ORIGINAL ARTICLE

INVESTIGACIÓN en DISCAPACIDAD
Vol. 11, No. 3
September-December 2025
pp 101-108
doi: 10.35366/123222

NGS panel for non-syndromic hearing loss: diagnostic yield and genotype-phenotype correlation in a Mexican population

Nykteja Dhamar Marín-Ríos,*,‡ Ariel Eduardo Jiménez-Romo,*,§ María de la Luz Arenas-Sordo*,¶

Keywords:
hearing loss, sensorineural, next-generation sequencing, connexin 26, genotype-phenotype correlation.

Abstract

Introduction: hearing loss is defined as the total or partial loss of auditory function, constituting a major cause of disability worldwide. It is the most common sensory disorder in humans, affecting approximately 1 in 3,000 newborns. Current molecular genetic studies report a diagnostic rate of around 50%. **Material and methods:** eleven patients with hearing loss were studied using a panel of 224 related genes. Sequencing (≥ 50 ×) was performed with Illumina technology, aligned to the GRCh37 human reference genome. Variants were reported according to the Human Genome Variation Society (HGVS) guidelines and confirmed through validated methods (Invitae). Results were correlated with patient phenotype and family history. **Results:** of the 11 cases (three males and eight females), one had a positive family history. Seven patients (63.6%) had a positive genetic result, three (27.3%) presented Variants of Uncertain Significance (VUS), and one had a negative result. Pathogenic variants (PV) were identified in the *GJB2* gene (three heterozygous and one compound heterozygous case). Compound heterozygous variants were found in *USH2A* and *ADGRV1*. Two patients showed inner ear malformations. **Conclusions:** the hearing loss gene panel (224 genes) demonstrated acceptable diagnostic yield, identifying PV in 63.6% of cases. *GJB2* was the most frequently involved gene, although not in the same proportion as observed in other populations, and often in heterozygous form. No direct correlation was found between the degree of hearing loss and the presence of PV; however, in some cases, there was an association with heterozygous or homozygous status. VUS may potentially contribute to Non-Syndromic Sensorineural Hearing Loss (NSHL), possibly modifying the phenotype. Segregation studies are necessary to improve diagnostic precision and support genetic counseling.

* Genomic Medicine Service. Instituto Nacional de Rehabilitación «Luis Guillermo Ibarra Ibarra». Mexico City, Mexico.
‡ Medical geneticist. Fellow. ORCID: 0009-0009-6946-1474
§ Medical intern. ORCID: 0009-0007-0548-6862
¶ Assigned physician. ORCID: 0000-0002-5429-0977

Correspondence:
María de la Luz Arenas-Sordo
E-mail: marenas@inr.gob.mx

Received: September 15, 2025
Accepted: January 15, 2026

Abbreviations:
ACMG = American College of Medical Genetics and Genomics
HGVS = Human Genome Variation Society
NGS = Next-Generation Sequencing
NSHL = Non-Syndromic Sensorineural Hearing Loss
VUS = Variants of Uncertain Significance

INTRODUCTION

The World Health Organization (WHO) defines hearing loss as a threshold exceeding 20 dB. It is a leading cause of global disability and the most common sensory disorder at birth, affecting 2-3 per 1,000 live births. Hearing loss is classified by type (conductive, sensorineural, mixed), origin (genetic, non-genetic), and onset (congenital, prelingual, postlingual). Up to 80% of prelingual cases are genetic, mostly non-syndromic (70%).[1]

How to cite: Marín-Ríos ND, Jiménez-Romo AE, Arenas-Sordo ML. NGS panel for non-syndromic hearing loss: diagnostic yield and genotype-phenotype correlation in a Mexican population. Invest Discapacidad. 2025; 11 (3): 101-108. https://dx.doi.org/10.35366/123222

Non-Syndromic Sensorineural Hearing Loss (NSHL) lacks external abnormalities but may involve inner ear alterations. Unlike syndromic forms, NSHL is typically autosomal recessive (77%), though dominant (19-22%), mitochondrial, or X-linked inheritance occur. Autosomal dominant *GJB2* variants usually present as postlingual, progressive high-frequency loss with variable penetrance. Specific variants like *c.109G>A* (*p.Val37Ile*) and *c.101T>C* (*p.Met34Thr*) show variable clinical presentations and controversial classifications.[1-4]

