in consanguineous families from Iran and Pakistan with two or more affected individuals, it is over 50% (Figure 2).

**Table 1:** Genes associated with non-syndromic hearing impairment.

| Gene <sup>1</sup> | DFN locus/ inheritance <sup>2</sup> | Gene <sup>1</sup> | DFN locus / inheritance <sup>2</sup> | Gene <sup>1</sup> | DFN locus / inheritance <sup>2</sup> |
|-------------------|-------------------------------------|-------------------|--------------------------------------|-------------------|--------------------------------------|
| <i>ABCC1</i>      | AD                                  | <i>GRAP</i>       | DFNB114                              | <i>PDZD7*</i>     | DFNB57                               |
| <i>ACTG1*</i>     | DFNA20/26                           | <i>GRHL2*</i>     | DFNA28                               | <i>PJVK</i>       | DFNB59                               |
| <i>ADCY1</i>      | DFNB44                              | <i>GRXCR1</i>     | DFNB25                               | <i>PLS1</i>       | AD                                   |
| <i>AIFM1*</i>     | DFNX5                               | <i>GRXCR2</i>     | DFNB101                              | <i>PNPT1*</i>     | DFNB70                               |
| <i>ATP2B2</i>     | Modifier; AD                        | <i>GSDME</i>      | DFNA5                                | <i>POU3F4</i>     | DFNX2                                |
| <i>BDP1</i>       | DFNB112                             | <i>HGF</i>        | DFNB39                               | <i>POU4F3</i>     | DFNA15                               |
| <i>BSND*</i>      | DFNB73                              | <i>HOMER2</i>     | DFNA68                               | <i>PIIP5K2</i>    | DFNB100                              |
| <i>CABP2</i>      | DFNB93                              | <i>IFNLR1</i>     | DFNA2C                               | <i>PRPS1*</i>     | DFNX1                                |
| <i>CCDC50</i>     | DFNA44                              | <i>ILDR1</i>      | DFNB42                               | <i>PTPRQ</i>      | DFNA73, DFNB84                       |
| <i>CD164</i>      | DFNA66                              | <i>KARS1*</i>     | DFNB89                               | <i>RDX</i>        | DFNB24                               |
| <i>CDC14A*</i>    | DFNB32                              | <i>KCNQ4</i>      | DFNA2A                               | <i>REST*</i>      | DFNA27                               |
| <i>CDH23*</i>     | DFNB12                              | <i>KITLG*</i>     | DFNA69                               | <i>ROR1</i>       | DFNB108                              |
| <i>CEACAM16</i>   | DFNA4B, DFNB113                     | <i>LHFPL5</i>     | DFNB66/67                            | <i>S1PR2</i>      | DFNB68                               |
| <i>CIB2</i>       | DFNB48                              | <i>LMX1A</i>      | DFNA7                                | <i>SERPINB6</i>   | DFNB91                               |
| <i>CLDN14*</i>    | DFNB29                              | <i>LOXHD1</i>     | DFNB77                               | <i>SIX1*</i>      | DFNA23                               |
| <i>CLDN9</i>      | AR                                  | <i>LRP5*</i>      | AR                                   | <i>SLC17A8</i>    | DFNA25                               |
| <i>CLIC5</i>      | DFNB103                             | <i>LRTOMT</i>     | DFNB63                               | <i>SLC22A4</i>    | DFNB60                               |
| <i>COCH</i>       | DFNA9/DFNB110                       | <i>MARVELD2</i>   | DFNB49                               | <i>SLC26A4*</i>   | DFNB4                                |
| <i>COL4A6</i>     | DFNX6                               | <i>MASP1*</i>     | AR                                   | <i>SLC26A5</i>    | DFNB61 [12]                          |
| <i>COL9A1*</i>    | AR                                  | <i>MCM2</i>       | DFNA70                               | <i>SLC44A4</i>    | DFNA72                               |
| <i>COL11A1*</i>   | DFNA37                              | <i>MET*</i>       | DFNB97                               | <i>SMPX</i>       | DFNX4                                |
| <i>COL11A2*</i>   | DFNA13, DFNB53                      | <i>METTL3</i>     | DFNM1, AD                            | <i>SPNS2</i>      | DFNB115                              |
| <i>CRYM</i>       | DFNA40                              | <i>MIR96</i>      | DFNA50                               | <i>STRC*</i>      | DFNB16                               |
| <i>DCDC2*</i>     | DFNB66                              | <i>MPZL2</i>      | DFNB111                              | <i>SYNE4</i>      | DFNB76                               |
| <i>DIABLO</i>     | DFNA64                              | <i>MSRB3</i>      | DFNB74                               | <i>TBC1D24*</i>   | DFNA65, DFNB86                       |
| <i>DIAPH1*</i>    | DFNA1                               | <i>MT-RNR1*</i>   | Mitochondrial                        | <i>TECTA</i>      | DFNA8/12, DFNB21                     |
| <i>DIAPH3</i>     | AUNA1, AD                           | <i>MT-TS1*</i>    | Mitochondrial                        | <i>TJP2*</i>      | DFNA51                               |
| <i>DMXL2*</i>     | DFNA71                              | <i>MYH14*</i>     | DFNA4A                               | <i>TMC1</i>       | DFNA36, DFNB7/11                     |
| <i>ELMOD3</i>     | DFNB88, AD                          | <i>MYH9*</i>      | DFNA17                               | <i>TMEM132E</i>   | DFNB99                               |
| <i>EPS8</i>       | DFNB102                             | <i>MYO15A</i>     | DFNB3                                | <i>TMIE</i>       | DFNB6                                |
| <i>EPS8L2</i>     | DFNB106                             | <i>MYO3A</i>      | AD, DFNB30                           | <i>TMPRSS3</i>    | DFNB8/10                             |
| <i>ESPN*</i>      | DFNB36                              | <i>MYO6*</i>      | DFNA22, DFNB37                       | <i>TMTC2</i>      | AD                                   |
| <i>ESRP1</i>      | DFNB109                             | <i>MYO7A*</i>     | DFNA11, DFNB2                        | <i>TOP2B</i>      | AD                                   |
| <i>ESRRB</i>      | DFNB35                              | <i>NARS2*</i>     | DFNB94                               | <i>TNC</i>        | DFNA56                               |
| <i>EYA4*</i>      | DFNA10                              | <i>NLRP3*</i>     | DFNA34                               | <i>TPRN</i>       | DFNB79                               |
| <i>RIPOR2</i>     | DFNB104, AD                         | <i>OSBPL2</i>     | DFNA67                               | <i>TRIOBP</i>     | DFNB28                               |
| <i>FOXF2</i>      | AR                                  | <i>OTOA</i>       | DFNB22                               | <i>TRRAP*</i>     | AD                                   |
| <i>GAB1</i>       | DFNB26                              | <i>OTOF</i>       | DFNB9                                | <i>TSPEAR*</i>    | DFNB98                               |
| <i>GIPC3</i>      | DFNB15/72/95                        | <i>OTOG</i>       | DFNB18B                              | <i>USH1C*</i>     | DFNB18A                              |
| <i>GJB2*</i>      | DFNA3A, DFNB1A                      | <i>OTOGL</i>      | DFNB84                               | <i>USH1G*</i>     | AR                                   |
| <i>GJB3*</i>      | DFNA2B, AR [12]                     | <i>P2RX2</i>      | DFNA41                               | <i>WBP2</i>       | DFNB107                              |
| <i>GJB6*</i>      | DFNA3B, DFNB1B                      | <i>PCDH15*</i>    | DFNB23                               | <i>WFS1*</i>      | DFNA6/14/38                          |
| <i>GPSM2*</i>     | DFNB82                              | <i>PDE1C</i>      | DFNA74                               | <i>WHRN*</i>      | DFNB31                               |

