QATAR UNIVERSITY

COLLEGE OF HEALTH SCIENCES

# NONSYNDROMIC HEARING LOSS IN QATAR: THE GENETIC BASIS AND THE DIAGNOSTIC YIELD OF GENETIC TESTING

BY

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ABSTRACT

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Title: Nonsyndromic Hearing Loss in Qatar: The Genetic Basis and The Diagnostic Yield of Genetic Testing

Supervisor of Project: Dr. Mashael Al-shafai.

*Background:* Hearing loss is the most predominant sensory defect worldwide with around 8% of cases occurring in children. Approximately 66% of childhood-onset hearing loss cases are attributed to genetic factors. The prevalence of hereditary hearing loss increases in consanguineous populations, and the prevalence of hearing loss in Qatar is 5.2%. *Aim*: We aim to investigate the genetic basis of nonsyndromic hearing loss (NSHL) in Qatar and evaluate the diagnostic yield of different genetic tests, including *GJB2* gene sequencing, chromosomal microarray, gene panel, whole exome sequencing, and mitochondrial genome sequencing and deletion testing. *Methodology*: A retrospective chart review was conducted for 128 eligible pediatric patients with NSHL referred to the Clinical and Metabolic Genetics department at Hamad Medical Corporation between 2014 and 2019, and who underwent at least one genetic test. *Results*: Our study revealed an overall diagnostic yield of 30.5%, attributed to 19 variants in 11 genes and two copy number variants. 36.8% of the causative variants were identified in *GJB2* gene, with the most common was c.35delG as it was seen in 9 out of 39 cases (23.1%) genetically diagnosed patients. We identified the known Qatari founder c.506G>A in *GJB2* gene in our cohort. Additionally, c.283C>T in *FGF3* gene was associated with NSHL for the first time worldwide. The reassessment of variants of unknown significance (VUS) resulted in identifying eight variants as being likely contributing to NSHL in our cohort, including *c*.3641G>A in *MYO15A*, c.6503T>G in *MYO15A*, c.599C>T in *WHRN*, c.2476G<A and c.4696A>T in *MYO7A*, c.-182G>A

and c.617-3_617-2dup in *TMPRSS3*, and *c*.98G>A in *OTOF*. Moreover, *GJB2* gene sequencing and gene panel testing were the two tests significantly associated with positive diagnostic yield. *Conclusion*: Our work adds new insight into the genetic basis of NSHL in Qatar. Based on our findings, we recommend performing *GJB2* gene sequencing as a first-tier genetic test for NSHL and gene panel as a second-tier genetic test for NSHL. We also encourage clinicians to consider reassessing VUS based on ACMG guidelines.

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ALFA: Allele Frequency Aggregator.

ACMG: American College of Medical Genetics and Genomics.

CNVs: Copy Number Variants.

HMC: Hamad Medical Corporation.

HL: Hearing loss.

HHL: Hereditary Hearing Loss.

NGS: Next-Generation Sequencing.

NSHL: Nonsyndromic Hearing Loss.

SHL: Syndromic Hearing Loss.

SNHL: Sensorineural Hearing Loss.

WES: Whole Exome Sequencing.

# CHAPTER 1: INTRODUCTION

The Global Burden of Disease (GBD) defines hearing loss (HL) as the minimum level a person can hear using their bare ear, taken as the pure tone average of audiometric thresholds of 0.5 kHz, 1 kHz, 2 kHz, and 4 kHz (1). HL is the most predominant sensory defect worldwide (2), and around 8% of the cases occur in children (3). In 2019, 1.5 billion people worldwide diagnosed with HL; 25.7% of these had moderate to severe HL. 37.9% of all incidences of HL in 2019 were in people below the age of 50 years old. The majority of moderate HL cases were in the Western Pacific region (127.1 million), followed by the South-East Asia region (103.4 million), and lastly, Americas (58·8 million) (1). The prevalence of HL in Qatar was estimated to be 5.2% (4).

There are many ways to classify HL, which can be done on the basis of its type, onset, severity, or etiology. Based on type, HL is classified into conductive, sensorineural, and mixed (5). Conductive HL primarily results from an abnormality in either the outer or the middle ear (conductive air structures). In comparison, sensorineural hearing loss (SNHL) results from an abnormality of the cochlea or the 8$^{th}$ cranial nerve. Mixed HL results from abnormalities in the conductive and the sensorineural compartments of the ear (6). Among those three types, SNHL is the most common sensory deficit in developed countries (7). Based on onset, HL is classified into pre-lingual (prior to speech development) or post-lingual (after speech development) (8). Considering etiology, HL can be classified into hereditary hearing loss (HHL), caused by genetic factors, or acquired HL, driven mainly by environmental factors (9). Overall, genetics explains 50-60% of the cases of HL (10, 11). Among the childhood-onset hearing losses, around 66% are due to genetic factors, while the remaining 33% are acquired (12).

HHL can be isolated, known as nonsyndromic hearing loss (NSHL), representing around 70% of HL cases (13, 14), or it can co-exist with other distinctive syndromic symptoms, collectively called syndromic hearing loss (SHL). To date, more than 400 syndromes have been associated with the development of HL (15). More than 6,000 causative variants in at least 150 genes are associated with HHL (16), most commonly in the *GJB2* gene (17). The use of next-generation sequencing (NGS) techniques facilitated the identification of genes and variants associated with HHL (18). Different types of variants have been associated with HHL, such as single nucleotide polymorphisms, microdeletions, duplications, and chromosomal abnormalities (19). The number of genes and variants associated with HHL is increasing. Therefore, several databases keep track of the newly discovered HL variants and genes, including the Hereditary Hearing Loss Website (https://hereditaryhearingloss.org/) and Deafness Variation Database (https://deafnessvariationdatabase.org/).

NSHL represents the most significant portion of HHL cases, and it is associated with variations in more than 90 genes (20). NSHL can be inherited in different modes: 80% of cases are inherited in an autosomal recessive (AR) manner, 15% of cases are inherited in an autosomal dominant (AD) manner, and 1-2% of cases are inherited in an X-linked (XL) or mitochondrial manner (21, 22).

In this project, we investigate NSHL by analyzing its genetic basis, identifying causative variants and genes associated with NSHL in the population of Qatar, and evaluating the diagnostic yield of the different genetic tests used.

*Study hypothesis*

Investigating the genetic basis of NSHL in patients from the understudied population of Qatar can reveal novel and/or founder NSHL variants (or genes). In addition, being genetically heterogeneous, with variation in *GJB2* as the most common cause of NSHL, *GJB2* gene comprehensive genetic testing will potentially have the highest diagnostic yield in Qatar.

*Study aim*

This study aims to investigate the genetic basis of NSHL in Qatar and evaluate the diagnostic yield of different genetic tests for NSHL used in Qatar.

*Study objectives*

a- To identify the spectrum of genetic variants associated with NSHL in the population of Qatar.

b- To identify novel/founder genetic variants/ genes associated with NSHL in the population of Qatar.

c- To perform genotype-phenotype correlation for the identified causative NSHL variants.

d- To re-assess the variants of unknown clinical significance (VUSs) (including copy number variants).

e- To compare the diagnostic yield of different genetic tests performed in Qatar, including *GJB2* gene sequencing, chromosomal microarray, gene panel, whole exome sequencing (WES), and mitochondrial genome sequencing and deletion testing.

# CHAPTER 2: LITERATURE REVIEW

Auditory system is anatomically divided into three structures: outer, middle, and inner ear. The first two compartments conduct external sound waves of different intensities and tones to the inner ear (23). The inner ear is the sensory portion of the ear, and it helps maintain body balance as well (24). Most of the genetic variants affect the inner ear's function, leading to the development of HHL (25).

## 2.1 Genetics of nonsyndromic hearing loss

### 2.1.1 *Hearing loss from genetics to the function of the ear*

Pathogenic variants in genes causing HHL affect at least one of the following cellular processes in the inner ear: cytoskeleton formation, cell-cell junctions, membrane transportation, or the function of regulatory elements (25).

### 2.1.2 *Genes involved in cytoskeleton formation*

The inner ear cytoskeleton is a group of filament proteins within the cell that help in providing the cell with the necessary support, mobility, and shape. Those filament proteins include three types: intermediate filaments, microtubules, and actin filaments (26). Moreover, motor proteins including kinesins and dyneins, are part of the cell's cytoskeleton system (27). Several genes associated with HHL encode proteins involved in forming or aiding the function of those cytoskeleton proteins, including *DFNA1*, *ACTG1*, *TRIOBP*, and *SLC26A5 (28, 29)*, all of which are known to be associated with NSHL (30-33). The *MYO7A* gene, which is associated with autosomal recessive nonsyndromic deafness-2 (DFNB2) and autosomal dominant nonsyndromic deafness (DFNA11), encodes a protein that plays a structural role in forming cochlear hair cells in the inner ear (34). However, *MYO7A* pathogenic variants are also associated with Usher syndrome type 1B, a type of SHL (35).

### 2.1.3 *Genes involved in cell-cell junctions*

The process of cellular communication between the different epithelial cells of the inner ear must remain intact. The proteins of cell-cell junctions include gap junction proteins, adherens junction proteins, and tight junction proteins (36). The function of the gap junction proteins is to facilitate cell to cell communication process, signaling pathways, and small molecules exchange between cells (37). The role of the adherens junction proteins is to form a connection between the proteins in one cell and the transmembrane adhesion proteins of the nearby cells or the extracellular environment. This connection is essential in many processes of the inner ear such as cochlear development, growth of auditory neurons, immune mediation, and planar cell alignment (38). Tight junction proteins are located on the sides of the cells; they regulate the transportation of soluble molecules across the epithelial cells of the inner ear, maintain ion concentrations, and regulate cellular movement (39, 40).

The connexin protein family includes key players for gap junction formation, which depends on the assembly of connexins from neighboring cells; thus, facilitating the above-mentioned processes in the inner ear (41). Here, two essential genes are discussed, *GJB2* gene, which encodes connexin 26, and *GJB6* gene, which encodes connexin 30, both constituting the majority of the gap junctions of the inner ear (42). Connexins 26 and 30 assemble to form a heteromeric gap junction channel, an integral gap junction structure; such a structure is formed through the action of NF-κB signaling pathway. The importance of the gap junction structure is to maintain the concentration of potassium across cells, which is essential for inner ear function in the transduction of sound signals (43). Variants that alter the protein structure of connexins 26 and 30 negatively impact the formation of gap junction, leading to disturbance in the ionic balance, which in turn leads to disruption of sound conduction (44). *GJB2* and *GJB6*

also contribute to calcium homeostasis and NF-κB signaling pathway (45, 46).

### 2.1.4 *Genes involved in membrane transportation*

The inner ear fluids are crucial for maintaining ion concentrations and absorbing the conduction of sound waves created in the conductive portion of the ear. The inner ear contains two primary extracellular fluids, endolymph and perilymph (47). To perform their function, those two fluids need to have specific volumes and ion contractions, which is maintained through the action of transporters and ion channels (48).

The *SLC26A4* gene encodes the pendrin protein, a transmembrane solute carrier that maintains the fluid volume. It facilitates the secretion of bicarbonate ions into the endolymph (49). Variants in *SLC26A4* are associated with either a decrease or increase of the volume of the inner ear fluids, thus, impairments in its function. Variants in *SLC26A4* are associated with NSHL and SHL (50).

The KCNQ protein family includes voltage-gated channels for potassium ions, expressed in different tissues and organs (51), they help by achieving balance of ion concentrations. Pathogenic variants of *KCNQ1* gene are associated with Autosomal Recessive (AR) and Autosomal Dominant (AD) forms of HL (52). Pathogenic variants of *KCNQ4* gene are also associated with progressive AD HL (53).

### 2.1.5 *Genes involved in regulatory elements*

The different cellular processes such as cellular respiration, growth, and apoptosis need to be precisely regulated. A major way to achieve this is through the regulation of gene expression during transcription. Pathogenic variants of *POU3F4* gene are associated with AR NSHL (88); this gene encodes a transcription factor that plays a vital role in neurons differentiation (89). Furthermore, the *EYA4* gene, which is associated with progressive AD HL, encodes a transcriptional activator required for the

proper development of the organ of Corti of the cochlea (90).

