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Original article

Genetic Screening of *GJB6* Large Deletions (*GJB6*-D13S1830 and *GJB6*-D13S1854) in Selected Libyan Families with Non-Syndromic Hearing Loss

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ABSTRACT
Hearing loss (HL) is one of the most common sensory defects, affecting approximately 1 in 1,000 newborns worldwide, while nearly 4% of individuals under 45 years of age experience some degree of hearing impairment, with a significant genetic contribution. Non-syndromic hearing loss (NSHL) is predominantly inherited in an autosomal recessive manner (75–80%), followed by autosomal dominant inheritance (20–25%), with X-linked and mitochondrial patterns accounting for a small proportion of cases (1–2%). The DeaFNess, autosomal recessive, locus B1 (DFNB1) is a major genetic contributor to autosomal recessive non-syndromic hearing loss (ARNSHL) globally. Two deletions in the Gap Junction Protein Beta 6 gene (GJB6), del(D13S1830) and del(D13S1854), are commonly implicated in this locus. The present study aimed to investigate the contribution of GJB6 deletions to hearing loss in four unrelated Libyan families. A total of 38 participants were involved, including 17 individuals diagnosed with congenital NSHL and 21 unaffected relatives from the same families. Following DNA extraction from blood samples, multiplex polymerase chain reaction (PCR) was performed, and the amplified products were visualized by agarose gel electrophoresis. Selected products were further analyzed by DNA sequencing. Neither the del(D13S1830) nor the del(D13S1854) GJB6 deletions were detected in any of the examined samples. However, DNA sequencing identified a heterozygous C/T variant in the GJB6 gene in one family, suggesting the presence of a rare or potentially novel variant associated with NSHL. The absence of these deletions in the studied families indicates that genetic factors other than GJB6 may play a role in the etiology of ARNSHL in the Libyan population. These findings highlight the genetic heterogeneity of NSHL and emphasize the need for further comprehensive genetic investigations to elucidate the full spectrum of causative genes in this population.

Introduction

Hearing loss (HL) is a heterogeneous condition characterized by partial or complete impairment of hearing and is the most common sensory defect present at birth [1]. According to the latest World Health Organization (WHO) update, HL is the most prevalent sensory impairment in both children and adults, affecting approximately 432 million adults and 34 million children worldwide, which accounts for over 5% of the global population [2]. Deafness is an etiologically heterogeneous trait that may result from environmental (acquired) factors or genetic (hereditary) causes.

There are several degrees of HL, including mild, moderate, and profound [3]. Genetically based HL can be classified as either syndromic or non-syndromic [4]. Syndromic hearing loss (SHL) is accompanied by additional clinical features that affect the ears as well as other parts of the body [5]. In contrast, non-syndromic hearing loss (NSHL) involves a partial or complete impairment of hearing without associated anomalies in other organs or systems [5].

NSHL is classified according to inheritance patterns into DFNA (autosomal dominant), DFNB (autosomal recessive), and DFNX (X-linked) categories [6, 7]. Genetic factors account for more than half of all cases of congenital (pre-lingual) deafness, including approximately 30% of syndromic and 70% of non-syndromic cases [4]. Among NSHL cases, the majority (approximately 80%) exhibit autosomal recessive inheritance, followed by autosomal dominant (12–15%), X-linked (~1%), and mitochondrial (~1%) patterns of inheritance [8].

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Hearing requires the maintenance of appropriate potassium ion levels in the inner ear to convert sound into electrical signals. Studies have indicated that gap junctions composed of connexin 30 help regulate potassium ion homeostasis by facilitating their transfer from supporting cells to hair cells through these gap junctions [9]. Connexin 26, connexin 30, and connexin 31, encoded by the gap Junction protein beta 2 (*GJB2)*, gap Junction protein beta 6 (*GJB6)*, and gap Junction protein beta 3 (*GJB3)* genes, respectively, form gap junctions in the inner ear [10].

