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Original Article

Investigation of Targeted Genes and Identification of Novel Variants with Next Generation Sequencing Method in Hearing Loss

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BACKGROUND: Hearing loss is a widespread condition throughout the world. It may affect patients from newborns to the elderly. There are too many reasons for hearing loss, including congenital hearing loss, virus infections, age-related situations, and traumatic situations, which may be related to the immune-mediated system. Fifty percent of hearing loss is related to genetic mutations and defects; genetic causes are highly heterogeneous, so the analysis of new variants are important for diagnosis. We aimed to describe the importance of detected gene variations by using targeted gene panels in the Next-Generation-Sequencing (NGS) platform.

METHODS: Eighty-one hearing loss targeted genes were investigated using Illumina NextSeq550 technology in 100 participants with hearing loss between 2017 and 2022 in our Genetic Diseases Evaluation Center.

RESULTS: Targeted genes were performed on 100 patients with hearing loss diagnosis. The total number of detected variants was 77. Forty-seven cases have likely pathogenic/pathogenic variants. Thirty of them have uncertain clinical significance variants, and from the detected variants, 8 are novel.

CONCLUSION: In this research, we highlighted that earlier detection of hearing loss using molecular genetic methods may help us understand the etiology and orient for a better prognosis. Results detected by using the NGS platform can assist and improve the diagnosis. In this study, the diagnostic rate with targeted genes was detected as 35.29%. It has an important role in clinical practice as the recommendation of cochlear implants. Clarifying the genotype and phenotype correlation helps us figure out the etiology of hearing loss and also the worth of genetic counseling in hereditary hearing loss.

KEYWORDS: Hearing loss, molecular diagnostic, targeted genes, next-generation sequencing, novel variants

INTRODUCTION

Hearing loss (HL) is a known risk in our lives. Affected persons may have problems with speech and language development delay problems.[1] Genetic defects and environmental influences can cause HL. Non-genetic and genetic combination factors are also related to HL. During early childhood, 1 in 1000 infants have HL problems.[2] Hearing loss is often divided into 3 types: conductive, sensorineural, and mixed.[3] Genetic and environmental factors can be the main reasons.[3] Sensorineural HL can be shown after an injury or defect in pathways of the cochlear nerve or cochlea, due to external, middle, and internal parts of the ear.[4] Factors causing congenital HL can be listed as congenital cytomegalovirus, structural abnormalities of the ear, inner part, and temporal bones. Fifty percent of factors were confirmed to be related to genes.[5]

Up to date, 140 genes reported are related to HL, the total number of non-syndromic HL (NSHL)-related genes is assigned as 124, 51 of them are shown with dominant inheritance, 78 of them with recessive inheritance, and 5 of them have an X-linked inheritance, and more than 1000 mutations are known to be the most genetically heterogeneous trait (https://hereditaryhearingloss.

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org/). Identification of related genes makes it easy to understand molecular functions in HL. Clinical examination is the basic and the first step test performed, standard diagnosis begins with looking into the family members, and proceeds by performing audiometric testing. After the clinical examination, patients can be divided into 2 groups; the first group is sporadic cases with autosomal recessive (AR) non-syndromic inheritance and the second group is patients with an autosomal dominant (AD) syndromic or non-syndromic inheritance of HL.[6]

The frequency of detected gene variation behind HL may vary from populations and ethnicities.[7]

The most widespread type of HL is NSHL which is usually inherited as an AR type. X-linked HL affects about 1%-2% of non-syndromic instances and many syndromic types.[8] The most common mutations reported until now related to HL are in *GJB2*, *OTOF, SLC26A4, TMC1,* and *CDH23* genes.[9] *GJB2* gene is the most known gene that causes HL; it is the first step recommended for diagnosis.[10]

Until now there are more than 600 syndromes reported that are related to HL like Waardenburg, Pendred, and Usher syndromes.[11] In SHL cases, there are some anomalies detected in the eye, and inner organs, defects in the nervous system, and different pigmentary disorders.[9]

MATERIAL AND METHODS

Herein we are presenting a retrospective study from the data of patients examined between 2017 and 2022 in our Medical Genetics Clinic. The patients' documents and family history were reviewed in our medical genetics department and written informed consent was obtained from patients. The ethical approval for this study was obtained from Trakya University ethics committee (Approval Number: 05/14, Ethical Committee Number: 2022/71, Date: March 7, 2022).

Clinical and physical examinations are the basis for molecular diagnosis. In the standard procedure, the first step taken are the patient's history and audiometric testing results. We included patients with congenital, bilateral, unilateral, sensorineural, and NSHL (including different types of severity).

Eighty-one HL targeted genes were collected in a panel according to Online Mendelian Inheritance in Man (OMIM) data and the literature review. The targeted hearing loss panel is commercially designed and is used by many institutions because of its high diagnostic rate. The patients were categorized according to the HL range and they have been classified into different HL degrees. The patients with 10-15 dB HL were considered to have a normal degree, slight HL between 16-25 dB, mild HL 26-40 dB, moderate HL 41-55 dB, moderately severe type between 56-70 dB, and higher than 91 dB HL with a profound degree.[12]

The exclusion criteria of our study are if the patient had trauma or used drugs that can affect the HL. If they had an infection from environmental factors and SHL patients.

In this study, we are reporting a targeted gene analysis of 100 HL patients with an age mean of 19.5 and a range of participants between 1-38 years old diagnosed with HL. As a method, we used new-generation sequencing and analyzed the results using the Illumina NextSeq Next-Generation-Sequencing (NGS) system. The detected variants were classified using American College of Medical Genetics and Genomics 2015 (ACMG) 2015 criteria and the and ClinVar database.

Targeted Next-Generation Sequencing Hearing Loss Panel and Next-Generation Sequencing Data Analysis

Peripheral venous blood samples were taken from the patients into Ethylenediaminetetraacetic acid (EDTA) tubes. The extraction of genomic DNA was performed using EZ1 DNA Investigator Kit protocol (Qiagen/Germany). For the prenatal patients, the extraction of DNA from amniotic fluid was performed using a Qiamp DNA mini kit (Qiagen, Germany). DNA quality was determined using NanoDrop (Thermo Fisher Scientific, Waltham, Mass, USA). DNA concentrations that have an A260/280 ratio and values between 1.8 and 2.0 values were included in our study. Onco-GeneSG Kit, IVD–CE (Sistemas Genomico, Valencia, Spain), and QIAGEN-targeted HL panel (Qiagen, Hilden, Germany) were performed according to the instructions of NGS. New generation sequencing contains 4 steps: extraction and fragmentation of genomic material, library preparation that includes the fragmentation of DNA, adapter ligation, the sequencing part, and finally the analysis part. The 81 targeted genes of this panel are shown in Figure 1. After we performed the wet-lab part, we converted the data from FastQ to bam-bai and vcf, and then using Genomize Seq Software (Genomize, Istanbul, Türkiye) we analyzed the detected variants. After the detection of variants, we performed a trio segregation analysis using the Sanger sequencing method.

Next-Generation Sequencing Data Analysis and Classification of Variants

After we converted the data using the Qiagen Clinical Insight (QCI) system, we used Integrative Genomics Viewer 2.4.8 (IGV) for visually detecting variants. We used the Human Genome Variation Society16 also for describing novel variants, and for the classification of variants, we used the criteria of the ACMG. ClinVar data and literature were considered for collecting information on known variants.

