# Targeted next-generation sequencing in children with bilateral sensorineural hearing loss : diagnostic yield and predictors of a genetic cause

# Targeted next generation sequencing in children with bilateral sensorineural hearing loss: diagnostic yield and predictors of a genetic cause.

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**Abstract**

**Objective:** To investigate the diagnostic yield of targeted next generation sequencing using hearing loss panels and to identify patient related factors that are associated with a definite genetic cause.

**Study Design:** Retrospective chart review

**Setting:** Tertiary referral center

**Patients:** Children with congenital or late onset, bilateral sensorineural hearing loss (SNHL)

**Intervention(s):** Diagnostic

**Main Outcome Measure(s):** The number of patients with a definite genetic diagnosis

**Results:** We report on 238 patients with hearing loss, 130 males and 108 females. About 55% had congenital hearing loss. A genetic cause was identified in 94 (39.5%) of the patients, with 72.3% of these showing non-syndromic and 27.6% syndromic hearing loss. The diagnostic yield was highest among North-African patients (66.7%). A multiple linear regression model shows that profound hearing loss, family history of hearing loss, congenital hearing loss and North African ethnicity are significantly related with identifying a genetic diagnosis.

**Conclusions:** Targeted next generation sequencing using a panel of hearing loss genes identified a genetic cause in almost 40% of children with bilateral SNHL. We describe predictors of a genetic diagnosis, and this information may be used during genetic counselling.

## Introduction

Recent evidence shows that about 60% of congenital hearing loss (HL) and 50% of childhood HL is of genetic origin.[1] Genetic testing based upon next generation sequencing (NGS) is now considered the first line diagnostic test in the etiological work-up of pediatric HL.[2] Although the techniques of molecular genetic testing for hearing loss are evolving, the diagnostic yield of the various methods ranges between 39%[3] up to near 60%.[4] In a large clinical sample of 1119 patients including both children and adults with congenital and/or late onset HL, several phenotypic variables were identified which affected the diagnostic yield.[3] A positive family history for HL, symmetric HL, Middle Eastern ethnicity, and age of onset (congenital) all increased the diagnostic rate.

Genetic testing based upon targeted NGS using gene panels was introduced in our hospital in 2015 and diagnostic yield ranged from 50% in children with severe to profound HL[5], to 58.4% in children identified through a neonatal hearing screening program. [4] The aim of this paper is to report on the diagnostic yield of targeted NGS in a large clinical sample of children with congenital and late onset HL and to identify patient related factors that are associated with a genetic cause.

## Methods

We performed a retrospective analysis on all children admitted at the multidisciplinary otogenetics clinic of the Antwerp University Hospital between January 2015 and December 2021. All children underwent a complete etiological work-up including a clinical examination by a pediatric otorhinolaryngologist and clinical geneticist, screening for congenital cytomegalovirus infection (cCMV), targeted NGS using gene

panels for known non-syndromic or syndromic deafness genes, magnetic resonance imaging, a vestibular screening, and ophthalmological assessment.[4] The analysis is limited to children with bilateral sensorineural hearing loss (SNHL). Children with unilateral SNHL and/or hearing loss caused by cCMV or other confirmed non-genetic causes were excluded.

Genetic testing based upon targeted NGS using a panel for either non-syndromic and/or syndromic deafness is performed after written informed consent of the parents and/or legal caregiver. In the first half of the study period, prescreening for *GJB2* mutations was done first and if negative, NGS testing was performed. Later, *GJB2* was included in the deafness panels and not tested separately.

Analysis was performed on stored blood or DNA samples and blood samples were collected from the proband, both parents (trio-testing) and/or unaffected sibs for additional variant segregation analysis when indicated.