The *c.101T>C* (*p.Met34Thr*) variant was initially reported to be associated with reduced penetrance dominant hearing loss and its classification has been controversial. One study identified it as a point mutation in a caucasian family with autosomal dominant deafness and palmoplantar keratoderma.[5]

The advent of Next-Generation Sequencing (NGS) has significantly advanced the identification of candidate genes implicated in disorders characterized by high genetic heterogeneity. Exome sequencing, in particular, has proven instrumental in detecting DNA variants associated with Mendelian conditions, such as NSHL. To date, over 80 genes and more than 1,000 pathogenic mutations have been implicated in NSHL, positioning it as an exemplary model for illustrating the diagnostic power of NGS technologies.[2] However, the diagnostic yield varies significantly based on clinical and genetic factors, including the severity of hearing loss, age of onset, family history, patient ethnicity, and the scope of the genetic panel employed. In general, higher diagnostic rates have been observed in individuals with a family history or when hearing loss is congenital and bilaterally symmetrical. Several studies have evaluated targeted gene panels for hereditary diseases, reporting limited diagnostic performance and complex interpretation; therefore, their use as screening tools in patients with low clinical probability is not recommended. Although targeted gene panels are widely used in clinical practice, they present important limitations, including variable diagnostic yield, challenges in variant interpretation (particularly Variants of Uncertain Significance [VUS]) and discrepancies in variant detection and filtering across laboratories. These limitations highlight the need to define population-specific mutational spectra to improve the accuracy and clinical utility of molecular diagnosis in hearing loss.[6]

OBJECTIVE

The aim of this study was to identify genetic variants associated with NSHL in a sample of the National Institute of Rehabilitation (INR LGII Instituto Nacional de Rehabilitación «Luis Guillermo Ibarra Ibarra» for its Spanish meaning) patients using a panel of 224 hearing loss-related genes (Invitae) and to evaluate their diagnostic performance. NGS enabled the assessment of genotype–phenotype correlations, allowing the analysis of genetic factors contributing to hearing loss in this population.

MATERIAL AND METHODS

Eleven Mexican patients with a clinical diagnosis of sensorineural hearing loss, under follow-up at the National Institute of Rehabilitation were included. All patients were referred by the Audiology or Speech-Language Pathology services, and selected by clinical geneticists due to the suspicion of genetic hearing loss, mostly bilateral and without other identifiable cause that could better explain the hearing loss. Molecular analysis was performed by NGS, using a targeted panel of 224 genes associated with syndromic and NSHL. Biological samples consisted of peripheral blood collected in EDTA tubes. Genomic DNA extraction was performed by Invitae Corporation, followed by enrichment by hybrid capture and sequencing with Illumina technology. All target regions were sequenced with an average minimum depth of coverage of $\geq$ 50×, or supplemented with additional analysis when necessary. Prior to the performance of genetic studies, written informed consent was obtained from all patients or, if applicable, from their legal representatives.

During processing, reads were aligned to the *GRCh37/hg19* reference genome, and variants in coding regions, 20 flanking intronic base pairs, and other regions previously associated with disease were evaluated. No promoter or coding regions were analyzed, except for clinically relevant regions known at the time of panel design. The interpretation of the identified variants was performed according to the Human Genome Variation Society (HGVS) nomenclature, and they were classified according to the guidelines of the American College of Medical Genetics and Genomics (ACMG). Validated in-house algorithms were used for the detection of copy number variants (CNVs). Clinically relevant variants were confirmed by orthogonal methods such as Multiplex Ligation-dependent Probe Amplification (MLPA), MLPA-seq or Single-Molecule Real-Time sequencing (SMRT) long read sequencing (PacBio), as appropriate. The technical protocol followed by Invitae is described in its institutional documentation (Invitae Corporation,

2021). All variants were again cross-checked by the researchers in different databases. The genotype-phenotype correlation was performed by integrating molecular findings with family history, clinical history and available paraclinical studies, including audiometry and computed tomography (CT). The classification of the degree of hearing loss was established according to the criteria of the World Health Organization (WHO, 2021), which allowed a more accurate clinical interpretation of the variants identified.