Pathogenic variants in genes that play a role in other inner ear components, such as *TECTA* and *COL11A2* are also associated with the onset of HL. Those genes encode proteins essential for the inner cell matrix structure (54, 55).

2.2 Genotype-phenotype correlation of nonsyndromic hearing loss

NSHL is variable both phenotypically and genetically, with pathogenic variants identified in more than 60 genes so far (56). Most cases of NSHL are described as SNHL, and only a few cases are conductive or mixed (57). The variability in the phenotype of NSHL is noted across different phenotypic parameters, including laterality, time of presentation, severity, and progression (58, 59)..

Most cases of NSHL are inherited in an AR manner (56), and there are at least 40 genes associated with this mode of inheritance. On the one hand, about 50% of the severe AR NSHL cases are attributed to genetic variation in *GJB2* gene, followed by genetic variation in *GJB6* gene. Both genes encode proteins that are part of the connexin family and are essential for forming Gap junction proteins (60). On the other hand, most of the moderate AR NSHL cases are caused by variation in the *STRC* gene (61, 62). The frequency of the variants in different genes causing AR NSHL is variable across different populations (60). For example, *GJB2* and *GJB6* contribute significantly to cases of NSHL in Caucasians, but less commonly to cases of NSHL in ethnic minorities (63),(64).

In AD NSHL, variations in few genes such as *KCNQ4 (65)* and *TECTA (66)* are recurrently described. Most variants are reported only in few sporadic cases or in families, majority with post-lingual presentation. Patients with *KCNQ4*-related disease usually present with progressive HL (65). Other genes associated with AD NSHL include *WFS1, MYO7A, and COCH* genes (67). Variants in *WFS1* are associated with

mild NSHL (68), while variants in *MYO7A* gene is associated with profound HL, variable age of onset , and post-lingual HL in some populations, including Chinese and Arabs (69, 70). Furthermore, AD *COCH*-related NSHL presents with profound and progressive HL (71).

The XL inheritance of NSHL is rare and associated with both pre-lingual and post-lingual onset, variable severity, and progressive disease (72). Genes reported to be associated with XL NSHL include *PRPS1(73), POU3F4 (74), SMPX (75), AIFM1 (76),* and *COL4A6* (77).

The mitochondrial inheritance of NSHL is also rare, constituting around 1% of pre-lingual NSHL (78) and 5% of post-lingual NSHL (79), and presents with variable severity (80). Several mitochondrial genes (*MT-RNR1, MT-TS1, MT-CO1, MT-ND1, MT-TH, MT-TI, MT-TK, MT-TL1, and MT-TS2)* are associated with mitochondrial NSHL, with the majority of the pathogenic variants are occurring in *MT-RNR1* and *MT-TS1* (81). Among patients with mitochondrial NSHL, m.1555A<G in the *MT-RNR1* gene is a common mitochondrial variant causing NSHL upon the administration of aminoglycosides (an antibiotic used mainly in the treatment of aerobic gram-negative bacilli infections) (82). Carriers of this *MT-RNR1* variant do not develop HL unless exposed to aminoglycosides, which affect the variant's penetrance level and lead to the development of HL (83). Generally, patients with mitochondrial NSHL have a good prognosis after cochlear implantation (84).

Interestingly, some of the genes associated with NSHL are also associated with syndromic forms of HL. For example, pathogenic variants in *CDH23* gene cause AR NSHL and Usher syndrome type 1D (85), variants in *SLC26A4* gene cause AR NSHL and Pendred syndrome (86), variants in *COL11A2* gene cause AD/AR NSHL and Stickler syndrome (87), and variants in *WFS1* gene cause AD NSHL and Wolfram

Syndrome (88).

## 2.3 Diagnosis of nonsyndromic hearing loss

### 2.3.1 Testing guidelines

HL is a heterogeneous condition in nature, which increases the complexity of establishing its etiology. However, the diagnostic yield of genetic testing significantly increases in cases with positive family history, childhood-onset HL, and bilateral HL (20). The complex nature of the condition makes it also challenging to choose the most appropriate diagnostic genetic test. As per the guidelines of the American College of Medical Genetics and Genomics (ACMG), clinical assessment should be made through the collection of audiometric and clinical symptoms as a first step. Secondly, acquired HL should be ruled out; if acquired HL cannot be ruled confidently, evaluation of HHL should be made through the appropriate genetic testing. If syndromic HL is suspected, genetic testing specific to suspected syndrome is of proper use. In contrast, if NSHL is suspected, single-gene testing of *GJB2* and *GJB6* gene should be conducted as first-tier genetic testing. If those first-tier genetic tests come back negative or inconclusive, more comprehensive genetic testing such as a gene panel or WES should be considered (89).

## 2.4 Management of hearing loss

### 2.4.1 Newborn hearing screening

The management of HL is essential, particularly in pediatric patients, since HL affects the development of speech, communication, and behavior (90). Newborn hearing screening is internationally recommended to allow for the early detection of HL. In the USA, 95% of all neonates get screened for HL (91), which results in better child development (92). In Qatar, the national program for newborn hearing screening was implemented in 2003. The program consists of three tests conducted at different ages: the first screening takes place at the age of 24 hours, the second screening is done

at the age of 3 months, and the third screening occurs before school admission (93). Early detection in Qatar positively affected the neonates and allowed for early management and intervention, and positively impacted speech and social skills development.

### *2.4.2 Treatment for hearing loss*

The treatment/management options of HL vary based on the etiology and the clinical presentation of the affected individuals. These options include:

1- Assistive hearing devices to help amplify the sounds to reach the levels of detection for people with HL. Hearing aids differ in their ability to amplify sound, their location of implementation, and their cost (94). The usage of hearing aids can be socially concerning to individuals due to their cost and associated stigmatization (95).

2- Surgical intervention, which is used when HL cannot be repaired with the use of regular hearing aids due to a traumatic event or a course of severe infection that results in separation of the middle ear ossicles. The surgical option restores the conductive function of the ears, thus improving hearing significantly (96).

3- Cochlear implantation is another option in some cases of severe to profound HL due to damaged cochlea. The cochlear implant helps in overcoming the poor function of the initially damaged cochlea (97). It should be noted that cochlear implantation is of clinical utility when the damage is restricted to the cochlea and does not impact the cochlear nerve (98).

4- Medication is sometimes indicated, not as a treatment for HL per se, but when other clinical symptoms accompany HL or when HL results from an infection (99).

All options discussed above have been found effective in enhancing

communication in children when implemented as early as possible (100, 101).

A new emerging treatment for HL, now under evaluation in animal models, involves the transplantation of pluripotent stem cell-derived progenitors to differentiate into different types of inner ear cell types and restore the function of the inner ear. However, the difficulty of this treatment lies in the lack of complete control over the fate of the stem cell differentiation process, leading to an overall low success rate (102). Currently, there are over 270 clinical trials related to different aspects of hearing loss in children, including prevention, treatment, and intervention efficacy (https://clinicaltrials.gov/ct2/home)

## **2.5** Hearing loss in Arab countries

In 2020, Sidenna *et al.*, systematically reviewed the genetic epidemiology of HHL in the Arab world that there are common and unique variants to Arab (103) . HHL variants were identified in 44 genes in 17 out of 22 Arab countries with 20% of variants in *GJB2* gene, 15% in *MYO7A* gene, 8% in *SLC26A4* gene, 5% in *MYO15A* gene, and 4% in *MYO6* gene, with only one or two variants identified in each of the remaining genes. The most commonly observed variant was c.35delG in *GJB2* gene, and it was found in half of the Arab countries. Of all the captured variants, 56 variants were found to be unique to Arabs NSHL patients. Even though mainly associated with severe to profound HL, variable clinical presentations were still observed in association with these variants. Of those 56 variants,12 variants were reported in patients from Qatar (103).

### *2.5.1 Nonsyndromic hearing loss in Qatar*

Six studies have already discussed HL in Qatar. Bener *et al.* (2005) reported that the prevalence of HL in Qatar is 5.2% and that HL is more common in consanguineous families (4). In another study, Girotto *et al.* (2014) highlighted the positive association between consanguinity and HHL in six families, as well as the minor contribution of

*GJB2* variation to HHL. The study also reported three genes, *BDP1*, *LOXHD1,* and *MYO15A*, and their association with variable clinical presentations of NSHL in Qatari patients (104). Moreover, another study conducted by Alkowari *et al.* (2012) on 126 Qatari patients with HHL revealed that the *MT-RNR1* mitochondrial variant (m.1555A<G) and variants in *GJB2* and *GJB6* genes are not common in the population of Qatar (105). Another study by Alkowari *et al.* uncovered the genetic etiology of 50% of their NSHL patients using a targeted sequencing approach that analyzed 81 genes concurrently. Causative variants were identified in six genes, including *CDH23, MYO6, GJB6*, *OTOF, TMC1,* and *OTOA.* Cases caused by pathogenic variants in *CDH23, MYO6,* and *GJB6* were inherited in an AR manner, while cases caused by pathogenic variants in *TMC1*, *OTOA*, and *OTOF* were inherited in an AD manner. The patients carrying variants in *TMC1*, and *OTOF* presented with severe to profound SNHL, while patients with the *OTOA* variants, were associated with moderate HL (106). The study identified c.6614C>T in *CDH23* as the first novel variant associated with severe to profound HL in Qatar. Vozzi *et al.* identified two additional novel variants in the population of Qatar: c.1588G>T in *LOXHD1* gene, which is associated with NSHL, and p.E152Gfs in *MYO15A* gene, which is associated with early-onset bilateral NSHL (107). A study performed by Girotto *et al.* (2014) found a fourth novel variant c.7873T>G in *BDP1* that i*s* associated with early-onset progressive NSHL in patients from Qatar (108).

The clinical approach for genetic testing in cases of suspected HHL follows a sequential order in Qatar, in line with the ACMG guidelines and recommendations. First-tier genetic testing includes *GJB2* gene sequencing with or without chromosomal microarray. If first-tier genetic testing is negative or inconclusive, second-tier genetic testing is offered via more comprehensive genetic tests such as gene panels, WES (with

or without mitochondrial genome sequencing and deletion testing).

Our study is the first to include a comprehensive chart review of pediatric cases of NSHL in Qatar. The study retrospectively analyzes pediatric patients with NSHL referred to the Clinical and Metabolic Genetics department at Hamad Medical Corporation (HMC), seen between 2014 and 2019, and underwent at least one genetic test. All with aim understand the spectrum of genetic variation associated with NSHL in the population of Qatar and to compare the diagnostic yield of the various genetic tests offered in the clinical setting.

# CHAPTER 3: MATERIALS AND METHODS

## *3.1 Ethical approval*

The project was approved by the Medical Research Center at HMC under the protocol number MRC-01-21-614 (Appendix A). The study was also approved by the Institutional Review Board (IRB) of Qatar University under the approval number QU-IRB 1578-E/21 (Appendix Figure B).

## *3.2 Study design*

A retrospective chart review was conducted at HMC for pediatric patients referred to the Clinical and Metabolic Genetics department at HMC due to their diagnosis of NSHL. The complete database contained more than 20,000 entries and was screened for eligible participants for this study.

### 3.2.1 Inclusion criteria

The patients who met all of the below criteria were included in this study:

1- Pediatric patient (< 18 years old) at the time of presentation
2- Established diagnosis of NSHL as per the chief complaint
3- Diagnosis of NSHL was made between 2014 and 2019
4- At least one genetic testing was conducted for the diagnosis of NSHL

### 3.2.2 Exclusion criteria

The patients who met at least one of the following criteria were excluded from the study:

1- Age older than 18 years
2- Having HL types other than NSHL such as acquired HL or SHL. The diagnosis of SHL was established based on the documentation of the patient presenting with HL and additional syndromic features.
3- No genetic tests were conducted for the molecular diagnosis of NSHL.

*3.3 Data collection*

The following data was extracted from the charts of the eligible patients: demographic information, audiometric, and genetic testing results. The diagnostic genetic tests considered in the study included all tests offered by the Clinical and Metabolic Genetics department at HMC: *GJB2* gene sequencing, chromosomal microarray, targeted familial variant testing, gene panel, WES, and mitochondrial genome sequencing and deletion testing. The first two above-mentioned genetic tests are offered in-house at HMC and free of charge. The other genetic tests are conducted in an external laboratory abroad (GeneDx, USA) and are offered free of charge only for Qatari patients. The hearing loss gene panel offered by GeneDx includes 146 nuclear genes and 6 variants in 4 mitochondrial genes, mainly aimed at detecting genetic causes of NSHL.