Mutation analysis was performed by NGS on a NextSeq500 sequencer (Illumina) after Haloplex enrichment of a non-syndromic and/or (depending on the phenotype), a syndromic hearing loss gene panel. Sequence data was analyzed with SeqNext analysis software (JSI medical systems). For all individual genes a minimal 30X coverage was obtained for more than 95% of the coding sequences, and for the total gene panel a minimal 30X coverage was obtained for more than 98% of the coding sequences. A minimal minor allele frequency threshold of 15% was used for variant detection. The composition of the gene panels is described in the online supplement 1 and 2. Classification of variants was performed according to the guidelines from the American College of Medical Genetics and Genomics and the Association for Molecular Pathology.[6]

The results of the genetic testing were classified in the following diagnostic categories:

*Definite genetic cause*: two (likely) pathogenic variants for autosomal recessive hearing loss (ARSNHL) and one (likely) pathogenic variant for autosomal dominant hearing loss (ADSNHL); *possible genetic cause*: one (likely) pathogenic and one variant of unknown significance (VUS) for ARSNHL; one VUS in an autosomal dominant gene for the index case and suggestive segregation with the hearing loss; *unknown*: all remaining findings

Hearing loss (HL) was diagnosed by auditory brain stem response audiometry (ABR) and/or by age-appropriate audiometric testing.

Pure tone average at 500-1000-2000-4000Hz (PTA4) was calculated. The thresholds in the best hearing ear were utilized to determine hearing loss severity according to the Gendeaf criteria: Mild 20-40dBHL, Moderate 41-70dBHL, Severe 71-95dBHL and Profound > 95dBHL.[7] Some children presented with normal hearing based upon PTA4 because of a very mild HL at 1 or 2 frequencies. These were classified as borderline and grouped together with the mild HL category for statistical analysis. In children who only underwent ABR, hearing loss severity was classified according to Madell: ABR threshold between 31-45dBnHL: mild; between 46 and 70dBnHL: moderate; between 71 to 90dBnHL: severe and ≥ 91dBnHL: profound hearing loss.[8] Hearing thresholds were obtained for the first audiometry and the most recent unaided audiometry. Hearing loss category between the first and last audiometry was compared and classified as stable, improvement or worsening.

Children who failed a neonatal hearing screening were classified as congenital hearing loss. Those presented with HL beyond the first month of life were classified as post-natal. Familial HL was defined as hearing loss present in a sibling or in one or both parents. Information was collected on consanguinity (present/absent) for non-European patients. Data on ethnicity were collected with the following categories: European, North African, Other parts of Africa, Middle Eastern, Asian, South American. When both parents originated from different regions, this was classified as mixed ethnicity.

Statistical analysis was performed by SPSS version 28. Pearson Chi-square analysis was used to explore the relationship between 2 categorical variables. Non-parametric Mann-Whitney U test was used to compare severity of hearing loss among subgroups. Logistic regression analysis was performed to investigate which independent variables (ethnicity, HL onset, Family history, HL severity and sex) could predict a confirmed genetic cause (dependent variable). As consanguinity was not questioned in the European patients, consanguinity was not included in this final model. However, a separate logistic regression model was fitted for the non-European patients only, to study the relation between consanguinity, ethnicity, and genetic cause of hearing loss. For all models, multicollinearity was checked by computing variance inflation factors (VIF). Statistical significance is concluded at $p < 0.05$.

The study was approved by the Local Ethics Committee. Project ID 2022-3026. Edge 002329.

**Results**

Data are available for 238 patients, 130 (54.6%) were males and 108 (45.4%) were females.

Patient characteristics are presented in Table 1. More than half of the patients (54.6%) had congenital HL and 33.5.% presented with post-natal HL. Seventeen patients did not undergo a neonatal hearing screening and data were unavailable for 11 patients. A family history of HL was present in 30.1%.

Regarding hearing loss severity, moderate HL was the most common category at baseline but about one third of the population (32.0%) presented with severe to profound HL. Eight patients were diagnosed with auditory neuropathy/dyssynchrony (ANSD). Follow-up data on hearing loss severity were available for 186 cases. In most patients (73.1%) HL remained stable, 15.6% had an improvement and 11.3% had worsening hearing thresholds.

The distribution of ethnicities and consanguinity is presented in Table 2. Information on consanguinity was not available for the European patients. Consanguinity was related to ethnicity. Among North African patients, 71.5% had consanguineous parents and 60.0 % of Middle Eastern patients had reported parental consanguinity, while only 20% was seen in other non-European ethnicities ($P_{\chi^2} < 0.001$).