To date, a total of 88 genes, including *GJB2* and *GJB6*, have been implicated in autosomal recessive non-syndromic hearing loss (ARNSHL) [11]. The DFNB1 locus (DeaFNess, B type 1) on chromosome 13q12 comprises the homologous genes *GJB2* (OMIM: 121011) and *GJB6* (OMIM: 604418)**,** which encode connexin 26 and connexin 30 subunits of gap junction proteins, respectively. Variants in the *GJB2* and *GJB6* genes account for approximately 50% of cases of this type of hearing impairment [12]. Despite the wide genetic heterogeneity of HL, the most frequently mutated genes in severe to profound ARNSHL are *GJB2* and *GJB6,* which encode gap junction beta-2 protein (connexin 26) and gap junction beta-6 protein (connexin 30), respectively.

The *GJB2* and *GJB6* genes are expressed in the cochlea, where they combine to form multi-unit hemi-channels in the cell membrane and function as integral components of the potassium regulation in the cochlea [13, 14]. Generally, mutations that result in a loss of gap-junction channel function are related to ARNSHL [15].-Mutations in the *GJB6* gene are among the key genetic contributors to NSHL. Several studies have focused on characterizing and updating the spectrum of *GJB6* deletions, highlighting their role in ARNSHL [16-18]. Large deletions in the *GJB6* gene, particularly del(*GJB6*-D13S1830) and del(*GJB6*-D13S1854), are important contributors to NSHL in some populations, but their prevalence is highly variable. Several studies across the Middle East and North Africa have reported low or absent frequencies of these deletions. In Syria, del(*GJB6*-D13S1830) was detected in homozygosity in only one proband, while del(*GJB6*-D13S1854) was not observed [19]. Similar studies in Algeria, Egypt, Morocco, Qatar, Saudi Arabia, and Iran found that *GJB6* deletions were extremely rare or absent**,** whereas the *GJB2* c.35delG mutation was frequently detected [20-24]. Only isolated cases, such as one homozygous *GJB6*-D13S1830 deletion in Saudi Arabia, were linked to NSHL without accompanying *GJB2* mutations [25].

GJB6 deletions have also been extensively studied in Europe and the Americas. In Spain, del(*GJB6*-D13S1854) was identified in 25.5% of *GJB2* heterozygotes, with similar deletions reported in other countries: 22.2% in the UK, 6.3% in Brazil, and 1.9% in northern Italy, suggesting population-specific differences [16, 17]. These findings suggest that the del(*GJB6*-D13S1854) mutation is generally rare but may occur more frequently in certain populations, particularly in Spain. In a related multicenter study, a founder effect for del(*GJB6*-D13S1830) was identified in European Jews, who shared a common haplotype extending over 464 kb, suggesting an older origin for the deletion in this population [16, 17]. In contrast, del(*GJB6*-D13S1830) is the most frequent *GJB6* deletion in Spain, France, the United Kingdom, and Brazil, accounting for 5.9–9.7% of all DFNB1 alleles. On the other hand, it is less frequent in the USA, Belgium, and Australia (1.3–4.5% of all DFNB1 alleles). Even though this deletion is absent in Austria, Turkey, and China, it is rare in southern Italy, and more common in northern Italy and Germany, at comparable frequencies to other European countries [13, 16, 17]. Moreover, founder effects have also been reported, such as for del(*GJB6*-D13S1830) in European Jews [17]. Studies from Venezuela, Argentina, and the United States confirm that while *GJB6* deletions are generally less frequent than *GJB2* mutations [26-30].

Taken together, the two *GJB6* deletions can contribute significantly to NSHL via compound heterozygosity or digenic inheritance with *GJB2* variants. These findings highlight the variable prevalence and clinical significance of *GJB6* deletions across populations and underscore the importance of including them in comprehensive genetic screening for NSHL. Therefore, the aim of this study was to investigate whether the two *GJB6* deletions are involved in NSHL cases in selected Libyan families and to determine their incidence in this population. We perform mutational screening for the *GJB6* deletions, del(D13S1830) and del(D13S1854), associated with NSHL in these selected families.