We also extracted the genetic findings from the genetic testing reports, including the gene name, the coding DNA (cDNA) description of the variant, the transcript number, the protein change, the zygosity status, the associated disorder, and the inheritance pattern. Then, genetic findings were classified into three main categories, based on their clinical interpretation provided by the Molecular Genetics laboratory or the Cytogenetic laboratory at HMC or by GeneDx, USA:

1- Positive findings (*i.e.* diagnostic findings):

    These include pathogenic or likely pathogenic variants in genes associated with NSHL with consistent zygosity status causing the disease, as well as VUSs considered likely pathogenic after family segregation analysis. This category represents the diagnostic findings.

2- Negative findings:

    These include two subcategories. The first subcategory refers to benign

findings including variants considered likely benign after family segregation analysis, variants in genes associated with NSHL with a zygosity status does not cause the disease (*i.e* variant only causes disease in homozygous state but identified in the study in heterozygous state), and findings in genes not associated with HL. The second subcategory refers to tests that yielded no genetic findings.

3- Findings of unknown clinical significance:

These include variants in genes associated with NSHL that are however classified as VUS by ACMG classification based on the available evidence at the time of testing.

*3.4 Analysis of findings of unknown clinical significance*

Using the variant Reference SNP (rs) number and the cDNA change or the protein change coupled with the gene name. The VUSs were further analyzed by searching public databases/archives such as the Single Nucleotide Polymorphism Database (dbSNP) (including ACMG classification) and ClinVar to acquire more information on the possible clinical significance of these variants and to capture any publications reporting or investigating these variants. We also used Ensemble (https://asia.ensembl.org/index.html) to acquire information on the allele frequencies in Genome Aggregation Database (gnomAD) and Allele Frequency Aggregator (ALFA), as well as the pathogenicity prediction scores of SIFT and polyphon (Appendix C).
The analysis of Copy Number Variants (CNVs) was performed differently by looking them up in search engines, including Google Scholar, PubMed, and ScienceDirect, to retrieve possible publications containing those CNVs.

This additional analysis aims to obtain further insights into the potential pathogenicity or clinical significance of the VUSs detected in our study cohort.

*3.5 Analysis of factors influencing diagnostic yield*

In the literature, some factors are reported to impact the diagnostic yield of genetic testing in the context of NSHL such as inheritance pattern, family history, consanguinity, severity, laterality, and onset (109). Here, we assessed the frequency of such factors among the patients with diagnostic findings.

*3.6 Statistical analysis*

Statistical analysis was conducted to assess the impact of the different genetic tests (*GJB2* gene sequencing, chromosomal microarray, gene panel, WES, and mitochondrial genome sequencing and deletion testing) on the overall diagnostic yield. Chi-square Tests (or Fisher exact tests for cells with less than five counts) were applied to look for significant associations. $P<0.05$ (2 tailed) was considered statistically significant. Stata, version 16 (StataCorp, College Station, TX), was used for the analysis. Targeted testing of specific familial causative variants was not included in this analysis, as it is not part of the sequential testing routinely performed at HMC but rather applicable only in families with a previously known genetic diagnosis of NSHL.

# CHAPTER 4: RESULTS

## *4.1 Eligible study participants and their demographic characteristics*

Initially, 20,000 entries from the database of the Clinical and Metabolic Genetics department were screened, of which 19,512 patients were excluded due to having diseases other than HL. The 488 remaining cases had HL and were further filtered based on the inclusion criteria mentioned in the methodology section. Accordingly, 360 cases were excluded for the following reasons: 36.4% were not referred within the selected time interval of the study, 21.4% were older than 18 years, 19.7% had no genetic testing conducted, 12.8% had SHL, 3.9% were suspected to have HL but later confirmed to have normal hearing assessment, 3.9% had cancelled appointments, and 1.9% had a family history of HL but were not affected (Figure 1). We analyzed a total of 128 (Qatari: N=39, 30.5%; Non-Qatari: N=89, 69.5%) individuals from 101 families with NSHL. The male to female ratio was 1:1 (Male: N = 65, 50.8%; Female: N = 63, 49.2%). Consanguinity and positive family history were observed in 79 (61.7%) and 76 (59.4%) of the NHLS cases, respectively. The analysis of patients' background revealed 19 nationalities (Algerian, American, British, Egyptian, Emirati, Ethiopian, Filipino, Indian, Iranian, Italian, Jordanian, Palestinian, Pakistani, Qatari, Saudi, Sudanese, Syrian, Tunisian, and Yemeni). The nationalities were distributed as follows, Qataris represented 30.5% (39 patients), Pakistanis accounted for 17.2% (22 patients), and Egyptians made up 11.7% (15 patients) of the study cohort; Syrians and Indians each represented 6.3% (8 patients), Sudanese, Palestinians, and Yemenis each represented 4.7% (6 patients), while Jordanians represented 2.3% (3 patients), Tunisians, Filipinos, Americans, and British each represented 1.6% (2 patients), and the remaining nationalities had a single patient each.

In terms of age of onset, 72 (56.3%) patients had congenital onset NSHL, i.e. HL

detected by the neonatal hearing screening program, while 47 (36.7%) patients had childhood-onset HL. In terms of type of NSHL, the most common type was SNHL seen in 113 (88.3%) cases, followed by auditory neuropathy in 4 (3.1%) patients and conductive HL in two (1.6%) patients.

In terms of disease severity, 43 (33.6%) patients presented with severe to profound HL, 34 (26.6%) patients had mild to moderate HL, 17 (13.3%) patients had moderate to severe HL, and 14 (10.9%) patients had progressive HL. 114 (89.1%) patients had bilateral HL, while 13 (10.2%) patients had unilateral HL. 74 (57.8%) patients used a hearing aid, 23 (18%) patients had a cochlear implant, 12 (9.4%) patients had both a hearing aid and a cochlear implant, while 19 (14.8%) patients did not use any hearing tool. 80 (62.5%) patients were reported to have speech delay, while 32 (25%) patients had no speech delay. 28 (21.9%) patients had school difficulties while 34 (26.6%) patients reported not to have school difficulties (Table 1).

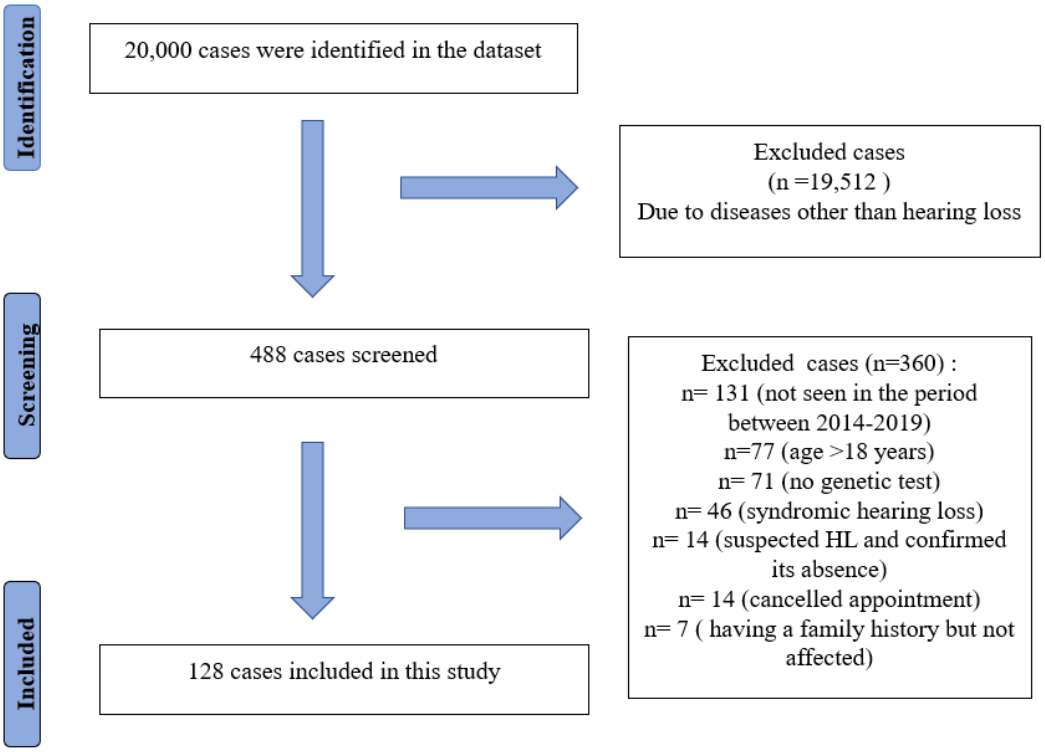


Figure 1. The process of case screening and selection of eligible patients for inclusion in the study.

Table 1. Demographic and Clinical Characteristics of the 128 Eligible NSHL Cases

| **Characteristics** | **N (%)** |
|---|---|
| **Gender** | |
| Male | 65 (50.8%) |
| Female | 63 (49.2%) |
| **Ethnicity** | |
| Qatari | 39 (30.5%) |
| Non-Qataris (18 Nationalities) | 89 (69.5%) |
| **Consanguinity** | |
| Yes | 79 (61.7%) |
| No | 24 (32.8%) |
| NR | 7 (5.5%) |
| **Family history** | |
| Yes | 76 (59.4%) |
| No | 48 (37.5%) |
| NR | 4 (3.1%) |
| **Age of onset** | |
| Congenital | 72 (56.3%) |
| Childhood | 47 (36.7%) |
| NR | 11 (8.6%) |
| **Type of hearing loss** | |
| Sensorineural hearing loss | 113 (88.3%) |
| Conductive | 2 (1.6%) |
| Auditory neuropathy | 4 (3.1%) |
| Not specified | 9 (7.0%) |
| **Severity** | |
| Mild to moderate | 34 (26.6%) |
| Moderate to severe | 17 (13.3%) |
| Severe to profound | 43 (33.6%) |
| Progressive | 14 (10.9%) |
| NR | 20 (15.6%) |
| **Laterality** | |
| Bilateral | 114 (89.1%) |
| Unilateral | 13 (10.1%) |
| NR | 1 (0.8%) |
| **Usage of hearing tools** | |
| Hearing aid | 74 (57.8%) |
| Cochlear implant | 23(18%) |
| Hearing aid and cochlear implant | 12 (9.4%) |
| No hearing tool | 19 (14.8%) |
| **History of speech delay** | |
| Yes | 80 (62.5%) |
| No | 32 (25%) |
| NR | 16 (12.5%) |

| Characteristics | N (%) |
|---|---|
| **History of school difficulties** | |
| Yes | 28 (21.9%) |
| No | 34 (26.6%) |
| NA | 31 (24.2%) |
| NR | 35 (27.3%) |

**NR: Not reported*

*4.2 Genetic findings and diagnostic yield*

In this study, 55 variants in 40 genes, in addition to 11 CNVs were identified. Out of these 55 variants, 36 (65.5%) variants were missense, 8 (14.5%) were frameshift, 5 (9.1%) variants were deletions, 4 (7.3%) were intronic, one (1.8 %) nonsense, and one (1.8%) duplication variants.

Among the available six genetic tests, the highest utilization frequency was for *GJB2* gene sequencing (103 patients, 80.5%), followed by chromosomal microarray (65 patients, 50.8%), WES without mitochondrial genome sequencing and deletion testing (31 patients, 24.2%), WES coupled with mitochondrial genome sequencing and deletion testing (20 patients, 15.6 %), gene panel (10 patients, 7.8%), and lastly, targeted familial variant testing (5 patients, 3.9%) (Table 2).

The overall diagnostic yield, which represents the number of times the utilized genetic test identified a causative variant, was 30.5%, equivalent to 39 solved cases out of the total 128 cases. The highest diagnostic yield per test was achieved by targeted familial variant testing (60%), followed by gene panel (50%), WES (41.9%), and *GJB2* gene sequencing (16.5%). In contrast, mitochondrial genome sequencing and deletion testing alone did not give any positive findings. Only three genetic tests produce significant yield (Table 2). The contribution of each genetic test to the overall positive findings of the study is indicated in Figure 4.