<sup>1</sup> Genes indicated by an asterisk (\*) are associated with different OMIM disease.<sup>2</sup> Abbreviations: DFNA = autosomal dominant locus, DFNB = autosomal recessive locus, DFNX = X-chromosomal locus; DFNM = modifier locus, AUNA = auditory neuropathy locus, AD = autosomal dominant gene; AR = autosomal recessive gene.



**Figure 2:** Worldwide diagnostic rates for HL that are attributed to NGS approaches, excluding *GJB2*. The unexplored genetic landscape for hereditary hearing loss is reflected by diagnostic yields less than 50 % for patients from European, African, North and South American, as well as a few Asian nations with data available. Some studies from the Middle East indicate diagnostic yields over 60 %. Created with mapchart.net [14, 16–30].

## Syndromes that mimic NSHL

One important challenge in exome and panel diagnostics is that a considerable proportion of children and sometimes adults who are clinically diagnosed with NSHL present with variants in genes associated with syndromic forms of HL. This is one challenge that must be specifically addressed in pre-NGS diagnostic genetic counseling. One obvious explanation is that additional features of a given syndrome are not yet present at the time when NSHL is diagnosed. For example, the retinitis pigmentosa in a child diagnosed with Usher syndrome or the goiter in Pendred syndrome may manifest many years after onset of hearing impairment. Many bona fide NSHL genes also have been associated with syndromic phenotypes, including Bartter (*BSND*), branchiootic (*SIX1*), Stickler (*COL11A1* and *COL11A2*), Usher (*CDH23*, *MYO7A*, *PCDH15*, *USH1G*, and *WHRN*), and other syndromes (Tables 1, 2). When identifying new pathogenic variants in these genes, it is not always possible to predict the phenotypic outcome, i. e., whether a child with NSHL will develop retinitis pigmentosa or not. Diagnostic screens of apparently NSHL patients [18, 31] will inevitably identify a growing number of clinically relevant undiagnosed syndromes, which initially mimic NSHL (Table 2). For example, we identified a

45 kb homozygous in-frame deletion in the gene *COL9A1* in two affected brothers with moderate to severe high-frequency HL. The index patient, who was last clinically evaluated at the age of 28 years, did not have any additional features of autosomal recessive Stickler syndrome. Although it is difficult to exclude a very mild, subclinical form of Stickler syndrome in this family, more likely and similar to *COL11A1* and *COL11A2*, *COL9A1* may also qualify as a bona fide NSHL gene [32]. In a sporadic case of NSHL in a consanguineous family, we identified a homozygous nonsense mutation in *KCNQ1*, which has been associated with Jervell–Lange–Nielsen syndrome (Table 2). This molecular diagnosis in a patient with isolated HL has immediate consequences for genetic counseling and prevention of sudden cardiac death. In prepubertal children, we regularly find syndromic forms of HL, which are associated with male (due to biallelic *STRC-CATSPER2* deletions in deafness-infertility syndrome or *CDC14A* variants in hearing impairment infertile male syndrome) or female infertility (Perrault syndrome) later in life (Table 2). In our experience, up to 30 % of *GJB2*-mutation negative children who are clinically identified as having NSHL and receive a NGS diagnosis consistent with HL exhibit pathogenic variants in syndrome-associated genes.

**Table 2:** Syndromic genes which can mimic NSHL.

| Gene <sup>1</sup> | Inheritance | OMIM <sup>2</sup> |  |
|-------------------|-------------|-------------------|--|
| <i>COL4A5</i>     | X-linked    | # 301050          | Alport 1   |
| <i>COL4A4</i>     | AR          | # 203780          | Alport 2   |
| <i>COL4A3</i>     | AR          | # 203780          | Alport 2   |
|                   | AD          | # 104200          | Alport 3   |
| <i>BSND*</i>      | AR          | # 602522          | Bartter 4A   |
| <i>SIX1*</i>      | AD          | # 608389          | Branchiootic 3   |