Methods

Subjects and Sample Collection

This study was approved by the National Center for Disease Control of Libya. The study was conducted in the Department of Biochemistry and Molecular Biology, Faculty of Medicine, University of Tripoli. Informed consent was obtained from all participating individuals and/or their parents.

The study included 38 Libyan participants from four unrelated families. Comprising 17 congenital NSHL and 21 unaffected family members. The 17 affected individuals, who had diagnostic audiologic evaluation for NSHL at the Department of Otorhinolaryngology /Tripoli Central Hospital and University of Tripoli Hospital in Libya and were diagnosed with a bilateral severe to profound sensorineural HL, were included in this study. The analyzed Libyan families originate from different geographic regions, including Al-Jabal

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Al-Garbi, Aljosh, and Tripoli. Written consent and questionnaire form were completed and signed by all participants on their behalf in this study before obtaining the blood samples.
A structured questionnaire was used to collect demographic data (age, sex, marital status, and number of children), clinical information (diagnosis and age at onset: pre-lingual or post-lingual), and family history, including parental consanguinity and the presence of affected relatives.

Family pedigrees

Family I consist of 20 members, 9 (45%) of whom are NSHL patients (2 females and 7 males), and 11 (55%) non-NSHL members, with one male having passed away. Eight out of 20 family members were selected to perform genetic analysis of *GJB6* del(D13S1830 and D13S1854). The samples included 3 NSHL (18, 24, and 25) and 5 non-NSHL (19, 20, 21, 22, and 23) members (Figure 1). **Family II** consists of 14 members, 8 (57.1%) of whom were NSHL (4 males and 4 females) and 6 (42.9%) non-NSHL, with one female passed away. Five out of 14 family members were selected to perform genetic analysis of *GJB6* del(D13S1830 and D13S1854). The samples include 3 NSHL (13, 14, and 17) and 2 non- NSHL (15, and 16) members (Figure 1). **Family III** consists of 50 members, 16 (32%) of whom were NSHL (9 males and 6 females) with one male having passed away, and 34 (68%) non-NSHL, with one male having passed away. Nineteen out of 50 family members were selected to perform genetic analysis of *GJB6* del(D13S1830 and D13S1854). The samples include 10 NSHL (1, 5, 7, 8, 9, 11, 26, 28, 37 and 38) and 9 non- NSHL (2, 3, 4, 6, 10, 12, 27, 35 and 36) members (Fig. 1). **Family IV** comprises of 20 members, 3 (15%) of whom were NSHL (3 males) and 17 (85%) non-NSHL with one male passed away. Six out of 20 family members were selected to perform genetic analysis of *GJB6* del(D13S1830 and D13S1854). The samples include 1 NSHL (34) and 5 non- NSHL (29, 30, 31, 32, and 33) members (Figure 1).