RESULTS

In this study, we are reporting 100 unrelated cases of HL. We performed the Targeted NGS panel related to HL. The HL patients are categorized according to the inheritance pattern of the genes. The patients were genetically diagnosed if they have a likely pathogenic/pathogenic heterozygous dominantly inherited variant or likely pathogenic/pathogenic homozygous recessively inherited variants. So after the NGS analysis, we detected the variants in 48 (1 of them is prenatal) HL cases.

MAIN POINTS

- The study has widened the genetic variation spectrum of targeted genes related to hearing loss and oriented the affected cases for a better prognosis.
- This study presents the molecular genetic data and diagnostic rate of hearing loss cases from the Thrace region.
- Eight novel hearing loss-related variants were described for the first time in this article.

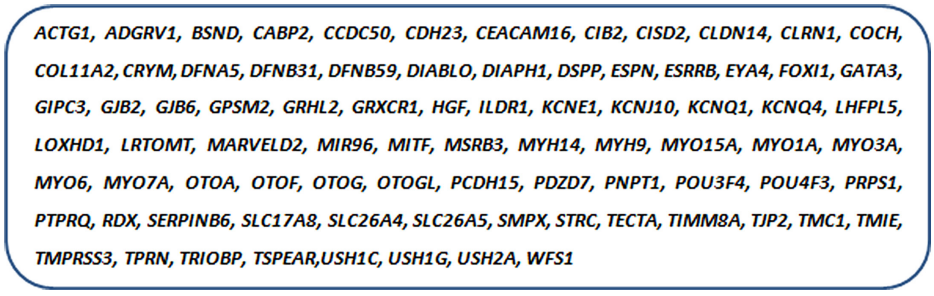

Figure 1. Eighty-one targeted genes related to hearing loss included in our study.

The total number of detected variants is 77. Forty-seven (61.03%) variants were detected as likely pathogenic/pathogenic in 34 patients, twenty-one cases (61.77%) have carrier variants for HL shown in Table 1. Of 34 patients, only thirteen (35.29%) are genetically diagnosed shown in Table 2. Some of the variants that cause HL are common for patients shown in Table 3.

The diagnosed patients have variants in *GJB2, MYO7A, TMIE, SLC26A4, MARVELD2,* LHFPL5, *TMC1, USH2A,* and *TECTA* genes. The state of the inheritance pattern (AD/AR) of detected variants decides the genetic diagnostic process. Targeted genes that are included in our panel are related to HL, they are performed in every patient that has HL. The targeted HL panel has been designed according to the published literature and OMIM database. As we know HL may be caused by many conditions, the genetic variants are one of the conditions. In our study, the most detected variants are related to the *GJB2* gene. Also in the published literature, *GJB2* variants are the most known cause of HL.

The *GJB2* gene has an AR inheritance; we detected 5 patients (P5, P15, P17, P21, P25) with NM_004004.5: c.35delG p.(G12fs*2) homozygous variant. The variant is pathogenic for HL in all databases as well as in the ClinVar database.

MYO7A genes are essential for hearing, until now in HGMD professional databases there are 511 variants reported, 132 of which are termination variants. Herein in our study, we have 2 cases (P8, P22) that are diagnosed with HL related to *MYO7A* variants. The *MYO7A* gene has in total 49 exons, NM_00260.3: c.3070C>T p.(Gln1024Ter) causes termination in the 24th exon of the gene, NM_00260.3:c.5293delG p.(Glu1765SerfsTer40) variant causes termination in the 38th exon. Both of the variants are termination ones. To understand the effect on the structure and function of the protein, a functional analysis must be performed.

Homozygous variants were detected in the *TMIE* gene (P11), *SLC26A4* gene (P18), *MARVELD2* gene (P23), *LHFPL5* gene (P30), and in the *TMC1* gene (P31). These genes have an AR inheritance, they are classified as pathogenic variants and cause HL. Patient 33 has 3 pathogenic variants, 2 of which are in the *USH2A* gene, the *USH2A* gene has an important place in hearing, and 1 pathogenic variant in the *TECTA* gene that has an AD inheritance. So, in this case, the cause of HL is related to the *TECTA* gene because of the inheritance state of the gene. We could not perform the family segregation; we performed the analysis only on his sister and we detected the same variants in her results; she has also sensorineural HL. Functional analysis was recommended for patients.

For the rest of the patients, the genetic diagnosis was inconclusive, as the genotype–phenotype comparison could not make a clear diagnosis. Whole genome or whole exome must be performed in carrier cases, as they may have new variants related to HL. Comprehensive studies must be performed in these cases, and functional analyses of these variants are also important in those cases. Cases with heterozygous, AR-inherited variants may have different etiological conditions related to HL instead of genetic causes.

Thirty of the variants (38.96%) were detected as having a Variant of Uncertain Significance (VUS) and from the detected variants 8 of them are novel. The detected variants were 82% heterozygous and 18% homozygous shown in Figure 2. From the detected variants, we are reporting 8 novel variants. We detected 2 novel pathogenic variants in *USH2A* and *MYO7A* genes. Three novel likely pathogenic variants in *OTOF, OTOG,* and *MYH14* genes, 3 novel variants are detected as VUS in *MSRB3, PTPRQ,* and 1 of them in *the OTOG* gene shown in Table 1 and 4.

After NGS sequencing analysis, we performed trio segregation in families of cases 13, 17, and 34. Case 34 is a prenatal patient, and we detected 4 different variants, just the pathogenic variant in the *OTOF* gene that is inherited from the parents, also the brother of the case has the same variant, and all family members have HL. In case 13 results, we detected that the case has inherited the variants from parents. Case 17 has a sibling, all of the variants that are detected are the same, and they both inherited the variants from the mother and father shown in Figure 3.

DISCUSSION

In recent years with the new era of genetic methods, we can easily see the benefits of the identification of genes and variants related to HL. These results led us to understand more detail about deafness and molecular mechanisms of HL. With all of this knowledge, it has been easier to confirm the diagnostic part of the population. In this study, we detected too many variants that are related to NHLS and SHL, 8 of these variants were classified as novel. The implications of new variants allow for the discovery of new candidates for disease association. The HL disease database is getting more valuable while using targeted gene analysis and multigene testing. Reporting new variants helps us with efficient molecular genetic screening and also helps us with characterizations of clinical findings of affected cases. Variant interpretation is an important part of clarifying the relationship between the variant and the patient's phenotype and also for diagnosis and treatment, even if the variants are not directly related to the diagnostic process of HL. They have a clinical significance in genetic counseling, about family inheritance, and also for the new

Table 1. Pathogenic and Likely Pathogenic Variants Detected with the Next-Generation Sequencing Analysis in the Carrier Cases