| <i>ACTG1*</i>     | AD          | # 614583          | Baraitser-Winter 2   |
| <i>CHD7</i>       | AD          | # 214800          | CHARGE   |
|                   | AD          | # 612370          | Hypogonadotropic hypogonadism 5 with/without anosmia                   |
| <i>FGF3</i>       | AR          | # 610706          | Congenital deafness with inner ear agenesis, microtia, and microdontia |
| <i>PSIP1</i>      | AD          | [4]               | Deafness and optic neuropathy  |
| <i>SLITRK6</i>    | AR          | # 221200          | Deafness and myopia  |
| <i>FITM2</i>      | AR          | # 618635          | Siddiqi  |
| <i>GPRASP2</i>    | X-linked    | [33]              | Hearing loss with inner ear abnormalities and facial dysmorphism       |
| <i>SLC7A8</i>     | AR          | [34]              | Age-related hearing loss; damage of cochlear structures                |
| <i>CDC14A*</i>    | AR          | # 608653          | DFNB32, with or without immotile sperm                                 |
| <i>PEX1</i>       | AR          | # 234580          | Heimler 1  |
|                   |             | # 214100          | Peroxisome biogenesis disorder 1A and 1B                               |
|                   |             | # 601539          |  |
| <i>PEX6</i>       | AR/AD       | # 616617          | Heimler 2  |
|                   |             | # 614862          | Peroxisome biogenesis disorder 4A and 4B                               |
|                   |             | # 614863          |  |
| <i>PEX26</i>      | AR          | #614873           | Peroxisome biogenesis disorder 7B<br>Heimler                           |
| <i>GATA3</i>      | AD          | # 146255          | Hypoparathyroidism, sensorineural deafness, and renal dysplasia        |
| <i>KCNQ1</i>      | AR          | # 220400          | Jervell–Lange-Nielsen  |
|                   | AD          | # 192500          | Long QT 1  |
| <i>KCNE1</i>      | AR          | # 612347          | Jervell-Lange-Nielsen  |
|                   | AD          | # 613695          | Long QT 5  |
| <i>KMT2D</i>      | AD          | # 147920          | Kabuki 1   |
| <i>PTPN11</i>     | AD          | # 151100          | LEOPARD 1  |
|                   |             | # 153950          | Noonan 1   |
| <i>FOXI1</i>      | AR          | # 600791          | Enlarged vestibular aqueduct (Pendred)                                 |
| <i>KCNJ10</i>     | AR          | # 600791          | Enlarged vestibular aqueduct, digenic (Pendred)                        |
|                   |             | # 612780          | SESAME   |
| <i>SERPINF1</i>   | AR          | # 613982          | Osteogenesis imperfecta VI   |
| <i>HSD17B4</i>    | AR          | # 233400          | Perrault 1   |
|                   |             | # 261515          | D-bifunctional protein deficiency                                      |
| <i>HARS2</i>      | AR          | # 614926          | Perrault 2   |
| <i>CLPP</i>       | AR          | # 614129          | Perrault 3   |
| <i>LARS2</i>      | AR          | # 615300          | Perrault 4   |
| <i>TWNK</i>       | AR          | # 616138          | Perrault 5   |
| <i>ERAL1</i>      | AR          | # 617565          | Perrault 6   |
| <i>SGO2</i>       | AR          | [35]              | Perrault   |
| <i>PHYH</i>       | AR          | # 266500          | Refsum   |
| <i>RAI1</i>       | AD          | # 182290          | Smith Magenis  |
| <i>COL2A1</i>     | AD          | # 108300          | Stickler 1   |
|                   |             | # 156550          | Kniest dysplasia   |
|                   |             | # 271700          | Spondyloperipheral dysplasia   |
|                   |             | # 132450          | Epiphyseal dysplasia multiple with myopia and deafness                 |
| <i>COL11A1*</i>   | AD          | # 604841          | Stickler 2   |
|                   | AR          | # 228520          | Fibrochondrogenesis 1  |
|                   |             | # 154780          | Marshall   |