RESULTS

Eleven patients with sensorineural hearing loss were studied. No relevant perinatal history or exposure to ototoxic drugs was found. The mean age was 24.3 years (range: 6-48 years), five were minors. The sex ratio was three males and eight females. One of the cases was familial. Pathogenic variants (PV) or likely pathogenic (LP) variants were identified in seven of the 11 patients analyzed, representing a diagnostic yield of 63.6%. Three patients (27.3%) had VUS and one had a negative result *(Table 1)*. Moreover, structural malformations of the inner ear were observed in two patients: one with a variant in *GJB2*, who presented absence of the cisternal and intracanalicular portions of the cochlear nerve (right CN VIII), along with hypodevelopment of the internal auditory canal; and another patient with a variant in *MYO7A* showed bilateral cochlear nerve agenesis on Magnetic Resonance Imaging (MRI), as well as a history of cochlear implantation in the left ear. *Table 1* summarizes the demographic characteristics, degree of hearing loss, imaging findings, and genetic results for each patient, including variant classification and zygosity, and their correlation with the expected phenotype described in the literature. The most frequently found gene with PV was *GJB2*, in four patients (three heterozygotes and one compound heterozygote). Variants in *USH2A* and *ADGRV1* (both in compound heterozygous state) were also identified. Of the two patients with variants in *USH2A*, only one had retinitis pigmentosa on ophthalmologic evaluation. In patients with PV, most of them presented bilateral moderate to profound hearing loss. The other genes we found are described in *Table 1*. During the analysis, no clinically relevant CNVs were identified in the patients analyzed.

DISCUSSION

Previous studies in the Mexican population have reported that although variants in the *GJB2* gene are less frequent compared to other populations (with proportions ranging between 10 and 30%), they continue to be the most prevalent in this population.[1,7,8]

We identified one patient with compound heterozygosity —a common finding— but also three cases with heterozygosity alone, a pattern that is increasingly reported. This was the case for patients 2, 3, and 4, who carried the following PV, respectively: *c.109G>A* (*p.Val37Ile*), *c.101T>C* (*p.Met34Thr*) and *c.34G>T* (*p.Gly12Cys*).[3-5] However, there is no clear correlation between heterozygosity and homozygosity or compound heterozygosity with the degree of hearing loss found.

In these patients, whose variants are found in heterozygosity (homozygosity is generally considered necessary for the pathological phenotype to manifest), we cannot fully explain it nor confirm that these heterozygous variants are the cause, and there is controversy in the literature. However, phenotypic expression in heterozygotes is plausible given the increasing prevalence of symptomatic carriers, or potentially due to unidentified modifier genes. Notably, we did not identify other variants (PV, VUS) to suggest digenic inheritance in these cases.[3-5]

The PV *c.109G>A* (*p.Val37Ile*) in the *GJB2* is frequently found in heterozygosity in normal-hearing populations, particularly in China.[3,4] Although initially classified as a benign polymorphism, it has been reevaluated due to its significant overrepresentation in hearing loss cohorts. Liang et al. reported marked phenotypic variability associated with this variant in both homozygous and heterozygous states, with most patients presenting mild-to-moderate hearing loss.[3,4,9]

In the case of patient 3, the variant *c.101T>C* (*p.Met34Thr*) can indeed cause hearing loss in heterozygosity, but there may also be an influence of some other genes. It has also been observed, that carriers of the *c.35delG* mutation in *GJB2* have significant hearing loss, and that in heterozygous women, hearing impairment may be more severe than in men.[10] Familial cases of heterozygous mutations in the *GJB2* gene have been reported with a high prevalence in individuals with the *1555A->G* mitochondrial mutation, suggesting it as an aggravating factor in the phenotypic expression of NSHL. Similarly, the combination of a heterozygous mutation in *GJB2* with deletions in the *GJB6* gene can cause severe hearing loss, demonstrating a di-genic inheritance pattern in some cases.[11]

Several case studies have documented the presence of atypical heterozygous variants of the *GJB2*

Table 1: Demographic and molecular characterization of the study cohort.