Bilateral Hearing Loss Cases										
Sampe ID	**Gender/ Age**	**HL type and Degree**	**Gene**	**Zygosity/ Inheritance**	**Variant Type**	**Nucleotide HGVS**	**Protein**	**dbSNP**	**ACMG**	**CLINVAR**
1**	M-11y	*Bilateral Severe (71 to 90 dB)*	*USH2A*	Heterozygous, AR	Missense	NM_206933.2:c.14453C>T	p.(Pro4818Leu)	rs143344549	Pathogenic (PP5, PMS2,PP3)	VCV000504509.5
2**	M-27	*Unilateral Slight (16-25 dB)*	*OTOG*	Heterozygous, AR	Nonsense	NM_001292063.2:c.3250A>T	p.(Phe1084Ile)	Novel	Likely pathogenic (PVS1,PMS2)	–
3**	F-2y	*Bilateral Severe (71 to 90 dB)*	*MYO15A*	Heterozygous, AR	*Missense*	NM_016239.3:c.5189T>C	p.(Leu1730Pro)	rs184435771	Pathogenic (PP5, PMS2,PP3)	–
			MYO15A	Heterozygous, AR	Missense	NM_016239.3:c.8183G>A	p.(Arg2728His)	rs184435771	Pathogenic (PP5, PMS2,PP3)	VCV000228276.12
			GJB2	Heterozygous, AR	Nonsense	NM_004004.5:c.71G>A	p.(Trp24Ter)	rs104894396	Pathogenic (PVS1,PM2,PP5)	VCV000017002.45
4**	F-33y	*Bilateral Severe (71 to 90 dB)*	*TMIE*	Heterozygous, AR	Missense	NM_147196.2:c.250C>T	p.(Arg84Trp)	rs28942097	Pathogenic (PP5, PMS2, PMS5, PP3)	VCV000003391.15
			SLC26A4	Heterozygous, AR	Missense	NM_000441.1:c.578C>T	p.(Thr193Ile)	rs111033348	Pathogenic (PP2,PP3, PP5,PM2)	VCV000004830.11
6**	M-34y	*Bilateral Severe (71 to 90 dB)*	*GJB2*	Heterozygous, AR	Frameshift	NM_004004.5:c.35delG	p.(G12fs*2)	rs80338939	Pathogenic (PVS1,PM2,PP5)	VCV000017004.66
7**	F-1y	*Bilateral Severe (71 to 90 dB)*	*USH2A*	Heterozygous, AR	Frameshift	NM_206933.2:c.9966_9967delCT	p.(F3322fs*23)	Novel	Pathogenic (PVS1,PM2)	–
			GJB2	Heterozygous, AR	Nonsense	NM_004004.5:c.71G>A	p.(Trp24Ter)	rs104894396	Pathogenic (PVS1,PM2,PP5)	VCV000017002.45
9**	F-10y	*Bilateral Severe (71 to 90 dB)*	*MYO15A*	Heterozygous, AR	Nonsense	NM_016239.3:c.4519C>T	p.(Arg1507Ter),	rs549138385	Pathogenic (PM1,PM2)	VCV000421664.3
10**	F-22y	*Bilateral Severe (71 to 90 dB)*	*GJB2*	Heterozygous, AR	Nonsense	NM_004004.5:c.71G>A	p.(Trp24Ter)	rs104894396	Pathogenic (PVS1,PM2,PP5)	VCV000017002.45
12**	F-30	*Bilateral Severe (71 to 90 dB)*	*GJB2*	Heterozygous, AR	Frameshift	NM_004004.5:c.35delG	p.(G12fs*2)	rs80338939	Pathogenic (PVS1,PM2,PP5)	VCV000017004.66
13**	F-8y	*Bilateral Severe (71 to 90 dB)*	GJB2	Heterozygous, AR	Missense	NM_004004.6:c.551G>C	p.(Arg184Pro)	rs80338950	Pathogenic (PM1,PM2,PP5, PP2, PP3, PP5)	VCV000017007.26
14**	F-23	*Bilateral Severe (71 to 90 dB)*	*SLC26A4*	Heterozygous, AR	Missense	NM_000441.2:c.1003T>C	p.(Phe335Leu)	rs111033212	Pathogenic (PVS1,PM2,PM5,PP2)	VCV000004842.23
16**	M-33	*Bilateral Severe (71 to 90 dB)*	*USH2A*	Heterozygous, AR	Missense	NM_206933.4:c.11864G>A	p.(Trp3955Ter)	rs111033364	Pathogenic (PVS1,PM2,PP5)	VCV000004842.23
			GJB2	Heterozygous, AR	Frameshift	NM_004004.5:c.35delG	p.(G12fs*2)	rs80338939	Pathogenic (PVS1,PM2,PP5)	VCV000017004.66

(*Continued*)

Table 1. Pathogenic and Likely Pathogenic Variants Detected with the Next-Generation Sequencing Analysis in the Carrier Cases (*Continued*)

Sampe ID	Gender/ Age	HL type and Degree	Gene	Zygosity/ Inheritance	Variant Type	Nucleotide HGVS	Protein	dbSNP	ACMG	CLINVAR
Bilateral Hearing Loss Cases										
19**	M-2	*Bilateral Moderate (41-55 dB)*	*USH2A*	Heterozygous, AR	Missense	NM_206933.4:c.11156G>A	p.(Arg3719His)	rs527236139	Likely pathogenic (PM2,PP5)	VCV000143170.17
20**	M-3	*Bilateral Severe (71 to 90 dB)*	*GJB2*	Heterozygous, AR	Nonsense	NM_004004.5:c.71G>A	p.(Trp24Ter)	rs104894396	Pathogenic (PVS1,PM2,PP5)	VCV000017002.45
24**	F-2	*Bilateral Moderate (41-55 dB)*	*OTOF*	Heterozygous, AR	Missense	NM_194248.3:c.1349A>C	p.(Asp450Ala)	Novel	Likely pathogenic (PM1,PM2, PP3)	–
26**	M-2	*Bilateral Moderate (41-55 dB)*	*USH2A*	Heterozygous, AR	Missense	NM_206933.4:c.5858C>G	p.(Ala1953Gly)	rs41302239	Likely pathogenic (PVS1,PM2)	VCV000048546.26
27**	M-38	*Bilateral Moderate (41-55 dB)*	*ADGRV1*	Heterozygous, AR	Missense	NM_032119.4:c.3443G>A	p.(Gly1148Asp)	rs200945405	Likely pathogenic (PM1,PM2,PP5)	VCV000046316.13
Unilateral Hearing Loss Cases										
28**	F-21y	*Unilateral Moderate (41-55 dB)*	*MYO15A*	Heterozygous, AR	Nonsense	NM_016239.3:c.5925G>A	p.(Trp1975Cys)	rs375290498	Likely- Pathogenic (PVS1,PM2)	VCV000203364.10
29**	F-7y	*Unilateral Moderate (41-55 dB)*	*CDH23*	Heterozygous, AR	Missense	NM_022124.5:c.7823G>A	p.(Arg2608His)	rs202052174	Likely pathogenic (PM1, PM2, PP5)	VCV000046040.16
Sensorineural Hearing Loss Cases										
32**	M-33y	*Sensorineural Severe (71 to 90 dB)*	*OTOGL*	Heterozygous, AR	Frameshift	NM_173591.3:c.4507_4508delTG	p.(Cys1503TrpfsTer12)	rs763898293	Pathogenic (PVS2,PM2,PP5)	–
Hearing Loss Type Unknown										
34**	Prenatal	–	*OTOF*	Heterozygous, AR	Missense	NM_194248.3:c.1469C>G	p.(Pro490Arg)	rs80356585	Pathogenic (PM1,PM2,PM5, PP3, PP5)	VCV000048170.4

ACMG, American College of Medical Genetics and Genomics; AD**,** autosomal dominant; AR, autosomal recessive; dB, decibel; HL, hearing loss; HGVS, Human Genome Variation Society.
**Carrier cases.