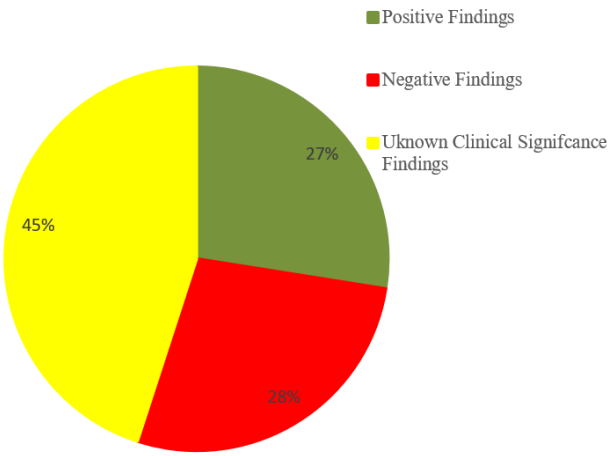


Figure 2. A pie chart representing the three categories of genetic findings identified in this study.

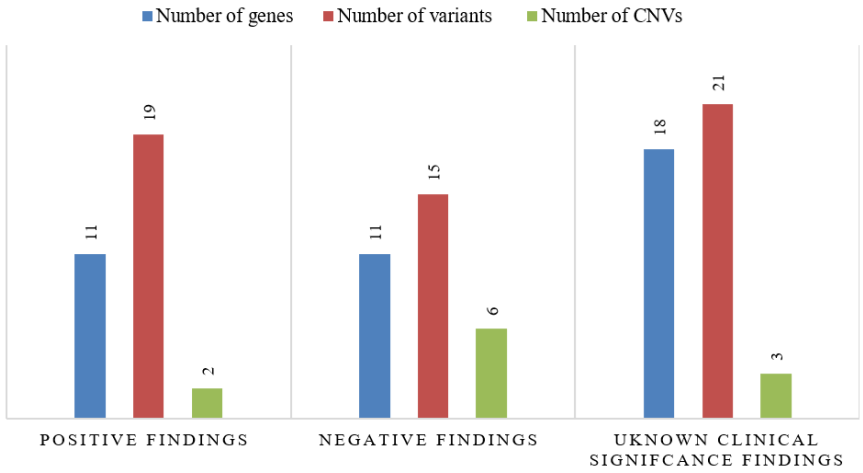


Figure 3. A bar chart illustrating the number of variants, genes, and copy number variants associated with each of the three categories of findings.

Table 2. The Utilization Frequencies of the Different Genetic Tests and their Associated Diagnostic Yields

| **Genetic test** | **Utilization frequency** | **Positive findings** | **Unknown significance findings** | **Negative findings** | |
|---|---|---|---|---|---|
| | | | | **Benign** | **No Finding** |
| *GJB2* gene Sequencing * | 103 (80.5%) | 17 (16.5%) | 0% | 3 (2.9%) | 83 (80.6%) |
| Chromosomal microarray | 65 (50.8%) | 1 (1.5%) | 4 (6.2%) | 3 (4.6%) | 57 (87.7%) |
| Targeted familial variant test | 5 (3.9%) | 3 (60%) | 0% | 0% | 2 (40%) |
| Gene panel * | 10 (7.8%) | 5 (50%) | 4 (40%) | 0% | 1 (10%) |
| WES | 31 (24.2%) | 13 (41.9%) | 8 (25.8%) | 1(3.2%) | 8 (25.8%) |
| Mitochondrial genome testing * | 20 (15.6%) | 0 | 0 | 4 (20%) | 16 (80%) |
| Total | - | 39 (30.5%) | - | - | - |

* Indicates a test that was statistically significant (p-Value of <0.05).
The Mitochondrial genome testing is conducted with WES.

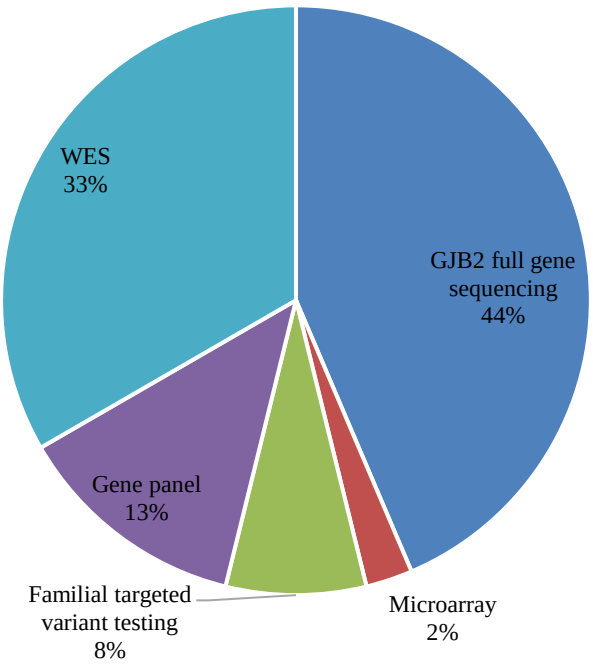


Figure 4. A pie chart showing the contribution of the different genetic tests to the overall diagnostic findings.

*4.3 Causative variants identified*

A total of 19 variants in 11 genes and two CNVs were causing NSHL in our study cohort (Tables 3 and 4). 36.8% of all causative variants associated with NSHL were found in *GJB2 (*7 variants), while 15.8% were found in the *OTOF* gene (3 variants). All other genes had a single causative variant, as given in Table 3. The two CNVs overlap the *STRC* gene, which is known to be associated with NSHL.

The causative variants were detected in patients from 11 nationalities (Qatari, Pakistani, Egyptian, Syrian, Indian, Palestinian, Jordanian, Tunisian, Filipino, Ethiopian, and Algerian). The distribution of the causative variants across the different nationalities is indicated in Figure 5.

Moreover, findings of unknown clinical significance were also obtained in this cohort, including 21 variants in 18 genes and 3 CNVs, which will be discussed in depth

in the next section. In addition, the negative findings category included 7 nuclear genes and 4 mitochondrial genes with a total of 15 variants (Appendix D) and 6 CNVs (Appendix E). The focus of our study is on the positive and unknown clinical significance findings.

Table 3. Causative Variants in Genes Associated with NSHL

| Gene | Amino Acid change | cDNA change | Variant class | Zygosity | Mode of inheritance | Classification* | Qatari cases | Non-Qataris |
|---|---|---|---|---|---|---|---|---|
| *GJB2* | p.G12Vfs | c.35delG | Fs | HM | AR | Pathogenic | 0 | 9 |
| | | c.−23G>T | Intronic | HM | AR | Pathogenic | 1 | 0 |
| | p.R143W | c.427C>T | M | HM | AR | Pathogenic | 0 | 1 |
| | p.C169Y | c.506G>A | M | HM | AR | pathogenic | 3 | 0 |
| | p.Tyr97* | 290dup | Fs | HM | AR | Pathogenic | 0 | 1 |
| | p. V37I | c.109G>A | M | HM | AR | Pathogenic | 0 | 1 |
| | - | c.−23+1G>A | Intronic | HM | AR | Pathogenic | 1 | 3 |
| *OTOF* | p.R1792H | c.5375G>A | M | HM | AR | Pathogenic | 1 | 0 |
| | p.E747X | c.2239G>T | Ns | HM | AR | pathogenic | 3 | 0 |
| | p.G541S and p.E747X | c.1621G>A and c.2239 G>T | M & Ns | CH | AR | Pathogenic | 1 | 0 |
| *ABHD12* | Partial deletion | - | Del | HM | AR | Pathogenic | 1 | 0 |
| *STRC* | 15q15.3 del | - | Fs | HM | AR | Pathogenic | 1 | 0 |
| *ESPN* | p.W753R | c.2257T>C | M | HM | AR | Likely pathogenic | 2 | 0 |
| *FGF3* | p.R95W | c.283C>T | M | HM | AR | Pathogenic | 1 | 0 |
| *MYO15A* | p.T2780== | c.8340G>A | M | HM | AR | Pathogenic | 0 | 1 |
| *SLC26A4* | p.C400VfsX32 | c.1198delT | Fs | HM | AR | Pathogenic | 0 | 3 |
| *TMIE* | p.E31G | c.92A>G | M | HM | AR | Pathogenic | 1 | 0 |
| *TMPRSS3* | p.V116M | c.346G>A | M | HM | AR | Likely pathogenic | 1 | 0 |
| *TRIOBP* | p.R399X | c.1195C>T | M | HM | AR | Likely pathogenic | 1 | 0 |

AR: autosomal recessive, CH: compound heterozygous, Del: deletion, Fs: frame shift, HM: homozygous, M: missense, Ns: nonsense.
* The classification of the variants is based on GeneDx report.

Table 4. CNVs Associated with NSHL

| **CNV** | **Cytogenetic Band** | **Zygosity** | **Mode of inheritance** | **Classification** | **Qatari cases** | **Non-Qatari cases** |
|---|---|---|---|---|---|---|
| Deletion of 51 KB involving *STRC* and *CATSPER2* | 15q15.3 | HM | AR | Pathogenic | 1 | 0 |
| Duplication of 71 kb containing *STRC* *CATSPER2* and *CKMT1A* genes | 15q15.3 | HM | NR | Pathogenic | 0 | 1 |

AR: autosomal recessive, HM: homozygous, NR: not reported.

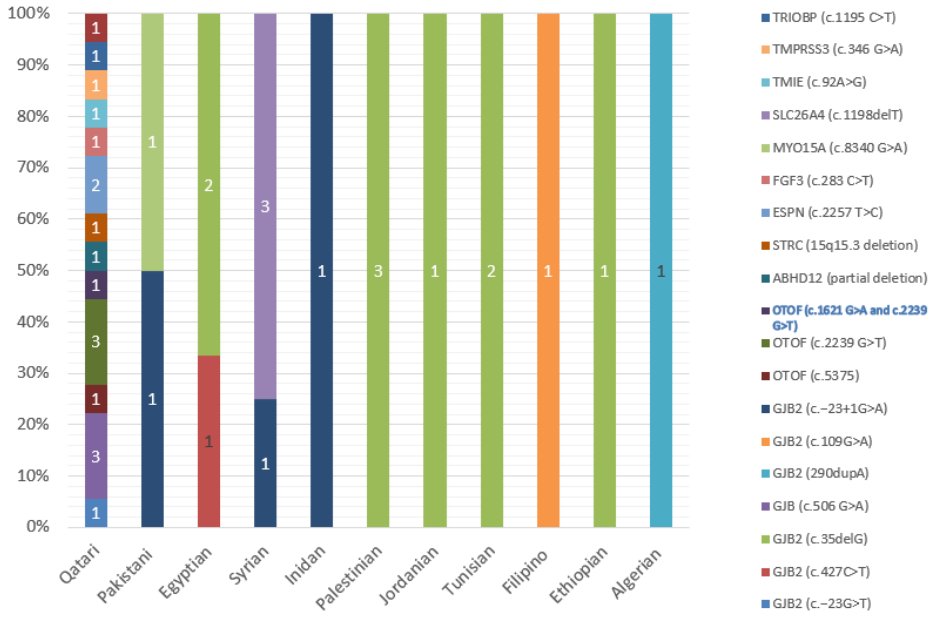


Figure 5. A bar chart representing the distribution of the 19 causative variants across different nationalities.

*4.4 Analysis of variants and copy number variants of unknown significance*

Initially, the findings of unknown clinical significance included 21 variants in 18 genes and 3 CNVs (Table 5 and 6). Those variants were re-analyzed to collect further evidence for possible re-classification, as indicated in the methodology section. Based on this analysis, eight variants could potentially contribute to NSHL our cohort, including *c*.3641G>A, p.R1214Q [NM_016239.4] in *MYO15A*, c.6503T>G, p.L2168R [NM_016239.4] in *MYO15A*, c.599C>T, p.T200I [NM_015404.3] in *WHRN*, c.2476G>A, p.A826T [NM_000260.4] in *MYO7A*, c.4696A>T, p.T1566S [NM_000260.3] in *MYO7A*, c.-182G>A and c.617-3_617-2 dup in *TMPRSS3*, and *c*.98G>A, p.R33Q [NM_194248.3,] in *OTOFI*. None of these variants were seen in more than one patient, and none of the potential causative VUS were reclassified using reclassified using family segregation studies, in which zygosity status of the parents and siblings are determined with aim to explain the association of the variant of interest to causing disease.