The results of the genetic testing are graphically displayed in Figure 1. A genetic cause could be identified in 39.5% of the patients. In contrast, in 56.7 % no genetic cause could be found. Analysis of the remaining 3.8 % revealed a possible genetic cause, but no definite conclusion could be drawn, mainly because of the presence of

one or more VUS. An overview of the causal genes identified in our study population can be found in Table 3. Among the patients with a genetic diagnosis, 72.3% had non-syndromic and 27.6% had syndromic hearing loss. In none of the ANSD patients, a genetic cause could be identified.

Mutations in the *GJB2* gene accounted for the most common cause of non-syndromic SNHL (21.3%). A homozygous *GJB2* pathogenic mutation was confirmed by direct *GJB2* screening in 13 patients and by non-syndromic panel analysis in 7 patients. Seven patients were homozygous for the c.35delG pathogenic variant, and another 6 patients were compound heterozygotes showing the c.35delG pathogenic variant in *trans* with another pathogenic *GJB2* variant. The distribution of the most common genes causing non-syndromic HL in our study population is presented in Table 4. Mutations in *GJB2* were most frequent among European patients while mutations in *TMPRSS3* were encountered mainly in North African patients.

Four patients had biallelic pathogenic variants in the *STRC* gene and in two of them (both male) this concerned a homozygous *STRC* whole gene deletion encompassing the nearby *CATSPER2* gene, resulting in the Deafness Infertility syndrome.

Three patients presented with pathogenic variants in *CDH23* (n=2) and *PCDH15* (n=1) which can be associated with non-syndromic hearing loss or Usher syndrome 1D and 1F, respectively. All three were classified as non-syndromic based upon clinical grounds. This is in contrast with three children, sibs from consanguineous parents, who presented with profound congenital HL, severe delay in motor development, bilateral vestibular dysfunction and retinitis pigmentosa in whom a diagnosis of Usher syndrome type 1F caused by biallelic pathogenic variants in the *PCDH15* gene, was established.

Ethnicity and consanguinity were both associated with a genetic cause. A genetic cause was more frequently detected in patients with North African (66.7%) or Middle Eastern (55%) ethnicity, while only 33.8% of the European patients had a definite genetic cause ($P_{\chi^2} < 0.001$) (Table 4). Of the non-European patients with a genetic cause 68.9% had consanguineous parents, while in patients without a diagnosis, this is only 32.6% ($P_{\chi^2} < 0.001$).

In a logistic regression model for the non-European patients, we see that after correction for consanguinity, ethnicity is no longer significantly related to a definite genetic cause (P = 0.083), while the effect of consanguinity remains significant after correction for ethnicity (P = 0.030). Furthermore, severity of hearing loss was associated with genetic cause, where more severe hearing loss was seen in the group where a genetic cause could be identified. ($P_{MWU} < 0.001$) Also, patients with congenital HL were more likely to have a genetic diagnosis (48.9% vs 25%, $P_{\chi 2} < 0.001$). In 42 cases with profound congenital HL at baseline, a genetic cause was identified in 34 (81%). A multiple linear regression model (presented in Table 5) shows that profound HL, family history of HL and congenital HL, are significantly related with a genetic diagnosis. North African patients have a higher odds for a genetic diagnosis ($OR_{N\text{-}Afr\ vs\ Eur} = 2.81$ (95% CI 1.03 – 7.70, P = 0.044), while no significant difference was seen between the other ethnicities.

## Discussion

Using targeted next generation sequencing, a genetic cause could be identified in 39.5% of children with congenital or late onset HL. Hearing loss severity (profound hearing loss), North African ethnicity, congenital HL and a family history of HL were predictors of a genetic cause.

Although these factors have been associated with a genetic cause in previous studies[3,9], our paper is the first report in a large clinical sample of children with bilateral SNHL for whom other non-genetic causes of HL were excluded.