Table 2. Pathogenic and Likely Pathogenic Variants Detected with the NGS Analysis in the Genetically Diagnosed Cases

Sampe ID	Gender/ Age	HL type and degree	Gene	Zygosity/ Inheritance	Variant type	Nucleotide HGVS	Protein	dbSNP	ACMG	CLINVAR
Bilateral Hearing Loss Cases										
5*	F-5y	*Bilateral Severe (71 to 90 dB)*	*GJB2*	Homozygous, AR	Frameshift	NM_004004.5:c.35delG	p.(G12fs*2)	rs80338939	Pathogenic (PVS1,PM2,PP5)	VCV000017004.66
8*	F-28y	*Bilateral Moderate (41-55 dB)*	*MYO7A*	Heterozygous, AD	Nonsense	NM_000260.3:c.3070C>T	p.(Gln1024Ter)	–	Likely pathogenic (PVS1,PM2)	–
11*	F-1y	*Bilateral Severe (71 to 90 dB)*	*TMIE*	Homozygous, AR	Missense	NM_147196.2:c.250C>T	p.(Arg84Trp),	rs28942097	Pathogenic (PP5, PMS2, PMS5, PP3)	VCV000003391.15
15*	M-23	*Bilateral Severe (71 to 90 dB)*	*GJB2*	Homozygous, AR	Frameshift	NM_004004.5:c.35delG	p.(G12fs*2)	rs80338939	Pathogenic (PVS1,PM2,PP5)	VCV000017004.66
			MYH14	Heterozygous, AD	Frameshift	NM_001145809.2:c.3339delG	p.(Lys1113AsnfsTer2)	Novel	Likely- Pathogenic (PVS1,PM2)	–
17*	F-4y	*Bilateral Severe (71 to 90 dB)*	*GJB2*	Homozygous, AR	Frameshift	NM_004004.5:c.35delG	p.(G12fs*2)	rs80338939	Pathogenic (PVS1,PM2,PP5)	VCV000017004.66
			GJB2	Heterozygous, AR	Nonsense	NM_004004.5:c.71G>A	p.(Trp24Ter)	rs104894396	Pathogenic (PVS1,PM2,PP5)	VCV000017002.45
			HGF	Heterozygous, AR	Nonsense	NM_000601.6:c.2183C>A	(Ser728Ter)	rs747516423	Likely pathogenic (PVS1,PM2)	VCV001065086.3
18*	M-18	*Bilateral Severe (71 to 90 dB)*	*SLC26A4*	Homozygous, AR	Nonsense	NM_000441.2:c.170C>A	p.(Ser57Ter)	rs111033200	Pathogenic (PVS1,PM2,PP5)	VCV001075541.3
21*	M-3	*Bilateral Severe (71 to 90 dB)*	*SLC26A4*	Heterozygous, AR	Frameshift	NM_000441.2:c.1198delT	p.(Cys400ValfsTer32),	rs397516413	Pathogenic (PVS1,PM2,PP5)	VCV000043494.23
			GJB2	Homozygous, AR	Frameshift	NM_004004.5:c.35delG	p.(G12fs*2)	rs80338939	Pathogenic (PVS1,PM2,PP5)	VCV000017004.66
			TMC1	Heterozygous, AR	Nonsense	NM_138691.3:c.645C>A	p.(Tyr215Ter)	rs964315149	Likely pathogenic (PVS1,PM2)	–
22*	M-2	*Bilateral Severe (71 to 90 dB)*	*MYO7A*	Homozygous, AR	Frameshift	NM_000260.4:c.5293delG	p.(Glu1765SerfsTer40)	Novel	Pathogenic (PVS1,PM2)	–
23*	M-2	*Bilateral Severe (71 to 90 dB)*	*MARVELD2*	Homozygous, AR	Splice donor	NM_001244734.2:c.1295+2T>C	-	rs772048719	Pathogenic (PVS1,PM2,PP5)	VCV000871776.15
25*	M-3	*Bilateral Severe (71 to 90 dB)*	*GJB2*	Homozygous, AR	Frameshift	NM_004004.5:c.35delG	p.(G12fs*2)	rs80338939	Pathogenic (PVS1,PM2,PP5)	VCV000017004.66
			RDX	Heterozygous, AR	In frame	NM_001260492.2:c.194_195insTGT	p.(Val65dup)	rs772389925	Likely pathogenic (PM2, PM4, PP3)	–
Unilateral Hearing Loss Cases										
30*	F-30	*Unilateral Mild (26-40 dB)*	*LHFPL5*	Homozygous, AR	Missense	NM_182548.4:c.472C>T	p.(Arg158Trp)	rs753739358	Likely- Pathogenic (PP5, PMS2, PP2, PP3)	VCV000505548.2

Table 2. Pathogenic and Likely Pathogenic Variants Detected with the NGS Analysis in the Genetically Diagnosed Cases (*Continued*)

Sampe ID	Gender/ Age	HL type and degree	Gene	Zygosity/ Inheritance	Variant type	Nucleotide HGVS	Protein	dbSNP	ACMG	CLINVAR
Sensorineural Hearing Loss Cases										
31*	M-5y	*Sensorineural Severe (71 to 90 dB)*	*TMC1*	Homozygous, AR	Nonsense	NM_138691.2:c.1534C>T	p.(Arg512Ter)	rs200171616	Pathogenic (PVS1,PM2,PP5)	VCV000545971.2
33*	M-16y	*Sensorineural Severe (71 to 90 dB)*	*USH2A*	Heterozygous, AR	Frameshift	NM_206933.4:c.12234_12235delGA	p.(Asn4079TrpfsTer19)	rs398124618	Pathogenic (PVS1,PM2,PP5)	VCV000096665.10
			USH2A	Heterozygous, AR	Splice acceptor	NM_206933.4:c.8559-2A>G	–	rs397518039	Pathogenic (PVS1,PM2,PP5)	VCV000048604.13
			TECTA	Heterozygous, AD	Splice acceptor	NM_005422.4:c.4977-1G>T	–	rs746386175	Pathogenic (PVS1,PM2,PP5)	VCV000421172.2

ACMG, American College of Medical Genetics and Genomics; AD, autosomal dominant; AR, autosomal recessive; dB, decibel; HL, hearing loss.
*Genetically diagnosed cases.

generations. The most common variants in our study were detected in *GJB, OTOF, OTOG, MYO7A, MYO15A, CDH23, TECTA, SLC26A4,* and *USH2A* genes. The likely pathogenic and pathogenic variants information are shown in Table 5.

In our study, we have in total 13 genetically HL-diagnosed cases. Twenty-one carrier cases have likely pathogenic/pathogenic variants that are not the cause of HL according to their inheritance state. We detected 18 cases that have VUS variants. In those cases, the variant effect is not pathogenic so we cannot say that it has a direct relation with HL. VUS variants must be controlled in databases every 6 months or 1 year period. If the variant pathogenicity appears after that we can say that it may be related to HL. Detection of VUS variants is important also when the variant is de novo according to ACMG criteria, for these cases family segregation is recommended. In VUS variants the inheritance of the genes does not change the result or genetic diagnosis of the patients. Variant of Uncertain Significance variants is important for scientific reports and also for databases that we use in the classification of variants. To understand VUS variants we need more extensive population data, functional studies, and tracing the variant in other family members who have or do not have the same health condition.