Table 5. Variants of Unknown Clinical Significance Identified in This Study

| Gene | Amnio Acid change | cDNA change | Variant class | Zygosity | Mode of inheritance | Classification by GeneDx | Qatari cases | Non-Qatari Cases |
|---|---|---|---|---|---|---|---|---|
| *COL11A1* | p.Q1509P | c.4526A>C | M | HT | AR/AD | VUS | 1 | 0 |
| *GJB6* | p.P70L | c.209C>T | M | HT | AR/AD | VUS | 1 | 0 |
| *TECTA* | p.F860I | c.2578T>A | M | HT | AR/AD | VUS | 1 | 0 |
| *TJP2* | p.R682W | c.2044C>T | M | HT | AR/AD | VUS | 1 | 0 |
| *WFS1* | p.A874T | c.2620G>A | M | HT | AR/AD | VUS | 1 | 0 |
| *COL11A2* | p.T323HfsX19 | c.966dupC | Fs | HT | AR/AD | Likely pathogenic | 1 | 1 |
| *COL4A4* | p.R724H | c.2171G>A | M | HT | AR/AD | VUS | 0 | 1 |
| *GJB3* | p.L218_D221del | c.652_663del12 | Del | HT | AR/AD | VUS | 0 | 1 |
| *MYO3A* | p.D227G | c.680A>G | M | HT | AR/AD | VUS | 0 | 1 |
| *PCDH15* | - | c.*9-*13delTTCTT | Del | HT | NR | VUS | 0 | 1 |
| *DSCAML1* | p.A181T | c.541G>A | M | HT | Not described | Not described | 1 | 0 |
| *KCNQ4* | p.F104L | c.310T>C | M | HT | AD | VUS | 1 | 0 |
| *MYO15A* | p. R1214Q | c.3641G>A | M | HM | AR | VUS | 0 | 1 |
| | p.L2168R | c.6503T>G | M | HM | AR | VUS | 1 | 0 |
| *WHRN* | p.T200I | c.599C>T | M | HM | AR | VUS | 1 | 0 |
| *MYO7A* | p.A826T | c.2476G>A | M | CH | AR/AD | Pathogenic | 0 | 1 |
| | p.T1566S | c.4696A>T | M | CH | AR/AD | VUS | 0 | 1 |
| *OTOF* | p.R33Q | c. 98G>A | M | HM | AR | VUS | 1 | 0 |

| Gene | Amnio Acid change | cDNA change | Variant class | Zygosity | Mode of inheritance | Classification by GeneDx | Qatari cases | Non-Qatari Cases |
|---|---|---|---|---|---|---|---|---|
| *TMPRSS3* | - | c.-182G>A | Intronic | CH | AR | VUS | 1 | 0 |
| | - | c.617-3_617-2dup | Dup | CH | AR | VUS | 1 | 0 |
| *SLC12A2* | p.N168D | c.502A>G | M | HT | NR | VUS | 1 | 0 |

HT: heterozygous, HM: homozygous, CH: compound heterozygous, AR: autosomal recessive, AD: autosomal dominant, VUS: variant of unknown significance, M: missense, Fs; frame shift, Del: deletion, Dup: duplication, NR; not reported.

Table 6. The CNVs of Unknown Clinical Significa

| CNV | Cytogenetic band | Zygosity | Mode of inheritance | Classification | Qatari cases | Non-Qatari cases |
|---|---|---|---|---|---|---|
| Deletion of 244 kb | 2p22.1 | HT | - | VUS | 0 | 1 |
| Duplication of 740 kb | 9q33.1 | HT | - | VUS | 0 | 1 |
| Duplication of189 kb | 15q13.2 | HT | - | VUS | 0 | 1 |

HT: heterozygous, VUS: variant of unknown significance.

Table 7. Frequencies of the VUSs with Pathogenicity Scores and Allele Frequencies

| **Gene** | **cDNA change** | **Rs ID** | **Zygosity** | **ClinVar** | **SIFT** | **PolyPhen** | **Allele frequency** | **ALFA (allele)** | **gnomAD (allele)** | **Evidence** |
|---|---|---|---|---|---|---|---|---|---|---|
| *COL11A1* | c.4526A>C | rs1057521422 | HT | VUS | Tol (0.3) | Benign (0.328) | 0.008 | 0.000165 (G) | 0.0000069 (G) | - |
| *GJB6** | c.209C>T | rs727505123 | HT | VUS | Delet (0) | Damaging (1) | 0.008 | 0.000041 (A) | 0.000 (A) | (110) |
| *TECTA* | c.2578T>A | - | HT | - | - | Benign (0.027) | 0.008 | - | - | - |
| *TJP2* | c.2044C>T | rs760622082 | HT | NR | Delet (0) | Damaging (1) | 0.008 | 0.000 (T) | 0.0000349 (T) | - |
| *WFS1** | c.2620G>A | rs200775335 | HT | VUS | Delet (0) | Probably damaging 0.9 | 0.008 | 0.0000 (A) | 0.00006 (A) | (111) |
| *COL11A2** | c.966dupC | rs748440351 | HT | Conf | - | - | 0.0016 | 0.0002459 | 0.000083 | (112) |
| *COL4A4* | c.2171G>A | rs753659852 | HT | VUS | Tol (1) | Probably damaging (0.995) | 0.008 | 0.000 (T) | 0.000006983 (T) | (113) |
| *GJB3** | c.652_663del12 | rs727503069 | HT | Conf | - | - | 0.008 | 0.001 (C) | 0.0002094 (C) | (114) |

| Gene | cDNA change | Rs ID | Zygosity | ClinVar | SIFT | PolyPhen | Alle frequency in the Study | ALFA (allele) | gnomAD (allele) | Evidence |
|---|---|---|---|---|---|---|---|---|---|---|
| *MYO3A* | c.680A>G | rs1842309816 | HT | - | - | Damaging (1) | - | - | - | - |
| *PCDH15* | c.*9-*13delTTCTT | - | HT | - | - | - | -- | - | -- | - |
| *DSCAML1* | c.541G>A | - | HT | - | - | - | - | - | - | - |
| *KCNQ4* | c.310T>C | rs866433910 | HT | - | Tol (0.3) | Benign (0.155) | 0.008 | - | - | - |
| *MYO15A* | c.3641G>A** | rs376676996 | HM | VUS | Tol (0.17) | Benign (0.003) | 0.008 | 0.000 (A) | 0.0000139 (A) | - |
| | c.6503T>G** | rs1567648703 | HM | Path | Delet (0) | Probably damaging (0.999) | 0.008 | - | - | (115) |
| *WHRN*** | c.599C>T | rs765757659 | HM | - | Delet (0) | Possibly damaging (0.906) | 0.008 | 0.000 (A) | - | - |
| *MYO7A*** | c.2476G>A | rs368341987 | CH | Path | Delet (0) | Benign (0.119) | 0.008 | 0.0004918 (A) | 0.0003069 (A) | (116, 117) |
| | c.4696A>T | rs552367391 | CH | - | Tole (0.85) | Benign (0.003) | 0.008 | - | 0.0000139 6 (T) | - |

| Gene | cDNA change | Rs ID | Zygosity | ClinVar | SIFT | PolyPhen | Alle frequency in the Study | ALFA (allele) | gnomAD (allele) | Evidence |
|---|---|---|---|---|---|---|---|---|---|---|
| *OTOF*** | c. 98G>A | rs56332208 | HM | Conf | Delet (0.01) | Probably damaging (1) | 0.008 | 0.0003181 (T) | 0.007 (T) | - |
| *TMPRSS3** * | c.-182G>A | rs147296608 | CH | VUS | - | - | 0.008 | - | - | - |
| | c.617-3_617-2dup | - | CH | - | - | - | 0.008 | - | -- | - |
| *SLC12A2* | c.502A>G | - | HT | - | - | - | 0.008 | - | - | - |

HT: heterozygous, HM: homozygous, CH: compound heterozygous, VUS: variant of unknown significance, Tol: tolerated, Delt:deleterious, Path: pathogenic, NR: not reported, Conf: conflicting

** Indicates the genes that are more likely contributing to NSHL after conducting the VUS analysis.

* Indicates the variants that are less likely contributing to NSHL after conducting the VUS analysis.

Variants with no asterisk are still VUS.

*4.5 Factors influencing diagnostic yield*

All patients with diagnostic findings had AR NSHL. 29 out of 39 (74.4%) patients had a positive family history of HL, while 9 (23.1%) patients had a negative family history. 27 out of 39 (69.2%) patients reported a history of consanguinity, while 10 (25.6%) reported no consanguinity. In terms of severity, 16 (41.0%) patients had severe to profound HL, while 10 (25.6%) patients had mild to moderate HL. All patients with diagnostic findings had bilateral SNHL, and 28 (71.8%) patients had congenital onset HL comparing to 9 (23.1%) patients has childhood onset HL (Figure 6).

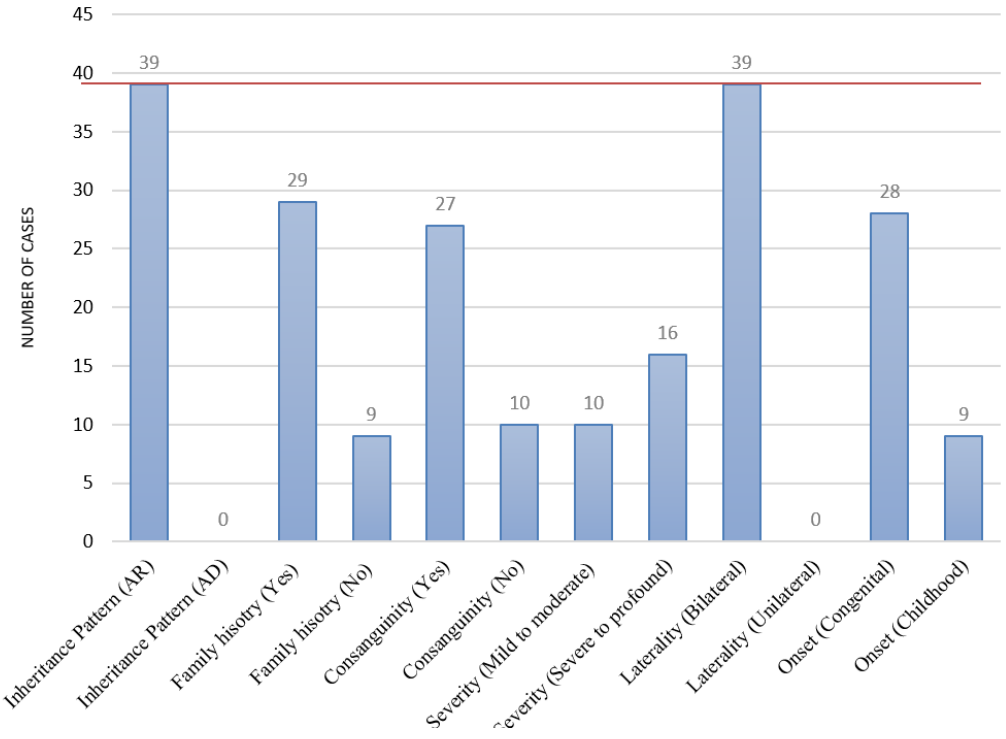


Figure 6. A bar chart indicating the number of positive cases identified across the different diagnostic factors assessed. The horizontal black line represents the total number of cases with positive findings (39 cases).

# CHAPTER 5: DISCUSSION

This project focused on analyzing the genetic basis of NSHL to identify causative variants and genes associated with NSHL in the population of Qatar and to evaluate the diagnostic yield of different genetic tests. 128 pediatric patients were eligible. The percentage of the excluded cases due to SHL (46 patients, 12.8%) was lower than the reported percentage in the literature (30%) (118), this might be due to the fact that SHL cases were not properly captured form the initial screening phase as it was not the main focus of the study. The study revealed 55 variants in 40 genes with 11 CNVs in the studied 128 pediatric patients. 39 cases (30.5%) were genetically diagnosed due to 19 causative variants in 11 genes and 2 causative CNVs in the population of Qatar, with the *GJB2* variant c.35delG, p.G12Vfs [NM_004004.6] being the most common pathogenic variant in the pediatric population of Qatar. This study also identified a previously known founder variant c.506G>A, p.C169Y [NM_004004.6] in *GJB2*. We also reported c.2257T>C, p.W753R in *ESPN* [NM_031475.3] as likely pathogenic variant for the first time globally. This study did not identify any novel genes.