The identification of a genetic cause in a child with HL had several clinical implications and may be used for prevention of hearing loss progression, therapeutic counselling, and genetic counselling providing a prediction of recurrence risk in future siblings.[10] Also when gene therapy or pharmacotherapy for specific genetic causes of SNHL becomes more standardized in the future, this is likely to change decision making for individual patients both in terms of treatment and parental counselling.[11]

We identified several patient related factors which are associated with a genetic cause. Hearing loss severity and more specifically, profound HL is associated with a genetic cause, a finding which has been reported earlier.[5,12] Ethnicity is a second patient characteristic associated with a genetic cause. Our study population had a diverse ethnic background. Although a vast majority was of European origin, nearly 40% of the patients had another ethnicity and North-African patients were most represented comprising 16.3% of the whole study population. Previous studies have demonstrated that the spectrum of genes involved in SNHL varies by race/ethnicity. Rouse et al. recently emphasized that Europeans and Asians are overrepresented in studies on genetic HL representing 96.4% of all reported subjects.[13]

We found a statistically significant association between a genetic cause and North-African Ethnicity. Seventy percent of North-African patients in our sample had consanguineous parents and in non-European patients, consanguinity was

associated with a genetic cause. Since the absolute number of North-African patients is relatively small (n=39), these findings should be confirmed in larger samples to support the clinical significance of these findings.

Florentine et al. recently published a study investigating diagnostic efficacy of comprehensive hearing loss gene panel testing in 240 children with SNHL.[14] The study included 35.8% children with congenital loss, 37.1% with postnatal onset and 27.1% for whom the onset of HL was unknown, 20.4% of the included children had unilateral HL. The overall diagnostic yield was 22% but Asian and White children had a higher rate of a definite diagnosis (26% and 46% respectively) compared to Black (10%) and Hispanic children (13%).

Congenital HL is a third factor associated with a genetic cause. It is assumed that about 60% of all congenital hearing losses have a genetic origin[1] and earlier studies using targeted NGS revealed a diagnostic yield of 44%[12] to 58.4%.[4] Usami et al. identified a genetic cause in 48.6% of 3877 children with congenital or early onset (~ 5 year) HL.[9]

Finally, having a first degree relative with HL was associated with a genetic cause. Information on the proportion of familial/sporadic cases in a study population is important from an epidemiological standpoint. Most causes of non-syndromic congenital hearing loss have an autosomal recessive inheritance with a recurrence risk of 25% in siblings and familial cases have a higher elucidation rate compared to sporadic cases.[15] The number of siblings included in this study is listed in Table 3.

Homozygous pathogenic variants in *GJB2* are the most common genetic diagnosis in our study population accounting for 21.3% of the definite cases. Pathogenic variants in *GJB2* are the most important cause of congenital autosomal recessive SNHL worldwide and the prevalence of specific variants is regionally dependent.[16] Worldwide, c.35delG accounts for 57% of the alleles in patients with biallelic *GJB2* related HL[16] and for 58-93% of mutant alleles in biallelic cases across Europe.[15] It is an inactivating mutation resulting in a loss of protein function and homozygous mutations cause a profound congenital HL.[17]

In a recent paper, del Castillo et al. summarized data on genetic causes for non-syndromic HL in Europe.[15] Apart from *GJB2* cases, the following genes had contributions higher than 2%: *MYO15A, MYO7A, LOXHDI, USH2A, TMPRSS3, CDH23, TMC1, OTOF, OTOA, SLC26A4, ADGRVI* and *TECTA*. Together with *STRC*, these genes explained 84% of the non-*GJB2* cases.[15] Although our sample size is much smaller, we see a similar distribution with most important contributions in non-*GJB2* cases, from *MYO15A, TMPRSS3, OTOGL* and *TMC1.* An X-linked inheritance pattern is found in less than five percent of non-syndromic HL cases. Despite its rarity, we found a deletion in the S*MPX* gene in 2 cases.

Screening for cCMV and comprehensive genetic testing based upon next generation sequencing are the key components of the etiological work-up for congenital HL.[1,2] A cCMV infection was excluded in all our patients using PCR analysis on a dried blood spot and none of the children included presented with other clinical signs of or findings on magnetic resonance imaging that suggested a cCMV infection.[18] Yet, the diagnostic yield of the gene panels was only 39.3%. Although this figure is in line with other reports in literature, it likely reflects the limitations inherent to targeted NGS.