Table 2: (continued)

| Gene <sup>1</sup> | Inheritance | OMIM <sup>2</sup> |  |
|-------------------|-------------|-------------------|--|
| <i>COL11A2*</i>   | AR/AD       | # 614524          | Fibrochondrogenesis 2  |
|                   | AD          | # 184840          | Otospondylomegaepiphyseal dysplasia                                      |
|                   | AR          | # 215150          | Otospondylomegaepiphyseal dysplasia                                      |
| <i>COL9A1*</i>    | AR          | # 614134          | Stickler 4   |
|                   | AD          | # 614135          | Epiphyseal dysplasia, multiple, 6  |
| <i>COL9A2</i>     | AR          | # 614284          | Stickler 5   |
|                   | AD          | # 600204          | Epiphyseal dysplasia, multiple, 2  |
| <i>COL9A3</i>     | AD          | # 600969          | Epiphyseal dysplasia, multiple, 3, with or without myopathy              |
| <i>ABHD12</i>     | AR          | # 612674          | Polyneuropathy, hearing loss, ataxia, retinitis pigmentosa, and cataract |
| <i>MYO7A*</i>     | AR          | # 276900          | Usher 1B   |
| <i>CDH23*</i>     | AR          | # 601067          | Usher 1D, 1D/F digenic   |
| <i>PCDH15*</i>    | AR          | # 602083          | Usher 1F   |
|                   | AR          | # 601067          | Usher 1D/F digenic   |
| <i>USH1G*</i>     | AR          | # 606943          | Usher 1G   |
| <i>CIB2</i>       | AR          | # 614869          | Usher 1J, refuted by [36]  |
| <i>WHRN*</i>      | AR          | # 611383          | Usher 2D   |
| <i>ADGRV1</i>     | AR          | # 605472          | Usher 2C   |
| <i>USH2A</i>      | AR          | # 276901          | Usher 2A   |
| <i>CLRN1</i>      | AR          | # 276902          | Usher 3A   |
| <i>HARS</i>       | AR          | # 614504          | Usher 3B, refuted by [37]  |
|                   | AD          | # 616625          | Charcot–Marie–Tooth, axonal, 2W  |
| <i>ARSG</i>       | AR          | # 618144          | Usher-like-4   |
| <i>CEP78</i>      | AR          | # 617236          | Cone-rod dystrophy and hearing loss                                      |
| <i>CEP250</i>     | AR          | # 618358          | Cone-rod dystrophy and hearing loss 2                                    |
| <i>PAX3</i>       | AD          | # 193500          | Waardenburg 1  |
|                   | AR/AD       | # 148820          | Waardenburg 3  |
|                   | AD          | # 122880          | Craniofacial-deafness-hand   |
| <i>MITF</i>       | AD          | # 193510          | Waardenburg 2A   |
|                   | AD          | # 103500          | Tietz-albinism-deafness  |
|                   | AR          | # 617306          | COMMAD   |
|                   | AD          | # 103470          | Waardenburg syndrome/ocular albinism, digenic                            |
| <i>SNAI2</i>      | AR          | # 608890          | Waardenburg 2D   |
|                   | AD          | # 172800          | Piebaldism   |
| <i>EDNRB</i>      | AR/AD       | # 277580          | Waardenburg 4A   |
|                   | AR          | # 600501          | ABCD   |
| <i>EDN3</i>       | AR/AD       | # 613265          | Waardenburg 4B   |
| <i>SOX10</i>      | AD          | # 613266          | Waardenburg 4C   |
|                   | AD          | # 611584          | Waardenburg 2E, with/without neurologic involvement                      |
|                   |             | # 609136          | PCWH   |
| <i>CISD2</i>      | AR          | # 604928          | Wolfram 2  |