The *GJB2* variants are the most commonly detected in HL. Herein in our study, the rate of detected *GJB2* variants is compatible with the literature. We also detected the novel variants and rare variants that are published in the literature. Pathogenic variant detection has an important part also in the integration of results in databases. Some of the variants are newly detected in patients, and we have to control them in databases to see the frequency in the population. In consanguineous marriages, it is important for genetic counseling.

The prevalence of gene variants associated with HL varies among different ethnic groups, the highest number of affected patients give more specific results according to variants and diagnostic processes. To improve the diagnostic part, functional analysis must be performed. In our study we used a targeted panel related to HL. Patients who are not diagnosed in this study may have different variants in other genes that cause HL so performing. Whole exome or whole genome sequencing can be more effective. After the analysis, genotype–phenotype factors can be revealed more clearly.

Here some literature results are similar to our study. Rabionet et al,[13] in their study, reported recessive and sporadic deafness in 576 unrelated HL cases. In 37% of their cases, they detected *GJB2* variations.[13] Until now in the HGMD database there are 300 missenses/nonsense, 59 small deletions, and 18 small insertions reported related to the *GJB2* gene. In our study, the prevalence of detected *GJB2* variants is 31.3%; patients have bilateral HL with severe degrees. We detected NM_004004.5(*GJB2*):c.71G>A p.(Trp24Ter) in 5 cases and NM_004004.5(*GJB2*): c.35delG p.(G12fs*2) variant in 7 cases, and NM_004004.6(*GJB2*):c.551G>C p.(Arg184Pro) in 1 case. Riza et al,[14] in their study, included children with bilateral congenital NSHL. They detected *GJB2* variants in 29 cases (9.67%) out of 124 cases. In HL cases, they detected compound heterozygous variants as *GJB2*:c.35delG, c.551G>C, c.35delG/c.269T>C, c.35delG/c.299_300delAT, c.35delG/c.101TG, c.35delG/c.370C>T, c.35delG/c.314_329del and c.299_300delAT/c.314_329del.[14]

Table 3. Genetic Causes of Hearing Loss and the Online Mendelian Inheritance in Man Phenotype

Sample ID	Gene	Zygosity/Inheritance	HGVS	ACMG	OMIM Phenotype
5	*GJB2*	Homozygous, AR	NM_004004.5:c.35delG	Pathogenic	Deafness, autosomal recessive
8	*MYO7A*	Heterozygous, AD	NM_000260.3:c.3070C>T	Likely Pathogenic	Deafness, autosomal dominant/recessive
11	*TMIE*	Homozygous, AR	NM_147196.2:c.250C>T	Pathogenic	Deafness, autosomal recessive
15	*GJB2*	Homozygous, AR	NM_004004.5:c.35delG	Pathogenic	Deafness, autosomal recessive
17	*GJB2*	Homozygous, AR	NM_004004.5:c.35delG	Pathogenic	Deafness, autosomal recessive
18	*SLC26A4*	Homozygous, AR	NM_000441.2:c.170C>A	Pathogenic	Deafness, autosomal recessive
21	*GJB2*	Homozygous, AR	NM_004004.5:c.35delG	Pathogenic	Deafness, autosomal recessive
22	*MYO7A*	Homozygous, AR	NM_000260.4:c.5293delG	Pathogenic	Deafness, autosomal dominant/recessive
23	*MARVELD2*	Homozygous, AR	NM_001244734.2:c.1295+2T>C	Pathogenic	Deafness, autosomal recessive
25	*GJB2*	Homozygous, AR	NM_004004.5:c.35delG	Pathogenic	Deafness, autosomal recessive
30	*LHFPL5*	Homozygous, AR	NM_182548.4:c.472C>T	Likely Pathogenic	Deafness, autosomal recessive
31	*TMC1*	Homozygous, AR	NM_138691.2:c.1534C>T	Pathogenic	Deafness, autosomal dominant/recessive
33	*TECTA*	Heterozygous, AD	NM_005422.4:c.4977-1G>T	Pathogenic	Deafness, autosomal, dominant

ACMG, American College of Medical Genetics and Genomics; OMIM, Online Mendelian Inheritance in Man.

In another study performed in Senegal, 129 affected HL cases were screened. The most commonly detected variant was *GJB2*: c.94C>T: p.(Arg32Cys) in cases (27.3%); they also defined a founder variant *GJB2*: c.427C>T: p.(Arg143Trp) for their population.[15]

The *OTOF* gene was reported for the first time in Lebanese families with a nonsense variant. Otoferlin is the protein encoded from the *OTOF* gene that is located in synapses of inner hair cells.[16] In our patients, we detected 3 missense (4.4%) variants in the *OTOF* gene, 2 of the variants (4.4%) are likely pathogenic, 1 of them is novel, and the other one is VUS according to the criteria.

OTOG gene is related to the non-progressive mild-to-moderate type of HL, the encoded protein is otogelin which has a very dynamic structure. Askari et al,[17] in their study, report missense rare variants from Iranian families (c.C2383T:p. R795C) in the *OTOG* gene. The gene's protein product may have a deleterious effect on the function and stability of the protein. Compound heterozygous variants of *OTOG* were reported as nonsense mutation, and loss-of-functions in the compound form confirm the HL.[18] In Ganaha et al,[19] they performed NGS in 7 HL families, and they identified a homozygous variant in the *OTOG* gene c.330C>G, p.(Tyr110*) in 4 unrelated families. The detected variant leads to a low-frequency HL and equilibrium dysfunction it was not shown. *OTOG* gene function is related more after birth periods. In the present study we are reporting 1 pathogenic and 3 VUS variants, 2 of the VUS variants are novel NM_001292063.2: c.3250A>T p.(Phe1084Ile) and NM_001292063.2:c.6331T>C p.(Arg 2111Trp).

MYO7A gene shows an AR inheritance (USH1B # 276900, DFNB2 # 600060) or AD (DFNA11# 601317) inheritance. *MYO7A* and *MYO15A* variants are related to HL and deafness.[20] In Bakhchane et al,[21] identified 5 variants in *MYO7A* related to DFNB2. In our study, we are reporting 1 pathogenic, 1 likely pathogenic, and 3 VUS variants related to *MYO7A*, but in our cases, we do not have any findings related to the syndromes.

Motovaf et al[22] reported a novel homozygous donor splice site variant, c.4596 +1G >A that was inherited from a consanguinity marriage. *MYO15A* variants were investigated in Iranian patients with nonsyndromic HL. One hundred forty families were included in the research, and they detected *MYO15A* variants were detected in 8 families.[23] Herein, in our study, a total 4 likely pathogenic/pathogenic variants were detected, with only 1 case having moderate unilateral HL and the others having severe bilateral HL.