Despite the wide implementation of comprehensive genetic testing in clinical practice, there are still many challenges on the technical and diagnostic level[19, 20] causing limited efficacy of current approach. Firstly, the analysis of some genes such as *OTOA* and *STRC* is complicated by the presence of pseudogenes or duplicated regions in the genome. The presence of these pseudogene sequences interferes with the gene-specific sequences, which can lead to failure of detection of the causative variants or to misdiagnosis. Secondly, the diagnostic analysis is currently limited to known HL causing genes, while there likely still exist several unidentified genes causing non-syndromic or syndromic HL. It can be assumed that the diagnostic yield of the current diagnostic approach can be further increased by identification of additional genes, identification of variants in complex genomic regions and better interpretation of putative splice site variants.[3]

Technologies like whole exome sequencing (WES) and whole genome sequencing (WGS) will play pivotal roles in novel gene discovery and in identifying causative mutations for complex heterogenetic diseases like HL in the coming years and have the potential to change the delivery of patient care.[20]

Variant interpretation that is accurate, disease specific and equitable representative is one of the major challenges in the clinical diagnosis of genetic HL.[14] Accurate clinical information is essential for the variant interpretation.[6] A possible genetic diagnosis was found in 3.8% of our study population. For these patients with a possible diagnosis, follow-up is recommended and if new clinical information becomes available or variants are reclassified the clinical significance of these variants may become clear.[6]

The majority (79%) of variants within hearing loss genes included in the Deafness Variation Database are classified as variants of unknown significance and are mostly missense and synonymous variants.[21] Counselling and explaining these findings to patients and caregivers may be delicate as there is no definite answer about a potential genetic cause and it is possible that the conclusions change over time when new data become available or new clinical signs emerge. Tayoun et al. suggested that presence of VUS may have a psychosocial impact on individuals and their family.[22]

Non-syndromic mimics (NMS) are syndromic forms of SNHL with hearing loss as the presenting feature and without any other concerns or syndromic features.[23] Typical examples of NMS are Usher syndrome, Deafness Infertility syndrome and Jervell-Lange Nielsen syndrome. Visual and balance problems in Usher syndrome are not apparent during infancy. Moreover, some of the genes involved in Usher syndrome may also cause non-syndromic hearing loss for example *CDH23*, *MYO7A* and *PCDH15*. Current knowledge on genotype-phenotype correlations for these genes does not allow to predict for sure whether a child with pathogenic variants in one of these genes will develop retinitis pigmentosa at a later stage.[24] In our study population, three patients presented with pathogenic mutations in *CDH23* (n=2) and *PCDH15* (n=1). In the absence of delayed motor development or other signs of vestibular dysfunction and at an age where signs of retinitis pigmentosa are not yet detectable, they were classified as non-syndromic cases. The detection of vision problems in patients with a possible diagnosis of Usher syndrome based upon genetic testing remains challenging. Ambrosio et al. concluded that tests of retinal function performed before age 10 years were normal in nearly half of the patients

with a genetically secured diagnosis of Usher syndrome.[25] The deafness-infertility syndrome is another NMS and was diagnosed in 2 male patients with congenital, moderately severe HL. This diagnosis has important implications for the future of the child. Despite normal clinical features, males are infertile whereas female patients only present HL.

Genetic testing for SNHL should be embedded in a multidisciplinary approach to the child with SNHL and its' caregivers and both pre-and post-testing genetic counselling should be provided.[26] During pre-test genetic counselling parental expectations and understanding of genetic testing should be addressed. In addition, parents should be informed that incidental findings may be revealed, especially when WES or WGS are used. Elander et al. utilized a patient reported experiences measure questionnaire to investigate the patient and parents experience with WES in a group of 11 children with severe bilateral SNHL.[27] Parents had a positive attitude and reported that genetic testing provided added value to their family. Nevertheless, 60% of the parents had difficulties to explain the findings to other relatives and two of the three families with children in whom only VUS were found interpreted the results as a confirmed genetic diagnosis.

Due to its retrospective nature, complete data on patient characteristics was not available for a subset of patients. A limitation is the lack of information on consanguinity for the European patients. The clinicians assumed that consanguinity is very rare in European patients and as such this was not always systematically asked for. However, we believe that this does not affect the results obtained in the

North-African population in whom a high prevalence of consanguinity was documented in the medical records.