Patient	1		2	3	4	5	6
Gender and age Gene/Locus	Female, 48 years USH2A / 1q41		Female, 45 years GJB2 / 13q12.11	Female, 36 years GJB2 / 13q12.11	Female, 15 years GJB2 / 13q12.11	Male, 6 years MITF / 3p13	Female, 10 years MYO7A / 11q13.5
ClinVar/ACMG criteria	Deletion (Exons 22-50)	c.5278del (p.Asp1760Metfs*10) Frameshift; Stop codon	c.109G>A (p.Val37Ile) Missense	c.101T>C (p.Met34Thr) SNV; Missense	c.34G>T (p.Gly12Cys) SNV; Missense	Deletion (complete coding sequence)	c.4117C>T (p.Arg1373*) SNV; Nonsense Stop codon
Classification	PV	PV	PV (Low penetrance)	PV	PV	PV	PV
Zygosity (Patient)	Compound Heterozygous		Heterozygous	Heterozygous	Heterozygous	Heterozygous	Heterozygous
Associated Inheritance Pattern	AR pattern	AR pattern	• AR pattern • AD pattern	• AR pattern • AD pattern	AD pattern	AD pattern	AR pattern
Findings in Present Cohort	Bilateral RE Moderate (47.5 dB) LE moderately severe (61.25 dB) No imaging studies Ophthalmologic evaluation: Pigmentary retinosis, severe myopia and astigmatism (R 20/80; L 20/100)		Bilateral sensorineural RE complete loss (120 dB) LE moderately severe (51.25 dB) CT: The absence of the cisternal and intracanalicular portions of the right VIII cranial nerve + hypodevelopment of the internal auditory canal	Bilateral sensorineural RE Moderate (47.5 dB) LE moderate (41.25 dB) No imaging studies	Bilateral RE Deep (82.5 dB) LE severe (76.25 dB) CT scan without evidence of structural ear pathology	Bilateral sensorineural RE complete loss (111.25 dB) LE complete loss (106.25 dB) CT scan without evidence of structural ear pathology	Bilateral sensorineural RE complete loss (120 dB) LE complete loss (120 dB) CT: Hypoplasia and/or agenesis of the cochlear nerve, left cochlear implant. MRI: In the right ear, no presence of the cochlear nerve is shown, but the inferior vestibular nerve is present. In the left ear, the cochlear nerve is not identified
Reported Phenotypes in literature	Exons 22-50 usually result in more severe phenotypes, with earlier visual impairment and more pronounced hearing impairment. (Varsome)	Exon 26, Truncating mutation. Reported as pathogenic in Usher syndrome cohorts. Causes loss of protein function[17]	Biallelic: Associated with progressive NSHL. Monoallelic: Associated with DFNA3A or syndromic forms (skin disorders)[5]	Mild, progressive HL; low penetrance. Highly controversial variant. May act as a hypomorphic allele in compound heterozygosity. Normal hearing was also reported[18]	NSHL, characterized by moderate to profound hearing loss. With a high frequency in the heterozygous state among Hispanic/ Mexican patients[19]	Congenital sensorineural deafness, usually bilateral, extensive depigmentation, similar to Waardenburg type 2A and Tietz syndrome. Susceptibility to malignant melanoma.[20] (OMIM)	Pathogenic variants in the MYO7A gene, responsible for Usher syndrome type 1B, may also manifest as NSHL with both AD and AR inheritance patterns[15]

ACMG = American College of Medical Genetics and Genomics. AD = autosomal dominant. AR = autosomal recessive. Cx26 = Connexin 26. HL = hearing loss. LE = left ear. LP = like pathogenic. NSHL = non-syndromic hearing loss. PV = pathogenic variant. RE = right ear. SNV = Single Nucleotide Variant. VUS = Variant of Uncertain Significance. ZSD = Zellweger spectrum disorder.
Demographic characteristics and study findings of the patient cohort.
* Stop codon.