CDH23 gene variants are related to non-syndromic and syndromic HL cases. Until now there are a lot of studies about the variants of *CDH23,* the variants may affect patients with congenital HL to high-frequency-involved progressive HL. Few of the *CDH23* variants may

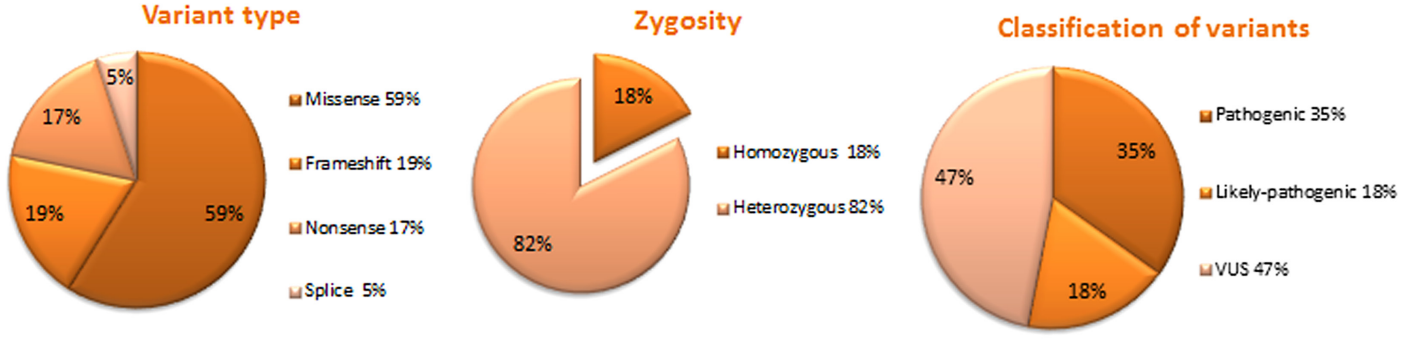

Figure 2. Percentage of type, zygosity, and classifications of variants detected in our study.

Table 4. Patients with Variant of Uncertain Significance

Sample ID	Sex/Age	HL Type and Degree	Gene	Zygosity/ Inheritance	Variant type	Nucleotide HGVS	Protein	dbSNP	ACMG	CLINVAR
Bilateral Hearing Loss Cases										
9	F-10	*Bilateral Mild (26-40 dB)*	*MYO7A*	Heterozygous, AD, AR	Missense	NM_000260.3:c.1868G>A	p.(Arg623His)	rs111033416	VUS (PM1, PM2, PP3)	–
11	F-1	*Bilateral Mild (26-40 dB)*	*COL11A2*	Heterozygous, AD, AR	Missense	NM_080680.2:c.1861C>A	p.(Pro621Thr)	rs121912952	VUS (PM2)	–
25	M-3	*Bilateral Mild (26-40 dB)*	*RDX*	Heterozygous, AR	Missense	NM_001260492.2:c.196A>T	p.(Thr66Ser)	rs766473708	VUS (PM2, BP4)	–
35	F-29	*Bilateral Mild (26-40 dB)*	*MARVELD2*	Heterozygous, AR	Missense	NM_001038603.2:c.454G>A	p.(Ala152Thr)	rs556047320	VUS (PMS2, BP4)	VCV000354081.4
			PCDH15	Heterozygous, AR	Missense	NM_033056.3:c.1649T>G	p.(Val550Gly)	rs61735482	VUS (PMS2)	VCV000300193.6
36	M-1	*Bilateral Mild (26-40 dB)*	*OTOG*	Heterozygous, AR	Missense	NM_001292063.2:c.6331T>C	p.(Arg2111Trp)	Novel	VUS (PM1, PM2, PP3)	–
			OTOG	Heterozygous, AR	Missense	NM_001292063.2:c.8512C>T	p.(Arg2838Cys)	rs1189461532	VUS (PM2, PP3)	-
37	M-3	*Bilateral Mild (26-40 dB)*	*PTPRQ*	Homozygous, AD, AR	Missense	NM_001145026.1:c.1961A>T	T p.(Tyr654Phe)	Novel	VUS (PM2, BP4)	–
38	F-4	*Bilateral Moderate (41-55 dB)*	*MSRB3*	Homozygous, AR	Missense	NM_198080.3:c.404G>A	p.(Cys135Tyr)	Novel	VUS (PMS2, PP3)	–
Unilateral Hearing Loss Cases										
39	M-34	*Unilateral Slight (16-25 dB)*	*USH2A*	Heterozygous, AR	Missense	NM_206933.2:c.14219C>A	p.(Ala4740Asp)	rs539192853	VUS (PM2, PP3)	VCV000429215.17
40	F-28	*Unilateral Mild (26-40 dB)*	*CDH23*	Heterozygous, AR	Missense	NM_022124.5:c.3146A>G	p.(Asp1049Gly)	rs752395991	VUS (PMS2)	–
			CLDN14	Heterozygous, AR	Missense	NM_012130.3:c.292G>A	p.(Ala98Thr)	rs759313432	VUS (PM2)	VCV001367577.1
			PTPRQ	Heterozygous, AD, AR	Missense	NM_001145026.2:c.6631A>G	p.(His2211Asp)	rs74971960	VUS (PMS2, BP4)	–
41	F-13	*Unilateral Mild (26-40 dB)*	*USH2A*	Heterozygous, AR	Missense	NM_206933.2:c.14017T>C	p.(Tyr4673His)	rs1040917329	VUS (PM2)	VCV000557599.2
			MYH9	Heterozygous, AD	Missense	NM_002473.5:c.5194G>A	p.(Ala1732Thr)	rs1050130268	VUS (PM2)	–
42	F-32	*Unilateral Mild (26-40 dB)*	*POU3F4*	Heterozygous, X-LR	Missense	NM_000307.4:c.191G>A	p.(Gly64Glu)	rs753206337	VUS (PM2,PP2, PP3)	–
			TECTA	Homozygous, AD, AR	Missense	NM_005422.2:c.2593G>A	p.(Gly865Ser)	rs370123961	VUS (PM2, PP3)	–
43	M-32	*Unilateral Slight (16-25 dB)*	*MITF*	Heterozygous, AD	Missense	NM_001354604.1:c.1493C>T	p.(Pro498Leu)	rs1425262191	VUS (PP2, PP3, BS2)	–
44	M-12	*Unilateral Slight (16-25 dB)*	*GJB2*	Heterozygous, AR	Missense	NM_004004.6:c.479G>A	p.(Gly160Asp)	rs1003116265	VUS (PMS2, PP2, PP3)	–
45	M-10	*Unilateral Mild (26-40 dB)*	*TECTA*	Heterozygous, AD, AR	Missense	NM_005422.2:c.4633G>A	p.(Val1545Ile)	rs377156351	VUS (PM1, PM2, BP4)	VCV000045333.10
46	F-30	*Unilateral Mild (26-40 dB)*	*KCNQ1*	Heterozygous, AD, AR	Missense	NM_181798.1:c.1504G>A	p.(Gly502Ser)	rs775608046	VUS (PM2, PP2, BP4)	VCV000427960.11

(*Continued*)

Table 4. Patients with Variant of Uncertain Significance (*Continued*)