## Conclusion

We found a genetic cause in 39% of children with bilateral, congenital, or late onset SNHL in whom other common causes (such as cCMV infection) had been excluded. Our current findings support the importance of genetic testing in the etiological work-up for bilateral SNHL in newborns and children and this information may guide clinicians when counselling parents about the value of genetic testing and the likelihood to establish a genetic cause.

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**Legend to Figures and Tables**

**Figure 1:** Results of genetic testing in 239 pediatric patients according to the final diagnosis.

**Table 1:** Patient characteristics

**Table 2:** Genetic causes in 94 children with congenital or late onset SNHL

**Table 3:** Genetic causes in 94 children with congenital or late onset SNHL

**Table 4**: Number (and percentage) of patients with a genetic diagnosis per ethnicity and number of patients for the most involved non-syndromic hearing loss genes.

**Table 5:** Multiple logistic regression model for predictors of a confirmed genetic diagnosis.

[a] reference group: European patients

[b] reference group: late onset

[c] reference group: borderline or mild hearing loss

**Online Supplement:**

**Supplement 1:** Composition of the DOOF_v13b_NS gene panel for non-syndromic hearing loss

**Supplement 2:** Composition of the DOOF_v13_SYN gene panel for syndromic hearing loss.

| Parameter | Number | Percentage |
|---|---|---|
| **Sex** | | |
| Male | 130 | 54,6 |
| Female | 108 | 45,4 |
| **HL onset** | | |
| *congenital* | 130 | 54,6 |
| *late onset* | 81 | 34,0 |
| *unknown* | 27 | 11,3 |
| **HL severity at baseline** | | |
| *borderline* | 6 | 2,5 |
| *mild* | 55 | 23,1 |
| moderate | 100 | 42,0 |
| *severe* | 18 | 7,6 |
| *profound* | 58 | 24,4 |

**Table 1.** Patient characteristics

| Ethnicity | Number (%[a]) | Consanguinous parents (N (%[b])) | Non-consanguinous parents (N (%[b])) | *Unknown consanguinity (N (%[c]))* |
|---|---|---|---|---|
| Europe | 144 (60.5%) | | | *144 (100%)* |
| North Africa | 39 (16.4%) | 27 (71.5%) | 11 (28.5%) | *1 (2.6%)* |
| Middle Eastern | 20 (8.4%) | 12 (60.0%) | 8 (40.0%) | |
| Asia | 15 (6.3%) | 5 (33.3%) | 10 (66.7%) | |
| Other African countries | 5 (2.1%) | 1 (25.0%) | 4 (75.0%) | |
| South America | 3 (1.3%) | | 2 (100%) | |
| Mixed | 8 (3.4%) | | 8 (100%) | |
| *Unkown* | *4 (1.7%)* | *2 (100%)* | | *2 (50%)* |

**Table 2.** Ethnicity and consanguinity in 238 children with bilateral sensorineural hearing loss.

[a] percentage in group of patients with known ethnicity

[b] percentage in group of patients with same ethnicity (row percentage) and known consanguinity