Continue Table 1: Demographic and molecular characterization of the study cohort.

7		8			9			10	11
Male, 8 years		Female, 37 years			Female, 30 years			Male, 12 years	Female, 21 years
ADGRV1 / 5q14.3		GJB2 / 13q12.11		PEX5 / 12p13.31	BSND / 1p32.3	CEACAM16 / 19q13.31	USH2A / 1q41	SLC26A4 / 7q22.3	Negative
Deletion (Exons 53-54)	c.15736C>T (p.Arg5246*)	c.516G>A (p.Trp172*) nonsense Stop codon	c.34G>T (p.Gly12Cys) SNV; Missense	c.317-2A>G (Splice acceptor)	c.859G>T (p.Glu287*) SNV; Nonsense Stop codon	c.631C>T (p.Arg211Cys) SNV; Missense	c.10993G>A (p.Gly3665Arg) SNV; Missense	c.1061T>C (p.Phe354Ser) Missense	Negative
PV	PV	PV	PV	LP	LP	VUS	VUS	VUS	Negative
Compound Heterozygous		Compound Heterozygous		Heterozygous		Probably Trigenic inheritance		Heterozygous	Negative
AR pattern	AR pattern	AR pattern	AD pattern	AR pattern	AR pattern	AD pattern	AR pattern	AR pattern	Negative
Bilateral sensorineural RE Severe (67.5 dB) LE moderately severe (58.75 dB) CT scan without evidence of structural ear pathology		Bilateral RE moderately severe (56.25 dB) LE severe (75 dB) CT: Subluxation of left hammer-anvil joint			Bilateral RE Severe (66 dB) LE Normal (7.5 dB) CT: Left labyrinthitis, vascular finding Ophthalmologic evaluation: No alterations			Bilateral RE Deep (86.25 dB) LE deep (81.25 dB) CT scan without evidence of structural ear pathology	Bilateral RE complete loss (120 dB) LE complete loss (120 dB) CT: Right tympanic glomus, probable adenoma at the pituitary level
The ADGRV1 gene is associated with autosomal recessive Usher syndrome type 2C, retinitis pigmentosa and nonsyndromic deafness. No specific clinical manifestations have been described in the literature about this variant (Varsome)	The c.15736C>T variant causes a loss of function by introducing a premature stop codon. In the homozygous state, this mutation is known to suggest Usher syndrome type 2C, which includes sensorineural hearing loss (Varsome)	Severe-to-profound congenital hearing loss[21]	NSHL, characterized by moderate to profound hearing loss. With a high frequency in the heterozygous state among Hispanic/ Mexican patients[19]	The PEX5 gene causes autosomal recessive ZSD, a syndromic disorder that involves deafness[22]	BSND is the causative gene for DFNB73 (NSHL), but mutations are also known to cause Bartter syndrome type IV. The HL phenotype may be accompanied by subclinical renal metabolic changes[23]	Bilateral NSHL with early onset and progressive course[24]	The available evidence is currently insufficient to determine the role of this variant in disease (Varsome)	Associated with NSHL and Pendred's syndrome, also reported in autoimmune thyroid diseases[25]	Negative

ACMG = American College of Medical Genetics and Genomics. AD = autosomal dominant. AR = autosomal recessive. Cx26 = Connexin 26. HL = hearing loss. LE = left ear. LP = like pathogenic. NSHL = non-syndromic hearing loss. PV = pathogenic variant. RE = right ear. SNV = Single Nucleotide Variant. VUS = Variant of Uncertain Significance. ZSD = Zellweger spectrum disorder.
* Stop codon.