<sup>1</sup> Genes indicated by an asterisk (\*) are associated with both NSHL and a syndromic phenotype(s).

<sup>2</sup> For gene-associated syndromic phenotypes without OMIM entries (#) a literature reference is given.

## Genetic diagnostics: past and present

### Diagnostic testing in the pre-NGS era

Genetic testing for HL has been performed since the late 1990s and was limited due to the low-throughput nature of Sanger sequencing or variant-specific screening using re-

striction fragment length polymorphism (RFLP) reactions [7]. Considering the extremely heterogeneous nature of NSHL, *GJB2* screening yielded an unexpectedly high diagnostic rate. Furthermore, the single-coding exon gene was simple to screen and could be easily analyzed using direct sequencing or RFLP testing. Genetic evaluation of *GJB2* in multiple large German hearing-impaired cohorts has shown diagnostic yields ranging from 17% [38] to 31% [39]. Two additional studies in German pa-

tients returned diagnostic yields of 21% [11] and 22% [40]. Following *GJB2* screening in the majority of undiagnosed patients, subsequent gene prioritization was difficult. Several groups tried to address this limitation in the pre-NGS era by developing more-inclusive resequencing arrays and single-primer extension microarrays [41, 42]. However, these methods were limited to previously identified pathogenic variants and did not provide an unbiased screening approach for all deafness genes [7]. Other groups developed Sanger sequencing assays for targeted sequencing of candidate genes based on clinical suspicion of a syndrome. This has been successfully performed in instances of Pendred syndrome in a series of German families with sensorineural HL and goiter [43], among other clinically distinct syndromes.

### State-of-the-art next generation diagnostics

Because *GJB2* is a single-coding exon gene accounting for roughly 20% of NSHL (DFNB1), Sanger sequencing and MLPA testing are still recommended as a first-line exclusionary diagnostic test prior to NGS testing [50]. In children with mild to moderate high-frequency HL, homozygous or compound heterozygous (together with a genetic point variant) deletions involving *STRC* (DFNB16) are a frequent cause of NSHL in 5%–10% of patients [51]. Because *STRC* and a pseudogene with 99.6% identical coding sequence reside in a tandemly duplicated region, one cannot rely on NGS for copy number variations (CNVs) and sequence analysis. Therefore, we developed a Sanger sequencing method with pseudogene exclusion for targeted analysis of *STRC* [52]. In Caucasian patients, DFNB1 and DFNB16 should be excluded before NGS analysis [50].

Similar to other molecular genetic testing approaches, diagnostic testing for HL takes the form of gene panels that usually contain over 100 genes, or whole-exome or whole-genome sequencing with subsequent targeted data analysis. Using whole-exome sequencing and targeted gene analysis, 20%–30% of the *GJB2* and *STRC* mutation-negative patients in our diagnostic cohort received a molecular diagnosis. In a conceptually related larger screening study [14], variants in eight genes, including *GJB2* (9% of cases), *STRC* (6%), *SLC26A4* (3%), *TECTA* (2%), *MYO15A* (2%), *MYO7A* (2%), *USH2A* (2%), and *CDH23* (2%) accounted for 296 (27%) of 1119 patients. The overall solve rate with a 66 and 89 deafness-associated gene panel identified pathogenic variants in 49 genes, accounting for a diagnostic rate of 39%. When comparing the most frequently implicated genes in HL diagnoses across several different studies, it appears that the most

frequently involved genes are recurrent in several populations (Table 3) and that roughly half of diagnoses can be explained by pathogenic variants in five to ten genes.