Sample ID	Sex/Age	HL Type and Degree	Gene	Zygosity/ Inheritance	Variant type	Nucleotide HGVS	Protein	dbSNP	ACMG	CLINVAR
Unilateral Hearing Loss Cases										
47	F-8	*Unilateral Slight (16-25 dB)*	*SMPX*	Heterozygous, X-LD	Missense	NM_014332.2:c.182A>G	p.(Lys61Arg)	rs201681071	VUS (PP2, BP4)	VCV000229261.3
			GATA3	Heterozygous, AD	Missense	NM_002051.2:c.295G>A	p.(Gly99Ser)	rs768583935	VUS (PM2,PP2)	–
			MYO7A	Heterozygous, AD	Missense	NM_000260.3:c.5021C>A	p.(Thr1674Asn)	rs766461538	VUS (PMS2)	VCV000991573.1
Sensorineural Hearing Loss Cases										
48	F-16	*Sensorineural Moderate (41-55 dB)*	*OTOF*	Heterozygous, AR	Missense	NM_194248.3:c.1630C>T	p.(Arg544Cys)	rs139954767	VUS (PM2, PP3)	VCV000048176.13
			CDH23	Heterozygous, AR	Missense	NM_022124.6:c.1520C>T	p.(Ser507Leu),	rs201584731	VUS (PM2, BP4)	VCV000045876.5
			MYO7A	Heterozygous, AD, AR	Missense	NM_000260.4:c.4351G>T	p.(Ala1451Ser)	rs754360346	VUS (PM2, BP4)	VCV000959378.2
Hearing Loss Type Unknown										
34	Prenatal	–	*GJB2*	Heterozygous, AR	Missense	NM_004004.6:c.380G>A	p.(Arg127His)	rs111033196	VUS (PM1, PM5, PP2, BA1,BP4, BP6)	VCV000044745.17
			MYO7A	Heterozygous, AD, AR	Missense	NM_000260.3:c.1868G>A	p.(Arg623His)	rs111033416	VUS (PM1, PM2, PP3)	–
			MYO6	Heterozygous, AD, AR	Missense	NM_004999.4:c.3607G>T	p. (Ala1203Ser)	rs764047739	VUS (PM2, PP3)	–

ACMG, American College of Medical Genetics and Genomics; AD, autosomal dominant; AR, autosomal recessive; dB, decibel; HL, hearing loss.

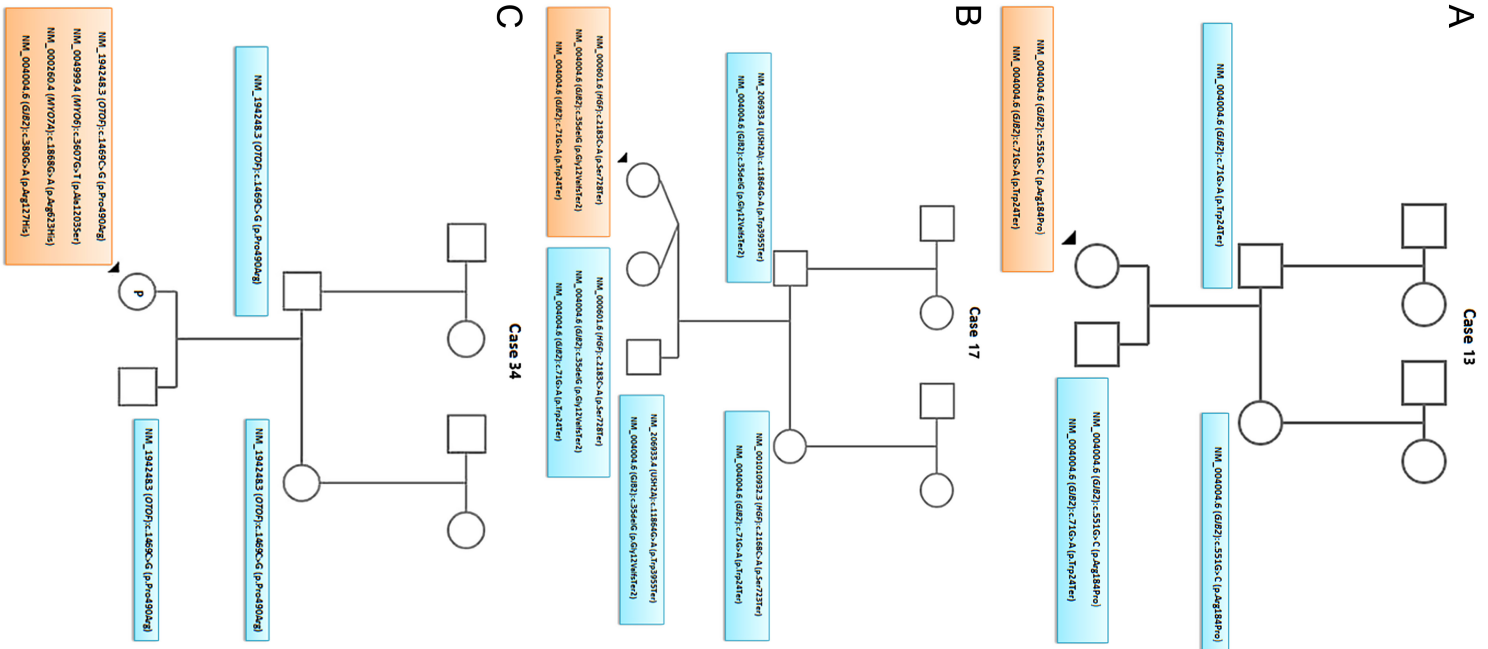

Figure 3. Family segregation of 3 patients. (A) Case 13 has 2 variants that are inherited from both parents also the brother of the case has the same variants. (B) Case 17 inherited 2 variants from the mother and 1 from the father; the patient's twin has the same genotype. (C) Case 34 is prenatal; both parents have the same variant in the *OTOF* gene, except this variant, the prenatal case has 3 different de novo variants that are related to hearing loss.