gene associated with specific and complex deafness phenotypes, challenging the purely recessive inheritance model. In one study, whole exome sequencing (WES) identified a pathogenic de novo variant *c.223C>T* (*p.Arg75Trp*) in *GJB2*, linked to autosomal dominant deafness and palmoplantar keratoderma. Similarly, another study described the heterozygous missense variant *c.370C>T* (*p.Gln124*)[I] in a patient with late-onset deafness and preserved speech. The latter variant showed extremely low allele frequencies (< 0.02%) in population genomic databases, supporting its likely pathogenicity. Finally, one study reported the heterozygous variant *p.Gly45Glu* in a patient with a lethal form of KID syndrome, a variant inherited from his mother, who in turn also carried it along with another nonsense variant, *p.Tyr136X*. These findings suggest that heterozygous variants of *GJB2* may manifest pathogenic effects through complex inheritance mechanisms, including de novo dominant inheritance and interaction with other genetic variants.[5,12,13] Other *GJB2* variants reported in heterozygosity have been: *c.224G>A* (*p.Arg75Gln*), *c.617A>G* (*p.Asn206Ser*).[3,4] These results confirm the clinical utility of the panel of genes for hearing loss and demonstrate the relevance of the *GJB2* gene in our population, despite the previously reported differences in frequency between different regions.

Of the remaining variants, very frequently in different studies, USHER-related genes such as *USH2A, USH1C, ADGRV1, MYO7A* and *CDH23* have been found to cause hearing loss. In general, no other manifestations were found that would allow considering that these were syndromic hearing losses and it is something very common that has been found in the different series of patients.[14] We identified three PV in *USH2A*, consistent with previous reports. As well as two cases of PV in *ADGRV1*, in a compound heterozygote, one of these variants associated with Usher syndrome type 2C. More than 200 mutations have been identified in *MYO7A* causing Usher type 1B, we describe a case in heterozygote in the *MYO7A* gene, but other unknown or undetected genes could be the responsible or acting together with *MYO7A*, since the pattern proposed is recessive.[13,15] Mutations in *MITF* have been associated with Waardenburg syndrome type 2A and with isolated hearing loss. In this study, we report a heterozygous case with a pathogenic complete coding sequence deletion presenting without syndromic features. While the hearing loss can be explained by this variant, it is also important to highlight the increased susceptibility to malignant melanoma associated with alterations in the same gene. This finding illustrates how exome sequencing may reveal clinically relevant information beyond the primary indication for testing, which should be considered during genetic counseling.[4]

Some mutations were reported in VUS, in the *BSND* gene, certain variants have been associated with Bartter syndrome type IV, in our case this association has not been described. In the same case a variant of the *CEACAM16* gene was reported in VUS, in the literature there is still not enough evidence to associate it directly with autosomal dominant NSHL. A mutation in the *SLC26A4* gene in VUS was reported in one case; this has been associated with Pendred syndrome and inner ear malformations.[16]

These results confirm the clinical usefulness of the panel of genes for hearing loss and not the single study of the *GJB2* gene. Overall, our results support the use of multigene panels as an effective diagnostic tool in patients with sensorineural hearing loss, and demonstrate the relevance of the *GJB2* gene and other genes such as those related to USHER syndrome in our population. The exome could even give us other important findings for patients. Likewise, it is necessary to highlight the need for segregation and clinical follow-up studies to clarify the phenotypic impact of VUS, as well as the genotype-phenotype correlation and to be able to provide adequate genetic counseling.

CONCLUSIONS

The 224-gene hearing loss panel demonstrated robust diagnostic performance, identifying PV in 63.6% of analyzed cases. The *GJB2* gene was the most frequently implicated, although at a lower proportion than reported in other populations and predominantly in the heterozygous state, which poses interpretative challenges regarding penetrance, inheritance patterns, and the possible presence of undetected variants in non-covered regions.

VUS may contribute to the phenotype of NSHL, particularly in the presence of family history or subtle clinical findings, as well as through the potential influence of epistatic interactions or modifier genes in certain cases.

The identification of PV in genes associated with Usher syndrome, such as *USH2A*, highlights the

[I] Stop codon.

importance of considering syndromic etiologies in the differential diagnosis, even in the absence of systemic features at initial evaluation. Similarly, in genes such as *CDH23*, the specific variant type may determine whether retinitis pigmentosa develops, underscoring the complexity of genotype–phenotype correlations.

Our findings, consistent with previous reports in Mexican patients, indicate that *SLC26A4* is a relevant contributor to sensorineural hearing loss after *GJB2*. The detection of diverse *SLC26A4* variants, without a recurrent mutation, reinforces the high genetic heterogeneity of hearing loss in this population.