Because of the smooth transition between non-syndromic and syndromic HL, it is important to genetically counsel patients before initiating NGS diagnostics and to clarify whether defined in silico gene panels for the most frequent forms of autosomal dominant or recessive NSHL or larger panels, including all NSHL genes, syndromic genes mimicking NSHL, a larger number of candidate genes, and/or newly identified genes with limited clinical validity, should be analyzed and if so, how variants that may cause phenotypes other than NSHL should be addressed. Ideally, children and, if conspicuous, also their parents should be evaluated by a clinical geneticist or expert in dysmorphology before testing.

### Factors that influence diagnostic rates in HL patients

#### Patient ethnicity

Diagnostic rates for NSHL are currently the highest for patients from Middle Eastern countries (Figure 2). This is largely attributed to high rates of consanguinity that not only enhance diagnostic screening in NSHL, a predominantly autosomal recessive disorder, but also make these populations appealing for research studies. Although molecular genetic studies in NSHL patients from African and South American countries are sparse in the literature, it appears that a diverse molecular landscape unlike what has been observed in other populations can be expected. For example, while pathogenic variants in the gene *GJB2* are common in most ethnic groups around the world, they appear to be rare in native African populations [53].

#### Hearing loss laterality

HL can manifest as bilateral, asymmetric, or unilateral. A genetic diagnosis is significantly more likely to be achieved in patients with bilateral forms of HL (44%) compared to asymmetric (22%) or unilateral (2%) [14].

#### Age at hearing loss diagnosis

There is a negative correlation between the age of HL onset in patients in whom environmental factors have been excluded and the diagnostic yield from molecular diagnostic testing. In a study reporting on the diagnostic yield of 1119 hearing-impaired patients, the authors correlated the age

**Table 3:** Genes frequently mutated in hearing loss according to diagnostics in the respective populations.

| Population     | European                      |             |        |         |                   |                | Asian |       | Middle Eastern |          |        |
|----------------|-------------------------------|-------------|--------|---------|-------------------|----------------|-------|-------|----------------|----------|--------|
| Reference      | Unpublished data <sup>1</sup> | [30]        | [17]   | [26]    | [18] <sup>2</sup> | [44]           | [45]  | [46]  | [47]           | [48]     | [49]   |
| Country        | Germany                       | Netherlands | France | Belgium | Spain             | Czech Republic | Japan | Korea | Iran           | Pakistan | Turkey |
| Patients (N)   | 213                           | 200         | 207    | 131     | 50                | 51             | 1025  | 32    | 302            | 321      | 29     |
| <i>GJB2</i>    | 1st                           | 1st         | 1st    | 1st     | 1st               | 1st            | 1st   | 1st   |                | 3rd      | 1st    |
| <i>STRC</i>    | 2nd                           | 3rd         | 2nd    |         | 3rd               | 2nd            | 5th   |       |                |          |        |
| <i>MYO15A</i>  | 4th                           | 5th         | 4th    | 2nd     |                   | 2nd            |       |       | 2nd            |          |        |
| <i>MYO7A</i>   |                               | 4th         | 3rd    | 2nd     |                   |                |       |       | 3rd            | 2nd      | 2nd    |
| <i>USH2A</i>   |                               | 2nd         |        |         | 5th               | 2nd            |       |       |                |          |        |
| <i>OTOA</i>    |                               |             | 5th    |         |                   |                |       |       |                |          |        |
| <i>CDH23</i>   | 5th <sup>3</sup>              | 4th         |        |         |                   |                | 3rd   |       | 4th            |          |        |
| <i>TECTA</i>   |                               |             | 4th    |         | 4th               |                |       |       |                |          |        |
| <i>TMC1</i>    |                               |             |        | 2nd     |                   |                |       |       |                |          | 3rd    |
| <i>SLC26A4</i> | 3rd                           |             |        |         |                   |                | 2nd   | 2nd   | 1st            | 1st      |        |
| <i>KCNQ4</i>   |                               |             |        |         |                   |                | 4th   |       |                |          |        |
| <i>TMPRSS3</i> |                               |             |        |         |                   | 2nd            |       |       |                |          |        |
| <i>PCDH15</i>  | 5th <sup>3</sup>              |             |        |         | 2nd               |                |       |       | 5th            |          |        |
| <i>LOXHD1</i>  | 5th <sup>3</sup>              |             |        |         | 2nd               |                |       |       |                |          |        |
| <i>POU3F4</i>  |                               |             |        |         |                   |                |       | 3rd   |                |          |        |
| <i>MYO6</i>    |                               |             |        |         |                   |                |       | 4th   |                |          |        |
| <i>COCH</i>    |                               |             |        |         |                   |                |       | 4th   |                |          |        |
| <i>CIB2</i>    |                               |             |        |         |                   |                |       |       |                | 4th      |        |
| <i>HGF</i>     |                               |             |        |         |                   |                |       |       |                | 5th      |        |
| <i>GJB6</i>    | 5th <sup>3</sup>              |             |        |         | 1st               |                |       |       |                |          |        |
| <i>ACTG1</i>   |                               |             |        |         | 2nd               |                |       |       |                |          |        |

<sup>1</sup>Diagnostic cases (index patients) from Würzburg.