[c] percentage of missing consanguinity in group of patients with same ethnicity

**Table 3. Genetic causes in 94 children with congenital or late onset SNHL**

| **Genetic cause (n=94)** | **Number** | **%** | **Number of siblings/isolated cases** |
|---|---|---|---|
| **Non-Syndromic hearing loss** | **68** | **72,3** | |
| *CABP2* | 1 | 1.1 | 0/1 |
| *CDH23* | 2 | 2.1% | 0/2 |
| *CIB2* | 1 | 1.1 | 0/1 |
| *GJB2* | 20 | 21.3 | 2/18 |
| *DIAPH1* | 1 | 1.1 | 0/1 |
| *ESSRB* | 2 | 2.1 | 2/0 |
| *GHRL2* | 1 | 1.1 | 0/1 |
| *KCNQ4* | 2 | 2.1 | 0/1 |
| *LOXHDI* | 1 | 1.1 | 0/1 |
| *MARVELD2* | 1 | 1.1 | 0/1 |
| *MYO15A* | 8 | 8.5 | 2/6 |
| *OTO-A* | 3 | 3.2 | 0/3 |
| *OTOG* | 2 | 2.1 | 0/2 |
| *OTOGL* | 4 | 4.3 | 2/2 |
| *PCDH15* | 1 | 1.1 | 0/1 |
| *POU3F4* | 1 | 1.1 | 0/1 |
| *SMPX* | 2 | 2.1 | 0/2 |
| *STRC* | 2 | 2.1 | 0/2 |
| *TMC1* | 4 | 4.3 | 2/2 |
| *TMPRSS3* | 6 | 6.4 | 3/3 |
| *TPRN* | 2 | 2.1 | 2/0 |
| *WHIRLIN* | 1 | 1.1 | 0/1 |
| **Syndromic hearing loss** | **26** | **27,6** | |
| *ABDH12* (PHARC) | 2 | 2.1 | 2/0 |
| *ATP6V1B1*(Renal tubular acidosis with deafness) | 3 | 3.2 | 2/1 |
| *GPR98* (USH2C) | 1 | 1.1 | 0/1 |
| *MITF (*Waardenburg 2A) | 2 | 2.1 | 0/2 |
| *MYO7A* (USH1) | 1 | 1.1 | 0/1 |
| *PAX3* (Waardenburg 1) | 1 | 1.1 | 0/1 |
| *PCDH15* (USH1D) | 3 | 3.2 | 3/0 |
| *SLC26A4 (*Pendred) | 3 | 3.2 | 0/3 |
| *SOX10* (Waardenburg-Shah) | 3 | 3.2 | 0/3 |
| *COL11A1* (Stickler) | 2 | 2.1 | 0/2 |
| *STRC/CATSPER2* (Deafness-Infertility) | 2 | 2.1 | 0/2 |
| *WFS1* (Wolfram) | 3 | 3.2 | 2/1 |

| Ethnicity (n) | Confirmed per ethnicity (%) | *GJB2* | *MYO15A* | *TMPRSS3* | *OTOG* | *TMC1* | *OTOA* |
|---|---|---|---|---|---|---|---|
| **European (144)** | 49 (33.8%) | 13 | 2 | 1 | 3 | 2 | 1 |
| **North African (39)** | 26 (66.7%) | 4 | 2 | 5 | 1 | 0 | 2 |
| **Middle Eastern (20)** | 11 (55%) | 2 | 3 | | | | |
| **Asian (15)** | 6 (40%) | | 1 | | | 2 | |
| **Other parts of Africa (5)** | 0 | | | | | | |
| **South America (3)** | 0 | | | | | | |
| **Mixed (8)** | 2 (25%) | 1 | | | | | |
| **Unknown (4)** | 0 | | | | | | |
| **Total (238)** | | | | | | | |

**Table 4:** Number (and percentage) of patients with a genetic diagnosis per ethnicity and number of patients for the most commonly involved non-syndromic hearing loss genes

| Factors | Odds ratio | 95%CI | P-value |
|---|---|---|---|
| Ethnicity[a] | | | 0.077 |
| North Africa | 2.81 | 1.03 – 7.70 | 0.044 |
| Middle Eastern | 0.74 | 0.22 – 2.56 | 0.64 |
| Other | 0.50 | 0.16 – 1.53 | 0.22 |
| Congenital hearing loss[b] | 2.24 | 1.01 – 4.95 | 0.048 |
| Hearing loss severity[c] | | | 0.001 |
| Moderate | 1.43 | 0.60 – 3.45 | 0.42 |
| Severe | 1.86 | 0.50 – 6.92 | 0.35 |
| Profound | 7.66 | 2.54 – 23.1 | < 0.001 |
| Family history of hearing loss | 3.59 | 1.69 – 7.62 | < 0.001 |
| Gender: M | 1.52 | 0.76 – 3.05 | 0.24 |

**Table 5.** Multiple logistic regression model for predictors of a confirmed genetic diagnosis.

[a] reference group: European patients

[b] reference group: late onset

[c] reference group: borderline or mild hearing loss

## Results of Genetic testing n=238