These results support the clinical utility of comprehensive multigene panels in patients with hearing loss, while emphasizing the need for family segregation studies, longitudinal follow-up, and population-specific databases to refine variant interpretation, establish stronger genotype–phenotype associations, and improve genetic counseling. Notably, a subset of patients remained without a molecular diagnosis, underscoring the need for broader genomic approaches, including non-coding regions and novel genes, in future research.

Unexpected or secondary findings, although not directly related to the primary genotype, may provide clinically relevant information by enabling preventive or anticipatory actions for other conditions. The identification of incidental or non-pathogenic variants can contribute to a more comprehensive genetic counseling approach, particularly when interpreted within an appropriate clinical and familial context.

Finally, this study provides population-specific insights into the genetic landscape of hearing loss in Mexican patients, contributing to the development of precision medicine, early diagnosis, and personalized management strategies, with potential implications for public health policies in hearing impairment.

References

1. Arenas-Sordo ML, Linares-Mendoza EP, Peñuelas-Romero KJ, Castro-Peña S, Agís-Ocaña JG. Hipoacusia no sindrómica de origen genético. Conceptos actuales. Otorrinolaringología. 2020; 65 (1): 43-58.
2. Atik T, Bademci G, Diaz-Horta O, Blanton SH, Tekin M. Whole-exome sequencing and its impact in hereditary hearing loss. Genet Res. 2015; 97: e4.
3. Imizcoz T, Prieto-Matos C, Manrique-Huarte R, Calavia D, Huarte A, Pruneda PC et al. Next-generation sequencing improves precision medicine in hearing loss. Front Genet. 2023; 14: 1264899.
4. Quaio CRDAC, Coelho AVC, Moura LMS, Guedes RLM, Chen K, Ceroni JRM, et al. Genomic study of nonsyndromic hearing loss in unaffected individuals: Frequency of pathogenic and likely pathogenic variants in a Brazilian cohort of 2,097 genomes. Front Genet. 2022; 13: 921324.
5. Posukh OL, Maslova EA, Danilchenko VYu, Zytsar MV, Orishchenko KE. Functional consequences of pathogenic variants of the *GJB2* gene (Cx26) localized in different Cx26 domains. Biomolecules. 2023; 13 (10): 1521.
6. Liu C, Huang Y, Zhang Y, Ding H, Yu L, Wang A et al. Next-generation sequencing facilitates genetic diagnosis and improves the management of patients with hearing loss in clinical practice. Int J Pediatr Otorhinolaryngol. 2022; 161: 111258.
7. Hernández-Juárez AA, Lugo-Trampe JDJ, Campos-Acevedo LD, Lugo-Trampe A, Treviño-González JL, de-la-Cruz-Ávila I et al. *GJB2* and *GJB6* mutations are an infrequent cause of autosomal-recessive nonsyndromic hearing loss in residents of Mexico. Int J Pediatr Otorhinolaryngol. 2014; 78 (12): 2107-2112.
8. Loeza-Becerra F, Rivera-Vega MDR, Martínez-Saucedo M, Gonzalez-Huerta LM, Urueta-Cuellar H, Berrruecos-Villalobos P et al. Particular distribution of the *GJB2*/*GJB6* gene mutations in Mexican population with hearing impairment. Int J Pediatr Otorhinolaryngol. 2014; 78 (7): 1057-1060.
9. Liang S, Li W, Chen Z, Yuan S, Wang Z. Analysis of GJB2 gene mutations spectrum and the characteristics of individuals with c.109G>A in Western Guangdong. Mol Genet Genomic Med. 2023; 11 (8): e2185.
10. Abe S, Kelley PM. Connexin 26 gene (GJB2) mutation modulates the severity of hearing loss associated with the 1555A-->G mitochondrial mutation. Am J Med Genet. 2001; 103 (4): 334-338.
11. Groh D, Seeman P, Jilek M, Popelár J, Kabelka Z, Syka J. Hearing function in heterozygous carriers of a pathogenic GJB2 gene mutation. Physiol Res. 2013; 62 (3): 323-330.
12. Van Heurck R, Carminho-Rodrigues MT, Ranza E, Stafuzza C, Quteineh L, Gehrig C et al. Benefits of exome sequencing in children with suspected isolated hearing loss. Genes. 2021; 12 (8): 1277.
13. Panigrahi I, Kumari D, Kumar BNA. Single gene variants causing deafness in Asian indians. J Genet. 2021; 100 (2): 35.
14. Rodriguez-Valero M, Pastolero A, Redfield S, Medrano A, Abreu-Gonzalez M, Gallardo-Ollervides JF et al. High prevalence of syndromic hearing loss in Mexican children undergoing cochlear implantation. Laryngoscope Investig Otolaryngol. 2024; 9 (3): e1291.
15. Jaijo T, Aller E, Oltra S, Beneyto M, Nájera C, Ayuso C et al. Mutation profile of the MYO7A gene in Spanish patients with Usher syndrome type I. Hum Mutat. 2006; 27 (3): 290-1.
16. Klarov LA, Pshennikova VG, Romanov GP, Cherdonova AM, Solovyev AV, Teryutin FM, et al. Analysis of