<sup>2</sup>Personal communication to clarify the lower than expected prevalence of *OTOA* in the Spanish population.

<sup>3</sup>Genes showing pathogenic mutations in 1 of 213 index patients.

of onset with diagnostic yield [14]. They observed a remarkable reduction in the diagnostic yield when comparing patients with a congenital onset (45%), patients diagnosed with a childhood onset (30%), and patients with an onset in adulthood (28%). These findings were replicated in a Dutch cohort of 200 patients who were grouped according to congenital onset (50%) or onset in the first (38%) or second (20%) decade of life [30].

### Other considerations in HL diagnostics

With approximately one out of every two people by the age of 75 years affected, age-related HL (presbycusis) is one of the most common conditions interfering with the quality of life in elderly populations [54]. Although age-related HL is generally thought to be multifactorial, resulting from a complex interplay of genetic susceptibility and adverse environmental (e.g., noise) and systemic (e.g., diabetes) factors, it is plausible to assume that a proportion of cases represent mild forms of autosomal dominant NSHL with very late onset. Age-related HL shows a

strong familial aggregation [55]. However, despite its high heritability, the genetic risk factors are largely unknown. A recent genome-wide association study for self-reported presbycusis phenotypes in 250,000 volunteers (between 40 and 69 years) identified 44 significant trait loci [56], including genes, e.g., *CLRN2*, which also has been associated with NSHL [57, 58]. Moreover, several genes, including *COL1A1* and *TGFB1*, have been associated with otosclerosis, one of the more common forms of adult-onset (conductive) HL [59]. The indigenous Filipino population has a 50% prevalence of middle ear infections which can cause HL. Apart from environmental factors such as smoking and swimming, several gene variants, i.e., in *FUT2* and *A2ML1*, were shown to confer susceptibility to familial otitis media [60]. Similar to the overlap between syndromic and non-syndromic HL genes, there may be an overlap between the many mainly monogenic forms of early onset HL and multifactorial age-related HL. For example, genes associated with presbycusis may act as modifiers for NSHL, and vice versa, variants in NSHL genes may predispose to presbycusis.

## Limitations of NGS

The biggest challenge in NGS diagnostics is the classification of numerous variants by various bioinformatic filtering strategies (e. g., by minor allele frequency) and prioritization of potentially pathogenic variants by manual interpretation. The Deafness Variation Database (DVD; <http://deafnessvariationdatabase.org>) provides information on >8000 pathogenic or likely pathogenic, >170,000 benign or likely benign, and approximately 700,000 variants of unclear significance (VUS) in 152 HL genes [61]. In diagnostics, new VUS are detected, even in well-studied genes. Moreover, >1000 variants have been re-classified in the past decade through expert variant curation according to the DVD. CNVs, most frequently in *STRC* (10 % of cases), *OTOA* (1 %), *GJB6* (0.5 %), *USH2A*, *MYH9*, *SLC26A4*, *TMC1*, and *TMPRSS3*, contribute to approximately 20 % of diagnoses [13]. Due to non-uniform sequence enrichment and low coverage, a substantial number of CNVs may remain undetected by exome analysis alone. Switching molecular diagnostics from whole-exome to whole-genome analysis may overcome this problem.

The many undiagnosed cases, the broad range of auditory pathophysiology, and systematic mouse screens [62] all suggest that a considerable number that is on the scale of several hundred HL genes remain to be elucidated. In addition, many pathogenic variants affecting known deafness genes may remain undetected using current diagnostic algorithms, because they reside in non-coding (intronic and regulatory) sequences or unannotated exons. Considering the many problems with classifying clinically relevant variants in coding and adjacent non-coding sequences, our understanding of the functional consequences of genetic changes outside the exome is even worse. Even if variants in these regions of genes are detected, our capacity for interpretation of these variants is extremely limited and requires experimental support. The integration of RNA sequencing in diagnostics may uncover the transcriptional consequences (reduced expression, aberrant splicing, mono-allelic expression) of genetic changes in genes with expression that is not limited to the inner ear, providing a potential avenue for improvement of variant prioritization and interpretation. Thus, the diagnostic yield should be improved by combined genomic and transcriptomic approaches, at least for disease genes and regulatory RNAs expressed in blood [63]. So far, DFNA50 is the only form of NSHL caused by variants in a regulatory RNA, *MIR96* [64].