## 5.1 Causative variants

36.8% of the identified causative variants were in *GJB2* gene and occurred in 51.3% of the patients genetically diagnosed (20 patients), making *GJB2* pathogenic variants the most common cause of NSHL in our pediatric cohort. Historically, variation in *GJB2* was found to be a significant cause of NSHL in different populations, such as in Germans (119), Northern Europeans (120), Mediterraneans (121), Indians (122), and Chinese (123). In the context of Arab countries, pathogenic *GJB2* variants have also been commonly identified in patients with genetically diagnosed Arab patients from the United Arab Emirates

(UAE) (18% of diagnostic findings) (124), Egypt (14.4% of diagnostic findings) (125), the Kingdom of Saudi Arabia (KSA) (10.1% of diagnostic findings) (126), and Mauritania (9.4% of diagnostic findings) (127). Moreover, in a systematic review assessing the prevalence of *GJB2*-related HL globally, pathogenic *GJB2* variants were found to be a common cause of HL with a different distribution of variants across ethnicities (128), which is in agreement with our study findings.

On the contrary, some previous reports from Qatar suggested that variation in *GJB2* is not a significant cause of NSHL (104, 105). Such a discrepancy over the contribution of *GJB2* variation to the genetic basis of NSHL in Qatar could be attributed to the genetic heterogeneity of the population of Qatar and the fact that the majority of participants in the studies contradicting ours were of Arabian origin or Bedouins (104), while our study participants were of a more diverse ethnic background. The frequency of *GJB2* variants in Qataris with positive genetic diagnosis is 21.1% (4 out of 19 patients), and 71. 4% (10 out of the 14 genetically diagnosed Arab's patients) had variants in *GJB2*.

Variants in other genes such as *LOXHD1*, *BDP1*, and *MYO15A* were previously reported as contributing to NSHL in patients of Qatari origin (104). However, no causative variants were detected in *LOXHD1* and *BDP1* in our study, reflecting the genetic heterogeneity of the condition and our population. At the variant level, c.35delG in *GJB2* was the most common variant in our cohort, observed in 23.1% of the patients with positive diagnostic findings (all in homozygous state). This variant is a frameshift deletion resulting in early termination of the protein polypeptide structure at codon number 13 (129). It was identified in 9 (23.1%) patients out of the 39 genetically diagnosed cases in our cohort; all are of non-Qatari origin, including three Palestinians, two Egyptians,

two Tunisians, one Jordanian, and one Ethiopian. Moreover, this variant was reported in other Arab countries including, Algeria (130), Mauritania (127), Egypt (131), and UAE (124). In Tunisia, c.35delG was the most common variant, seen in 35% of NSHL patients and accounting for 85.4% of all variants identified in *GJB2* gene (132). Likewise, c.35delG was the most common variant in Morocco, observed in 35.80% of the patients with *GJB2* variants (133). Similarly in Kuwait, c.35delG was the most commonly identified variant, seen amongst 80% of patients with *GJB2* variants (134) (135). Additionally, this variant represents around 66.7% of *GJB2* variants in Europeans (141), and 70.7% of *GJB2* variants in American Caucasians (136). Thus, our study is in agreement with other findings from the Arab region as well as other regions regarding the predominance of the *GBJ2* c.35delG variant in patients with NSHL.

The second most common causative variant was the c.-23+1G>A [NM_004004.6] in *GJB2,* seen in four patients from different nationalities (Qatari, Syrian, Pakistani, and Indian). This variant was also reported to be the second most common variant in a cohort of Iranian patients (137). The variant has also been observed in patients from Egypt (125), France (138), Palestine (139), China (140), and India (141). This variant causes aberration in the mRNA splicing, leading to the absence of RNA production or the production of non-stable RNA (139). In our cohort, this variant was disease causing in homozygous state. However, in some other populations such as the Chinese population, the c.-23+1G>A variant in *GJB2* explained NSHL in around 2% of the patients in a heterozygous state. Similarly, this variant was reported in a heterozygous state in patients from Turkey (142). Given all publications, the variant contributes to the disease in both AR and AD manners (140). This variant is not commonly seen in

the literature in patients of Arab origin.

The variant c.2239G>T, p.E747X [NM_194248.3] in *OTOF* was also seen in four patients; three Qatari patients had it in a homozygous state and one Sudanese patient had it in a compound heterozygous state with another variant. The *OTOF* gene encodes otoferlin, a calcium-binding protein that has an integral role in exocytosis in the inner hair cell (143). This variant was observed in a homozygous state in patients from Libya (144). In addition, variations in *OTOF* gene were observed in patients with AR NSHL from several countries, including Japan (145), Pakistan (146), and Korea (147). The variant p.E747X in *OTOF* gene has been identified in a single family from Qatar before (106).

Considering our patients of Qatari origin, the most common causative variants were c.2239 G>T in *OTOF* gene and c.506G>A in *GJB2* gene, each was observed in three patients. Both variants were seen in 35.3% of genetically diagnosed Qatari patients. The variant c.506G>A in *GJB2* was identified in 7.7% of the patients (Qatari origin); all had it in homozygous state. The variant was reported in patients from Qatar previously as a founder variant, in which it was initially known globally to to be a polyparasitism (148), but in Qatari families it is diseases causing. Moreover, this variant was not observed in patients of Arabinan origin in the literature. We support the founder impact of this variant since it was only identified in Qatari patients.

The variant c.1198delT, p.C400VfsX32 in *SLC26A4* gene was observed in three Syrian patients in homozygous state (149). In some populations such as Europeans (150), Brazilians (151), and South Asians (152), pathogenic variation in the *SLC26A4* gene is known to be the second most common cause of NSHL

after *GJB2* variation. However, in our cohort, variation in *SLC26A4* ranks as the third most common cause of NSHL after variation in *GJB2* and *OTOF*. The c.1198delT variant identified in *SLC26A4* causes a frameshift that leads to a premature stop codon, resulting in the production of a nonfunctional protein (153). This variant was reported in patients with NSHL from Iran (154), Turkey (155), and Egypt (156) with no reported detailed phenotype.

The variant c.2257T>C in *ESPN* gene was reported in two of our 39 (5.1%) genetically diagnosed patients, both of whom had it in homozygous state and belonged to the same Qatari family. Variation in *ESPN* is associated with AD and AR NSHL (157, 158). c.2257T>C variant is reported to be of unknown clinical significance in ClinVar (rs869312937). However, in our cohort, the report issued by GeneDx classified the variant as likely pathogenic.

Variation in *ABHD12* has been associated with rare syndromic forms of HL such as polyneuropathy, hearing loss, ataxia, retinitis pigmentosa, and cataract syndrome (PHARC) (OMIM 612674) (159) and Usher syndrome type 3 (OMIM 276902) (160). However, in our cohort, *ABHD12* variation was detected in a patient with NSHL due to a partial deletion of *ABHD12*, which confirms the involvement of the gene in both SHL and NSHL (161).

The 15q15.3 deletion encompassing *STRC* gene was reported in a single patient in our cohort, in a homozygous state. Partial deletions of *STRC* gene were seen in 10% of patients from the Czech Republic and was the second most common genetic variation in that population after *GJB2* variation (61).

The variant c.283C>T, p.R95W [NM_005247.4] in *FGF3* was reported in a single Qatari patient in our cohort in homozygous state. The *FGF3* gene encodes

the protein fibroblast growth factor 3, which is important for the development of the inner ear during the embryonic period (162). The involvement of this variant in HL was only recently described in the literature in a Somali family. The variant c.283C>T was associated with HL, microdontia, and variable inner ear malformations (163). Historically, the c.283C>T variant was related to a syndromic form of HL of variable clinical presentation known as congenital deafness with labyrinthine aplasia, microtia, and microdontia also called LAMM syndrome (OMIM 610706) (164). However, we reported this variant to be associated with NSHL in Qatar for the first time.

The variant c.8340G>A, p.T2780= [NM_016239.4] in *MYO15A* was reported in our cohort in a single patient from Pakistan in homozygous state. The patient had severe to profound NSHL. Variation in *MYO15A* is considered as the third or fourth most common cause for NSHL (165). Moreover, this variant has been reported to cause NSHL in Arab patients in North Israel (166).

The variant c.92A>G, p.E31G [NM_147196.3] in *TMIE* was reported in our cohort in a single Qatari patient in in homozygous state. The *TMIE* gene encodes the transmembrane inner ear protein, which plays a role in the sensory development in both stereocilia bundle and sensory transduction in auditory neurons (167). Moreover, this variant has been reported in a family from Pakistan and Jordan with NSHL (168).

The variant c.346G>A, p.V116M [NM_032404.2] *TMPRSS3* was seen in a single Qatari patient in a homozygous state. This gene encodes the transmembrane serine protease 3, which plays a role in the development of cochlear hair cells through the potassium Kcnma1 channels (169). Variation in this gene is reported

to significantly contribute to the overall incidence of NSHL in China (170). There is disparity in the contribution of *TMPRSS3* variants to NSHL across different populations. For example, in the European population, *TMPRSS3* variants account for 0.45% of cases of NSHL in patients negative for *GJB2* variant 35delG (171). In contrast, *TMPRSS3* variants account for 5% of cases of profound AR NSHL in Tunisia (172). The c.346G>A variant was reported for the first time in Indian NSHL patients (173).

The variant c.1195C>T, p.R399X [NM_001039141.2] in *TRIOBP* gene was reported in one Qatari patient in homozygous state. The patient presented with congenital severe to profound NSHL. The gene encodes trio- and factin-binding protein, which is involved in cytoskeleton organization (174).

The two CNVs identified encompass the *STRC* and *CATSPER2* genes, although one CNV is a duplication and the other CNV is a deletion. The deletions of *STRC* and *CATSPER2* are reported in the literature as a common CNV associated with NSHL (34). CNVs involving *STRC* gene are considered the most common CNV associated with NSHL, representing almost two-thirds of all CNVs related to NHSL (62).

Moreover, we did not detect any causative variants in *GJB6* gene, which is a common cause for NSHL in different populations (175). Our finding is in agreement with a previous report from Qatar suggesting a minor role for *GJB6* variation in NSHL in Qatar (105). Moreover, some other genes associated with NSHL in Qatari patients based on other reports such as *BDP1 (108), CDH23 (106), LOXHD1* (107) were not identified as causative genes in this cohort.

## 5.2 Genotype-phenotype correlation of causative variants

Establishing significant genotype-phenotype correlation might be challenging since the number of patients harboring a given variant is small. Herein, we only addressed the variants that were identified in more than one patient.

In our cohort, the variant c.35delG in *GJB2* is associated with childhood-onset NSHL in 55.5% of patients who harbor this variant and with congenital onset NSHL in the remaining patients. All patients presented with bilateral SNHL. However, 55.5% of the patients presented with moderate to severe SNHL, while 33% of the patients had severe to profound SNHL (data not shown). In comparison, patients from KSA with the c.35delG variant in *GJB2* presented mainly with profound HL (126). In Algeria, the variant was associated with congenital profound HL in all patients (130). However, in terms of severity, 63% of the patients harboring the variant presented with profound HL, while 29% presented with severe HL, and around 9% of the patients presented with mild to moderate HL (130). Also, there was a significant association between the c.35delG variant and profound HL (176). Given all, this variant seems to be associated with a less severe presentation in our cohort compared to what has been observed in other populations.

Most of our patients with the variant c.-23+1G>A in *GJB2* presented with bilateral, mild to moderate, congenital onset SNHL, while only one patient presented with progressive SNHL. Previously, the variant was reported in a Palestinian patient with congenital onset HL (139). In France, this variant was associated with profound HL (138).

In our cohort, the *OTOF* variant c.2239G>T was associated with auditory

neuropathy in 50% of patients and severe SNHL in the other 50%. This is consistent with previous a report associating OTOF gene variants with auditory nephropathy or severe to profound SNHL (144).

The variant c.506G>A in *GJB2* is associated with congenital, severe to profound SNHL in our cohort. Similarly, it was previously detected in two Qatari families with severe SNHL (148).

The variant c.1198delT in *SLC26A4* gene is associated with childhood-onset, severe bilateral SNHL in our cohort. However, in one patient the presentation was different, in which the patient presented with congenital severe to profound SNHL in one ear and moderate SNHL in the other ear.