SLC26A4, FOXI1, and KCNJ10 gene variants in patients with incomplete partition of the cochlea and enlarged vestibular aqueduct (EVA) anomalies. Int J Mol Sci. 2022; 23 (23): 15372.
17. Garcia-Garcia G, Aparisi MJ, Jaijo T, Rodrigo R, Leon AM, Avila-Fernandez A et al. Mutational screening of the USH2A gene in Spanish USH patients reveals 23 novel pathogenic mutations. Orphanet J Rare Dis. 2011; 6 (1): 65.
18. Lameiras AR, Goncalves AC, Santos R, O'Neill A, Reis LRD, Matos TD et al. The controversial p.Met34Thr variant in GJB2 gene: two siblings, one genotype, two phenotypes. Int J Pediatr Otorhinolaryngol. 2015; 79 (8): 1316-1319.
19. Tang H, Fang P, Ward PA, Schmitt E, Darilek S, Manolidis S et al. DNA sequence analysis of GJB2, encoding connexin 26: Observations from a population of hearing impaired cases and variable carrier rates, complex genotypes, and ethnic stratification of alleles among controls. Am J Med Genet A. 2006; 140A (22): 2401-2415.
20. Izumi K, Kohta T, Kimura Y, Ishida S, Takahashi T, Ishiko A et al. Tietz syndrome: unique phenotype specific to mutations of MITF nuclear localization signal. Clin Genet. 2008; 74 (1): 93-95.
21. Felix F, Ribeiro MG, Tomita S, Zalis MG. Frequency of GJB2 mutations in patients with nonsyndromic hearing loss from an ethnically characterized Brazilian population. Braz J Otorhinolaryngol. 2019; 85 (1): 92-98.
22. Steinberg SJ, Raymond GV, Braverman NE, Moser AB. Zellweger Spectrum Disorder. 2003. In: Adam MP, Feldman J, Mirzaa GM et al., editors. GeneReviews®. Seattle (WA): University of Washington, Seattle; 1993-2024. Available from: https://www.ncbi.nlm.nih.gov/books/NBK1448/
23. Riazuddin S, Anwar S, Fischer M, Ahmed ZM, Khan SY, Janssen AGH et al. Molecular basis of DFNB73: Mutations of BSND can cause nonsyndromic deafness or Bartter syndrome. Am J Hum Genet. 2009; 85 (2): 273-280.
24. Zhang D, Wu J, Yuan Y, Li X, Gao X, Han M et al. A novel missense variant in CEACAM16 gene causes autosomal dominant nonsyndromic hearing loss. Ann Hum Genet. 2022; 86 (4): 207-217.
25. Wolf A, Frohne A, Allen M, Parzefall T, Koenighofer M, Schreiner MM et al. A novel mutation in SLC26A4 causes nonsyndromic autosomal recessive hearing impairment. Otol Neurotol. 2017; 38 (2): 173-179.

Funding: this study did not receive any funding.
Conflict of interests: the authors declare there are not any conflict of interest.