The human inner ear may be the least accessible organ for gene expression analyses. The SHIELD database (<https://shield.hms.harvard.edu>) provides gene expression data

for many cell types in the mouse inner ear, but typically for only two embryonic and four postnatal developmental time points [65]. This resource is particularly useful for the prioritization of new candidate genes, many of which are found in only a single patient or family.

At least in former times, assortative mating was common among hearing-impaired individuals. This may explain the enrichment of potentially pathogenic variants in different HL genes in individuals/families with NSHL [66]. Many current diagnostic reporting approaches only consider pathogenic or likely pathogenic dominant or biallelic recessive variants as disease-causing in a given patient and neglect the four or five variants (mainly recessive or VUS) in other HL-associated genes which are often also present. So far, there are few examples for digenic inheritance of HL, including *CDH23/PCDH15* [67]. However, one such example of digenism, *TMPRSS3/GJB2*, has recently been refuted in a study presenting three families with discordant segregation of pathogenic variants in each of the two genes [68]. This study highlighted how overinterpretation of NGS data in families of limited size may lead to erroneous associations. A modifier variant in *METTL3* suppresses DFNB26 due to biallelic variants in *GAB1* [69]. Since such complex disease etiologies are difficult to unravel, their role in HL may be largely underestimated, accounting for the highly variable expressivity and reduced penetrance of NSHL within families.

## Do patients benefit from molecular diagnoses?

To date, comprehensive genetic testing for deafness leads to a diagnosis in approximately 50 % of patients. This removes uncertainty, e. g., whether hearing impairment is genetic or acquired by congenital infection. In many instances, the molecular diagnosis has important implications for treatment (e. g., hearing aids vs cochlear implants [CIs]), individual management (e. g., of additional symptoms of a syndromic disease), prognosis (progressive or stable HL), and family planning. Although cochlear implantation is the treatment of choice for children with severe to profound and/or progressive HL, the outcomes can vary considerably between different genetic forms. Recent research has suggested that patients with variants in *PCDH15* (DFNB23) and *PJVK* (DFNB57) have poor post-implantation CI outcomes, whereas patients with variants in *GJB2* (DFNB1) and *SLC26A4* (DFNB4) have good CI outcomes [70, 71]. Patients with inner ear malformations, e. g., due to variants in *POU3F4* (DFNX2), have an increased risk

for sudden loss of perilymph fluid (Gusher phenomenon) during cochlear implantation [72] or stapes surgery [73].

In murine models, a variety of gene therapy approaches using adeno-associated viral vectors for gene delivery, antisense oligonucleotides, or injection of genome editing agents into the cochlea have already yielded promising results for *Tmc1* [74, 75], *Otof* [76], *Slc17a8* [77], *Ush1c* [78], *Ush1g* [79], and *Whrn* [80]. According to the US National Library of Medicine (<https://clinicaltrials.gov>) several clinical trials using drug and gene therapies for treating HL are underway or in planning stages for 2020. Although initiating therapies in babies with HL already present at birth will be challenging, as there is already damage to the inner ear, it may be more realistic to initiate therapies for genes with a delayed disease onset and a greater therapeutic window to reduce progression of inner ear damage. In principle, NGS technologies enable comprehensive screening of newborns/children before manifestation or severe progression of sensorineural HL and, thus, prevention and management of HL by classical, and maybe in the future, gene-targeted therapies [8].

## Conclusions

Unraveling the genetic diagnosis of HL is important to guide genetic counseling, to support prognostic outcomes and decisions with currently available treatment modalities (e. g., hearing aids versus CIs), and for patient eligibility in future therapy trials. A genetic diagnosis can provide tremendous value by diagnosing syndromes in patients who are pre-symptomatic, allowing for patients to be under the care of respective specialists before symptoms are present. Many challenges remain with respect to obtaining higher diagnostic yields, which may mean that variants in not yet discovered genes or those that reside in regions that are challenging to interpret need to be addressed by the field.

## Bullet points for clinical practice

- Genetic testing for HL should be the next test to follow a clinical diagnosis of HL by audiometric testing.
- *GJB2* and *STRC* are highly valuable to pre-screen in patients of European ethnicity before NGS testing is initiated.
- Genetic testing can potentially uncover sub-clinical or pre-symptomatic syndromes in patients, which may

be powerful for timely interventions with other medical specialists. This must be addressed in pre-NGS diagnostic counseling.

- The achievement of a diagnosis is heavily influenced by several aspects such as patient ethnicity, laterality of HL, and age at diagnosis.

**Patients' rights and animal protection statement:** The article does not contain any studies with human or animal subjects.

**Conflict of interest:** The authors declare that they have no competing interests.

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