The variant c.2257T>C in *ESPN* is not previously reported to be pathogenic by ClinVar. However, in our cohort, it was reported by GeneDx as likely pathogenic, and it was confirmed through family studies. The two relatives in whom this variant was identified presented with congenital onset, severe bilateral SNHL.

The two CNVs identified in our cohort involved mainly two genes: *STRC* and *CATSPER2*. Our patients presented with congenital onset, mild to moderate SNHL. Patients with CNVs involving *STRC* gene were reported to have mild SNHL in previous reports (177) (62).

5.3 Analysis of variants and copy number variants of unknown clinical significance

For variants of known significance, we collected further evidence to reassess whether they could play a role in NSHL pathogenesis

Out of the 21 investigated VUSs, 8 are likely contributing to NSHL in our cohort, including *c*.3641G>A in *MYO15A,* c.6503T>G in *MYO15A*, c.599C>T in *WHRN,*

*c.2476*G<A, and c.4696A>T in *MYO7A (*compound heterozygous), c.-182G>A and c.617-3_617-2 dup in *TMPRSS3 (*compound heterozygous), and *c.* 98G>A in *OTOF*. On the contrary, c.209C>T variant in *GJB6* gene, c.2620G>A in *WFS1* gene, c.652_663del12 in *GJB3*, and p.T323HfsX19 in *COL11A,* are less likely to be contributing to NSHL in our cohort.

The contribution of the following variants to NSHL in our cohort was based on them being inherited in either homozygous or compound heterozygous state: c.3641G>A and c.6503T>G in *MYO15A* (reported by the Department of Genetics, SQUH-Genetics Sultan Qaboos University Hospital in Oman to cause NSHL in homozygous state (115)), c.599C>T in *WHRN*, c.2476G>A and c.4696A>T in *MYO7A* (compound heterozygous*),* c.-182G>A and c.617-3_617-2dup in *TMPRSS3* (compound heterozygous) and c.98G>A in *OTOF*. Moreover, two of those variants, c.599C>T in *WHRN* and .98G>A in *OTOF* indicated a damaging impact on the protein structure using SIFT and PolyPhen. On the contrary, the only variant that showed benign and tolerated impact on the protein structure using PolyPhen and SIFT was c..4696A>T in *MYO7A*. Moreover, c.2476G>A in *MYO7A* is a known disease-causing variant in homozygous state, however, in our cohort it was identified in a compound heterozygous state with a VUS (4696A>T in *MYO7A).*

We have identified four variants that are less likely to be contributing to NSHL in our cohort for different reasons. First, the variant c.209C>T,p.P70L [NM_001110220.2] in *GJB6,* which has been reported to be a novel causative variant for NSHL in patients from Qatar in a homozygous state (110). However, in our study cohort, this variant was identified in a heterozygous state. Second, the variant c.2620G>A, p.A874T [NM_001145853.1] in *WFS1,* which was reported to cause NSHL in the homozygous state (111), while it was presented in a heterozygous state in

our patient. Third, the c.652_663del12 variant in *GJB3*, which has been associated with AD erythrokeratodermia variabilis (114), a phenotype unrelated to HL. Fourth, the variant p.T323HfsX19 in *COL11A2*, which was reported to have conflicting interpretation in ClinVar. The variant was identified in our cohort in a Qatari patient in the heterozygous state, although variation in *COL11A2* is associated with both AD (178) and AR NSHL (179), the patient in our cohort inherited it from a asymptomatic mother. The genetic testing of the patient in our cohort was conducted at GeneDx in 2018; however, GeneDx reclassified the variant as likely pathogenic in 2019.

For the CNVs of unknown clinical significance, the first CNV (2p22.1 deletion) did not involve any genes. Furthermore, 2p22.1 deletion was observed in three generations of a family with AD thoracic aortic aneurysm (180). The second CNV (9q33.1 duplication) was reported in association with neurodevelopmental disorders (181), autism spectrum disorder (181), attention deficit hyperactivity disorder (182), and developmental delay (183). The third CNV (15q13.2 duplication) was reported in association with developmental delay when involving the *CHRNA7* gene (184). However, a single report identified this CNV in a female patient with hearing impairment and additional clinical features of the prominent nasal bone and brachydactyly (185). Accordingly, the three CNVs are less likely to be contributing to NSHL in our cohort.

Through the analysis of findings of unknown clinical significance, 38.1% (8 out of 21) of the VUSs are likely contributing and explaining the NHSL, and 19.0 % (4 out of 21) of the VUSs are less likely contributing to NSHL in our cohort. The remaining variants are still considered as VUSs even after conducting the analysis (Table 7). The variants identified as likely contributing to NSHL shall be considered for further family segregation studies and functional studies. Moreover, the reassessment attempt for the

CNVs revealed no clear contribution to their incidence of NSHL in our cohort.

5.4 Test utilization and diagnostic yield of different genetic tests

*GJB2* gene sequencing and chromosomal microarray had the highest utilization rate, estimated at 80.5% and 50.8%, respectively. This could be attributed to the fact that these two tests are conducted at HMC as a first-tier workup and free of charge for all patients regardless of nationality. 54 (42.2%) patients did the full first-tier genetic workup, including *GJB2* gene sequencing and chromosomal microarray, of which 61.1% (33 out of 54 patients) had negative results. Among the patients with negative first-tier genetic testing, only 27.3% (9 out of 33 patients) pursued more comprehensive testing such as a gene panel (2 patients) or WES (7 patients). In our cohort, we had a total of 65 patients who ended up with negative genetic workup, of which four patients conducted both WES and a gene panel, and only one patient did WES without a gene panel.

Generally, the utilization of comprehensive genetic testing such as gene panel and WES was higher in Qatari patients as opposed to non-Qatari patients. For example, amongst the 31 patients who underwent WES, 21 (67.7%) patients were Qatari. Moreover, 80% of patients who had a gene panel were Qataris.

The overall diagnostic rate of our cohort was 30.5%, which is similar to a study that clinically evaluated the different genetic tests for patients with HL and identified a diagnostic yield of 39% (192).

As predicted, the highest diagnostic yield (60%) was obtained using targeted familial testing that relies on the presence of a known causative variant for HL in the family. However, this test and its diagnostic yield will not be considered in our interpretation and statistical analysis. Besides, we will focus on the diagnostic yields of the other genetic tests.

Besides targeted familial testing, the highest diagnostic yield (50%) was reached using a gene panel for HHL. This is agreeing with a study done in Qatar by Alkowari et al. (2017) that achieved a total diagnostic yield of 50% using a panel of 81 genes for HHL (108). Moreover, in another study from Qatar by Vozzi el at., the diagnostic yield using a panel of 96 genes for HLL was 33% (109).

Moreover, comprehensive genetic tests used for the genetic diagnosis of HHL, such as gene panel and WES, are associated with an increased diagnostic yield, ranging from 10% to 83%, with an average of 41% (188). However, not all include the analysis of CNVs as part of the genetic testing for HHL, although CNVs involving genes associated with HL have been identified as contributing factors to HHL in 13% of cases (189). Those comprehensive genetic tests provide the highest sensitivity and specificity compared to single gene testing (e.g. *GJB2* gene sequencing) account for the ethnic variation associated with HHL (190). However, the use of comprehensive genetic tests has limitations, including high cost, and the frequent absence of CNV analysis (191).
The statistical analysis identified three genetic tests that have a significant association with diagnostic rate: *GJB2* gene sequencing, gene panel, and mitochondrial genome sequencing and deletion testing. *GJB2* gene sequencing and gene panel are positively associated with diagnostic yield. However, mitochondrial genome sequencing and deletion testing is negatively associated with NSHL. In other words, in patients with no genetic diagnosis of NSHL due to negative or inconclusive genetic test results, the mitochondrial genome testing will either be negative or have a benign change; this is expected since mitochondrial changes account for 0.7-14% of NSHL (192). These findings highlight the importance of *GJB2* gene sequencing as a first-tier genetic test and the importance of gene panel testing as a second-tier genetic test.

5.5 Factors influencing the diagnostic yield

In the literature, many factors were assessed relative to their association with the diagnostic yield such as clinical presentation of HL, ethnicity, family history, consanguinity, and pattern of inheritance.

In terms of clinical presentation, it has been reported that the diagnostic yield was significantly higher in patients with severe to profound HL, congenital onset, and bilateral or symmetrical presentation of HL (20, 193). In our cohort, 41% (16 out of 39) of the patients with a positive genetic diagnosis presented with severe to profound NSHL, while 25.6% (10 out of 39 patients) presented with mild to moderate NSHL. 71.8% (28 out of 39) of the patients presented with congenital onset HL as compared to 21.1% (9 out of 39 patients) who presented with childhood HL. All patients with positive/diagnostic genetic testing results presented with bilateral NSHL. Our findings are concurrent with what has been previously reported in the literature about the presence of those factors in patients with a genetic diagnosis of NSHL.

The second factor is ethnicity, in which it is postulated that ethnicity contributes to the identification of the genetic etiology of HHL. The highest diagnostic yield of genetic testing for HHL has been reported in Asians, followed by Arabs, and then Ashkenazi Jews; however, lower diagnostic yields were observed in African patients (20) (194, 195). The increased diagnostic yield for the above-mentioned ethnicities is attributed to their relatively high coefficient of inbreeding resulting from high consanguinity or genetic isolation (196). This highlights the association between consanguinity and diagnostic yield, in which consanguinity was significantly associated with HHL regardless of its type (106, 197). Furthermore, the diagnostic yield is higher in patients with a positive family history of HL (20, 193). In our cohort, 87.2% (34 out of 39) of the patients were Arabs, two were Africans, two were South Asians, and one

was Iranian. Also, consanguinity was reported in 69.2% of the patients with positive/diagnostic findings, which is consistent with another report from Qatar indicating that consanguinity presented in 60.5% of the patients with HHL (106). Moreover, a positive family history of HL was reported in 74.4% of our solved cases.

5.6 Uptake of genetic testing

In this study, 71 out of the 360 excluded patients (19.7%) had no genetic test performed and therefore no genetic results, although the families were seen in the Clinical and Metabolic Genetics department by a genetic counselor, or a geneticist and pre-testing counseling was provided to one or both parents. The reason behind rejecting genetic testing was not noted in the medical records. However, many factors have been discussed in the literature that could be attributed to refusing genetic testing. One of the determinants of undergoing genetic testing is how patients and families perceive the severity of the condition. Patients who perceive the condition as severe are more likely to undergo genetic testing (198). Some parents might have perceived HL as a condition that is not painful. Another determinant is the perceived impact of the genetic diagnosis on disease management (199). In the context of HL, pediatric management and interventions are delivered through a multidisciplinary team involving audiologists, pediatricians, speech therapists, and geneticists (200). The management is not entirely dependent on identifying the genetic etiology (205), although insights to therapeutic means such as gene therapy are only possible with determining the genetic etiology (201). Parents of children with NSHL might perceive HL as a disease that is relatively manageable without the need for genetic testing.

Furthermore, the cost of testing could be a hindering factor for the uptake of comprehensive genetic testing for non-Qatari patients. This is reflected by the low number of non-Qatari patients who pursued comprehensive genetic testing. In fact, only

3 out of 56 non-Qatari patients with negative first-tier genetic testing underwent an additional more comprehensive genetic test.

Moreover, social factors such as parental education level, socioeconomic status, and fear of stigmatization associated with the genetic disease might affect parents' perception and acceptance of genetic testing (202-204).

It is unclear to which extent each of these factors influenced the uptake of genetic testing in our cohort and further studies are needed on this topic.

5.7 Recommendations for genetic testing and the role of the genetic counselor

Based on our findings, we recommend using *GJB2* gene sequencing, as a first-tier genetic test for NSHL. We also recommend using gene panel as a next step for comprehensive genetic testing as it is significantly and positively associated with the diagnostic yield. The combination of these two tests (*GJB2* gene sequencing and gene panel) might be a powerful approach in assessing NSHL in the pediatric patient population Qatar. We also recommend that genetic counselors and medical geneticists to search the literature and public databases for additional evidence on any detected VUSs, as this could bring important insights on the role of these variants in the pathogenesis of the disease and could prompt them to seek further reassessment for such variants based on the new evidence.