Table 5. The Summary of Pathogenic Variants Detected in Our Cases

Gene	Pathogenic Variant	Variant Prevalence	Total variant Detected	Heterozygous	Homozygous	Type of HL	Degree of HL
GJB2	NM_004004.5:c.71G>A		5	5	–	Bilateral	Severe
	NM_004004.5:c.35delG	31.1%	8	5	3	Bilateral	Severe
	NM_004004.6:c.551G>C		1	1	–	Bilateral	Severe
USH2A	NM_206933.2:c.14453C>T		1	1	–	Bilateral	Severe
	NM_206933.2:c.9966_9967delCT		1	1	–	Bilateral	Severe
	NM_206933.4:c.11864G>A		1	1	–	Bilateral	Severe
	NM_206933.4:c.11156G>A	15.5%	1	1	–	Bilateral	Moderate
	NM_206933.4:c.5858C>G		1	1	–	Bilateral	Moderate
	NM_206933.4:c.8559-2A>G		1	1	–	Sensorineural	Severe
	NM_206933.4:c.12234_12235delGA		1	1	–	Sensorineural	Severe
SLC26A4	NM_000441.1:c.578C>T		1	1	–	Bilateral	Severe
	NM_000441.2:c.1003T>C	8.8%	1	1	-	Bilateral	Severe
	NM_000441.2:c.170C>A		1	–	1	Bilateral	Severe
	NM_000441.2:c.1198delT		1	1	–	Bilateral	Severe
MYO15A	NM_016239.3:c.5189T>C		1	1	–	Bilateral	Severe
	NM_016239.3:c.8183G>A	8.8%	1	1	–	Bilateral	Severe
	NM_016239.3:c.4519C>T		1	1	–	Bilateral	Severe
	NM_016239.3:c.5925G>A		1	1	–	Unilateral	Moderate
OTOF	NM_194248.3:c.1349A>C	4.4%	1	1	–	Bilateral	Moderate
	NM_194248.3:c.1469C>G		1	1	–	–	–
TMIE	NM_147196.2:c.250C>T	4.4%	2	1	1	Bilateral	Severe
TMC1	NM_138691.3:c.645C>A		1	1	–	Bilateral	Severe
	NM_138691.2:c.1534C>T	4.4%	1	1	–	Sensorineural	Severe
OTOGL	NM_173591.3:c.4507_4508delTG	2.2%	1	1	–	Sensorineural	Severe
TECTA	NM_005422.4:c.4977-1G>T	2.2%	1	1	–	Sensorineural	Severe
CDH23	NM_022124.5:c.7823G>A	2.2%	1	1	–	Unilateral	Moderate
LHFPL5	NM_182548.4:c.472C>T	2.2%	1	–	1	Unilateral	Mild
MYH14	NM_001145809.2:c.3339delG	2.2%	1	1	–	Bilateral	Severe
HGF1	NM_000601.6:c.2183C>A	2.2%	1	1	–	Bilateral	Severe
MYO7A	NM_000260.3:c.3070C>T	2.2%	1	1	–	Bilateral	Moderate
MARVELD2	NM_001244734.2:c.1295+2T>C	2.2%	1	–	1	Bilateral	Severe
RDX	NM_001260492.2:c.194_195insTGT	2.2%	1	1	–	Bilateral	Severe
ADGRV	NM_032119.4:c.3443G>A	2.2%	1	1	–	Bilateral	Moderate

have a correlation with age and noise-induced HL.[24] In a patient from Saudi Arabia, a compound heterozygous variant p.(Asp918Asn); p.(Val1670Asp) was detected in the *CDH23* gene, in their three-dimensional structure of the peptide, they confirm that it has a direct affected contact of calcium ions.[25] In our study, we are reporting 3 different variants in the *CDH23* gene, 1 of them pathogenic and 2 of them VUS.

We detected 3 variants in the *TECTA* gene: a homozygous and heterozygous missense variant, and a heterozygous splice acceptor. Variants in the *TECTA* gene can be inherited as AD DFNA8/A12, and AR DFNB 21 related to NSHL. In a study, 134 targeted genes were analyzed and 2 novel variants p.(C1332F) and p.(T1873I) were detected in the *TECTA* gene.[26] In a study performed in 812 Japanese unrelated AD HL patients, the prevalence of the *TECTA* gene was reported as 3.2%.[27]

SLC26A4 mutations are usually related to Pendred syndrome, the mutation spectrum changes among ethnic populations. In a study 2353 unrelated NSHL patients were included, hot spot regions of the *SLC26A4* gene were analyzed and they report 86 variations and 47 of them were novel.[28] Also in our study we detected 4 pathogenic variants on the *SLC26A4* gene with 8.8% prevalence, all of the cases have bilateral severe HL. We detected p.(Thr193Ile), p.(Ser57Ter), p.(Cys400ValfsTer32), p.(Phe335Leu) that are related to Pendred syndrome.

In our study, the second highest prevalence after *the GJB2* gene was *USH2A* with 15.5%. We detected 9 variants related to the *USH2A* gene, 7 of which are likely pathogenic/pathogenic.

Three of the cases that have *USH2A* variants have bilateral severe HL, 2 of them bilateral moderate HL, and 2 of them with sensorineural severe HL.

USH2A gene has a relation with inherited deaf-blindness, if there are biallelic variants they can be related to Usher syndrome type 2.[29] ZHU et al,[30] performed NGS in 284 unrelated Chines families, and identified 230 variants in the *USHA2* gene from them 90 were novel. *USH2A* gene variants were the most frequent ones in HL cases. In a Korean study, 2 families with severe sensorineural HL were analyzed. In both probands, they detected *USH2A* variants, in the first case they detected a missense (c.1823G>A: p.C608Y) and nonsense (c.8176C>T: p.R2723X) variant. In the second case a compound heterozygosity for (c.8176C>T: p.R2723X), (c.1823G>A: p.C608Y) and compound heterozygosity for 2 frameshift variants (c.14835delT: p.S4945fs & c.13112_13115delAAAT: p.G4371fs).[31]

In a HL case, a compound heterozygous variant c.8559-2A>G and c.4749delT was detected in a 5-year-old girl. After the trio analysis, they detected the parents as heterozygous carriers, and 2 variants were pathogenic. In line with the detected results, they performed the variant analysis taken from amniotic fluid for prenatal diagnosis. After the examination, they found that the fetus carries only the c.4749delT variant. After the post-natal period, they confirmed that the baby was healthy.[32] Also in our study, we have an amniotic fluid sample shown in Figure 3 (Case 34). We detected 4 different variants in *GJB2, OTOF, MYO6, and MYO7A. OTOF* variants were inherited as heterozygous from the parents, and it is pathogenic. We have to follow up the infant for 3 other VUS variants.

DNA sequencing, targeted multi-gene analysis, or whole-exome sequencing is the primary usable data for the diagnosis of deafness and HL.[33]

There are some limitations of this study. Further studies with large-scale numbers need to be performed to detect new genes and variations. Genetic results may help us in the diagnosis and treatment of patients, until now there are a lot of genes reported related to HL also there are too many VUS variations, also in our study we presented some of them. The relation of VUS is not necessarily correlated with HL, for this reason, the variant position and protein function are important. For VUS variants, functional studies must be performed.

In recent years NGS technologies have been acceptable for the prognosis of different syndromes, illnesses, and also in HL cases. In our study, we aimed to underscore the multi-gene analysis and contribution to the diagnosis of patients with HL. The HL patients are categorized according to variants inheritance. The patients were genetically diagnosed if they have a likely pathogenic/pathogenic heterozygous dominantly inherited variant or likely pathogenic/pathogenic homozygous recessively inherited variants. The detection and identification of new variants help us to understand the etiology of HL and also the prognosis of patients. Whole exome and whole genome sequencing analysis were recommended if no variant was detected to explain the clinic of the patients as a result of targeted panel analysis. Targeted gene panels in NGS are effective for usage so we can have the result faster with a fair price.

Data availability: The data that support the findings of this study are available in the article. If additional data were required, it may be requested from the corresponding author.

Ethics Committee Approval: This study was approved by the Ethics Committee of Trakya University (Approval Number: 05/14, Ethical Committee Number: 2022/71, Date: March 7, 2022).

Informed Consent: Informed consent was obtained from the patients who agreed to take part in the study.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept – D.Z., S.Y.; Design – D.Z., H.S.G., S.Y., S.D.; Supervision – D.Z., S.Y., H.G.; Resources – D.Z., E.A., E.I.A.; Materials – D.Z., S.Y., H.G., H.S.G.; Data Collection and/or Processing – D.Z., H.S.G., S.Y., H.G.; Analysis and/or Interpretation – D.Z., H.S.G., S.Y., S.D., E.A., E.I.A., H.G.; Literature Search – D.Z., H.S.G., S.Y.; Writing – D.Z., H.S.G., S.Y.; Critical Review – S.Y., S.D., E.A., H.G.

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