Furthermore, since CNVs involving *CATSPER2* gene are associated with male infertility in some reports, we recommend that male patients with such a CVN have a formal evaluation by a urologist when they reach the reproductive age.

The role of genetic counselors is quite integral in the context of genetic testing for NSHL both in pre-test and post-test encounters, given that the information and guidance they provide are key to facilitate patients' decision making. Genetic counselors have the needed skills to simplify complex genetic information for patients and families and

to discuss challenging aspects of HHL such as: the concept of genetic heterogeneity, the fact that genetics contribute to 50-60% of HL cases, the limitations of genetic testing, the possibility of having a negative genetic testing result, the risk of identifying VUSs, the difficulty of interpreting VUSs and understanding their implications, the individual and familial implications of a genetic diagnosis, and the limited utility of genetic testing in informing disease prognosis and the treatment/management plan.

*Limitations*

This study has some limitations, including:

i) The sample size was relatively small, which impacted the ability to conduct some statistical analysis and the ability to make generic conclusions.

ii) The lack of some information in medical charts such as the pre-lingual and post-lingual onset of NSHL, which might influence the genotype-phenotype correlation.

iii) The lack of reported reasons for refusing genetic testing.

iv) the absence of comprehensive genetic testing for some patients given the due to financial, social or personal preferences.

*Future directions*

In the future, we aim to:

i) Increase the length of the investigation period beyond 2014-2019 to increase sample size.

ii) Assess the approach of VUS analysis from a statistical point of view, conduct family segregation studies on the families harboring those VUSs, further study VUSs and their possible pathogenic contribution to NSHL, and establish clear genotype-phenotype correlations if VUSs are proven to be pathogenic.

iii) Statically analyze the factors associated with the diagnostic rate.

iv) Explore the reasons for not conducting or pursuing genetic testing.

v) Obtain the allele frequency of all variants identified in this study from Qatar Genome Project (QGP).

# CHAPTER 6: CONCLUSION

In conclusion, we investigated NSHL by analyzing the genetic basis of NSHL to identify causative variants and genes associated with NSHL in the population of Qatar and by evaluating the diagnostic yield of the different genetic tests used for NSHL in Qatar.

We revealed 19 causative variants (in 11 genes) and 2 CNVs in 30.5% of our pediatric patients with NSHL. Variants in the *GJB2* gene were the most common genetic cause of NSHL in Qatar, consistent with several studies in many other populations. The *GJB2* variant c.35delG was the most commonly identified variant in our cohort and it seems to be associated with a less severe presentation of NSHL than in other populations. Furthermore, we have identified the known founder variant c.506G>A in *GJB2*. Our report shed light on 8 VUSs potentially causative of NSHL in our cohort, including *c*.3641G>A and c.6503T>G in *MYO15A*, c.599C>T variant in *WHRN*, *c*.2476G<A and c.4696 A>T in *MYO7A (*compound heterozygous), c.-182G>A and c.617-3_617-2dup in *TMPRSS3 (*compound heterozygous), and *c*.98 G>A in *OTOF*. The identified VUSs merit further confirmation through family segregation and functional studies. Additionally, c.283C>T in *FGF3* was associated with NSHL for the first time.

In a clinical setting, we recommend performing *GJB2* full gene sequencing as a first-tier genetic workup and gene panel as a second-tier genetic workup since they positively correlated with positive diagnostic findings.

Identifying the causative variant/gene in patients with HL is essential and has crucial implications on the prediction of disease prognosis, the accurate assessment of recurrence risk, and genetic counseling for the patients and their family members. Moreover, the genetic characterization of understudied population shall help in

offering screening programs, modifying existing programs such as premarital screening program, and facilitate the establishment of precision medicine services.

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# APPENDICES

## Appendix A: The approval letter of the medical research center at HMC.

9/19/21, 12:13 AM

مؤسسة حمد الطبية
Hamad Medical Corporation

**APPROVAL LETTER**
**MEDICAL RESEARCH CENTER**
**HMC, DOHA-QATAR**

**Ms. Karen El-Akouri**
**Genertic Counselor**
**Genetics**
**Sidra Medical and Research Center, Qatar**

**Date: 12th August 2021**

| | |
|---|---|
| **Protocol No.** | MRC-01-21-614 |
| **Study Title** | Nonsyndromic Hearing loss in Qatar: the genetic basis and the diagnostic yield of genetic testing |
| The above titled research study has been approved to be conducted in HMC and is summarized below: | |
| **Study type:** | Data Review |
| **Data Collection Period:** | 01/01/2014 to 31/12/2019 |
| **Team Member List:** | Dr. Houssein Khodjet Elkhil, Dr. Mashael Al-Shafai, Dr. Nader Izz Eddin Saleem Aldewik, Dr. Tawfeg I M Ben Omran, Ms. Karen El-Akouri, Ms. Shaza DiaAldin Alkhidir |
| **Review Type:** | 'Exempt' under MOPH guidelines<br>Category 3: Research involving the collection or study of existing: Data, documents, records and the information is recorded by the investigator in such a manner that subjects cannot be identified, directly or through identifiers linked to the subjects. |
| **Decision:** | Approved |
| **Hospitals/ Facilities Approved:** | Hamad General Hospital (HGH) |

This study must be conducted in full compliance with all the relevant sections of the Rules and Regulations for Research at HMC and the Medical Research Center should be notified immediately of any proposed changes to the study protocol that may affect the 'exempt' status of this study. Wherever amendments to the initial protocol are deemed necessary, it is the responsibility of the Principal Investigator to ensure that appropriate reviews and renewed approvals are in place before the study will be allowed to proceed.

Appendix B: Ethical approval of Qatar University IRB

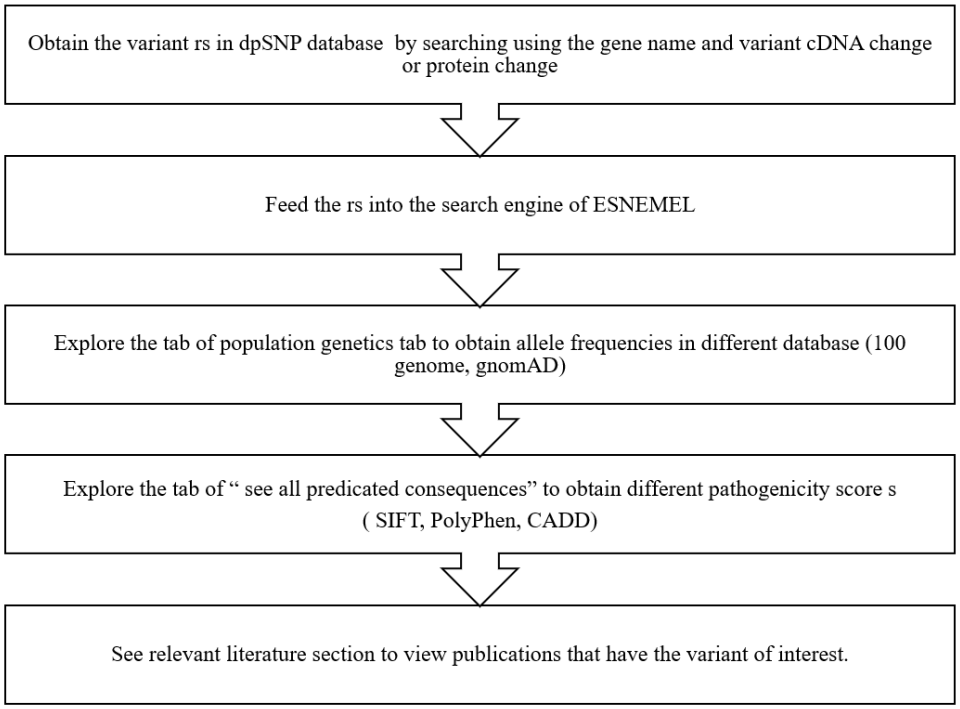
جامعة قطر
QATAR UNIVERSITY

Qatar University Institutional Review Board **QU-IRB**

QU-IRB Registration: IRB-QU-2020-006, QU-IRB, Assurance: IRB-A-QU-2019-0009

| | |
|---|---|
| DATE: | September 6, 2021 |
| TO: | Mashael Alshafai, PhD |
| FROM: | Qatar University Institutional Review Board (QU-IRB) |
| PROJECT TITLE: | 1801911-1Nonsyndromic Hearing loss in Qatar: the genetic basis and the diagnostic yield of genetic testing |
| QU-IRB REFERENCE #: | QU-IRB 1578-E/21 |
| SUBMISSION TYPE: | New Project |
| ACTION: | DETERMINATION OF EXEMPT STATUS |
| DECISION DATE: | September 6, 2021 |
| REVIEW CATEGORY: | Exemption category # 3 |

Appendix C: The stepwise manner of the analysis of variants of unknown clinical significance.



Appendix D. Negative single gene findings.

| Gene | Amino acid change | cDNA change | Zygosity | Mode of inheritance | Classification | Qatari cases | Non-Qatari Cases | Justification |
|---|---|---|---|---|---|---|---|---|
| HBA2 | - | partial deletion | - | AR/AD | Pathogenic | 1 | 0 | Associated with other disease |
| HBB | p.E7V | c.20 A>T | HT | AR/AD | Pathogenic | 1 | 0 | Associated with other disease |
| CRYAB | p.R56W | c.166 C>T | HT | AR/AD | VUS | 1 | 0 | Associated with other disease |
| MT-ND6 | p.M64V | m.14484 T>C | HOMP | - | Pathogenic | | | Mother is HOMP (healthy) |
| MT-TQ | - | m.4380 C>T | HOMP | | benign | 0 | 1 | Mother is HOMP (healthy) |
| MT-TH | - | m. 12174 C>T | HOMP | | benign | 1 | 0 | Mother is HOMP (healthy) |
| MT-RNR2 | - | m.3156 A>G | HOMP | | VUS | 0 | 1 | Mother is HOMP (healthy) |
| TECTA | p.T1891M | c.5672 C>T | HT | AR | VUS | 1 | 0 | inconsistent zygosity status |
| | p. A567T | c.1699 G>A | HT | AR | VUS | 2 | 0 | inconsistent zygosity status |
| PCDH15 | p.P1789LfsX52 | c.5364-5373del10 | HT | AR | Pathogenic | 1 | 0 | inconsistent zygosity status |

| Gene | Amino acid change | cDNA change | Zygosity | Mode of inheritance | Classification | Qatari cases | Non-Qatari Cases | Justification |
|---|---|---|---|---|---|---|---|---|
| GJB2 | p.G12Vfs | c.35delG | HT | AR | Pathogenic | 0 | 1 | inconsistent zygosity status |
| | p.V37I | c.109G>A | HT | AR | Pathogenic | 0 | 1 | inconsistent zygosity status |
| | p.K112EfrX2 | c.334_335 delAA | HT | AR/AD | Pathogenic | 0 | 1 | Parent is a healthy carrier |
| | p.M163V | c.487A>G | HT | AR | pathogenic | 1 | 0 | inconsistent zygosity status |
| MYO3A | - | c.1359+1 G>T | HT | AR | pathogenic | 0 | 1 | inconsistent zygosity status |

HOMP: Homoplasmic, AR: Autosomal recessive, AD: autosomal dominant.

Appendix E: Negative CNVs.

| CNV | Classification | Qatari Cases | Non-Qatari cases | Justification |
|---|---|---|---|---|
| Deletion of 157 kb within the band 7p21.2 causing intragenic deletion of AGMO gene | Benign | 0 | 1/89 | One parent is a healthy carrier |
| Duplication of 493 Kb involving the band 2p25 involving SNTG2 gene. | Benign | 0 | 1/89 | One parent is a healthy carrier |
| Deletion of 244 kb in the band 2p22.1 | Likely Benign | 0 | 1/89 | One parent is a healthy carrier |
| Duplication of 597 kb within 2p22 involving CRIM1 and FENZ genes. | Benign | 0 | 1/89 | One parent is a healthy carrier |
| Two copies gain of ~53 (KB) within 2q31.1 causing partial duplications of KLHL41 gene | Pathogenic | 1 | 0 | Associated with a disease other than NSHL (Nemaline myopathy 9) |
| Del of ~32 (kb) in Xq13.1 involving EDA gene | VUS | 1 | 0 | Associated with a disease other than NSHL (X-linked hypohidrotic ectodermal dysplasia) |