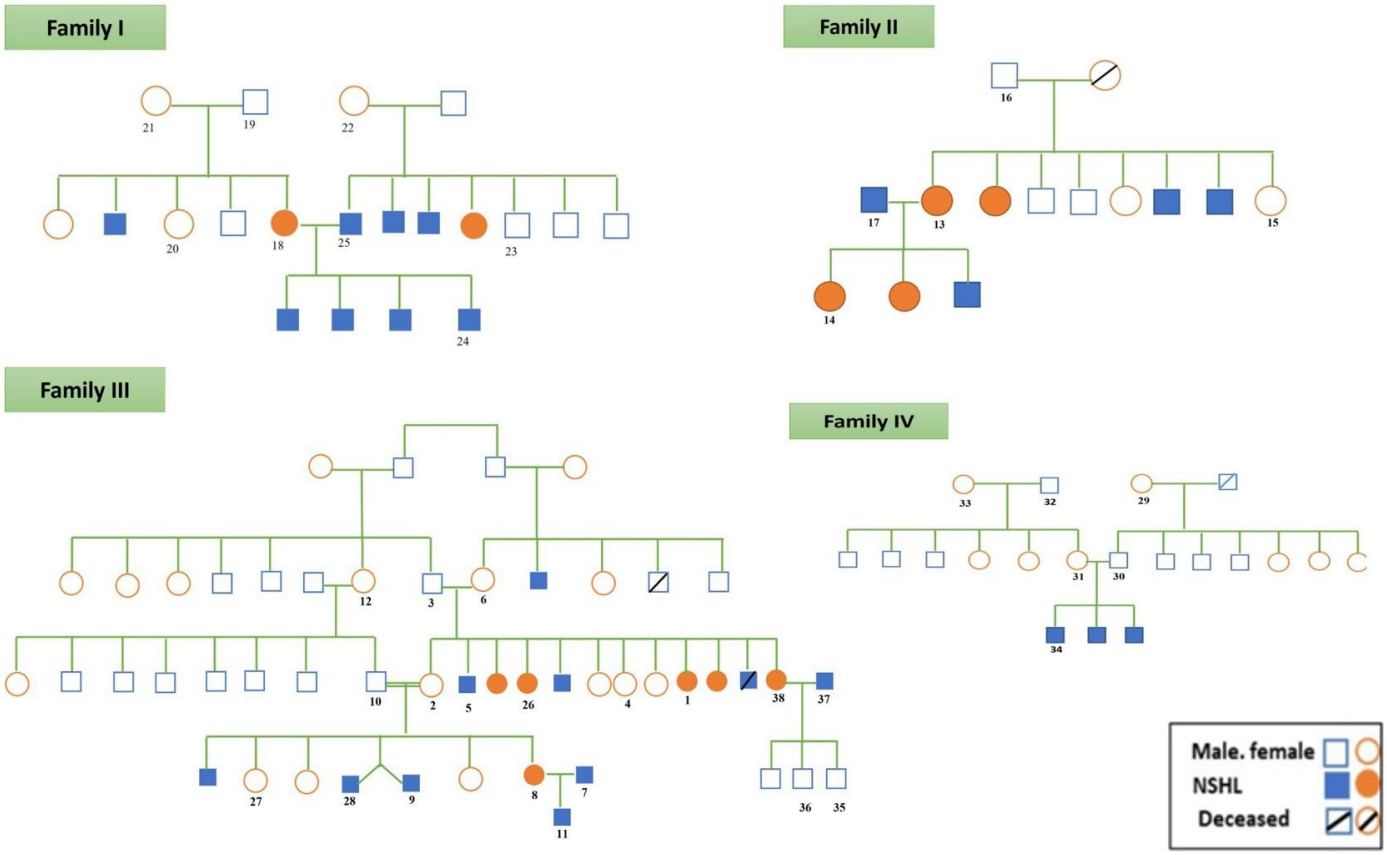

Figure 1. Pedigrees of the four studied families. Pedigree diagrams of Families I, II, III & IV. Squares indicate males, and circles indicate females. Filled symbols represent affected individuals, empty symbols represent unaffected individuals, and symbols crossed by a diagonal line denote deceased individuals.

Molecular testing

Two milliliters (mL) of blood were collected from affected patients, their parents, and unaffected siblings, as shown in the pedigree (Figure 1). The DNA extraction, PCR reaction, gel electrophoresis, and visualization

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was carried out in the biochemistry and molecular biology department at the Faculty of Medicine, University of Tripoli. Additionally, the sequencing of DNA analysis of selected samples was carried out in (Ribosite Biotechnology for advanced molecular biology, technical support, and customized services, Tunisia).
Genomic DNA was extracted using a DNA extraction kit (Epicenter Master Pure™ Complete DNA and RNA Purification Kit Bulk Reagents, China) following the manufacturer's protocol. The deletion and wild type fragments of selected samples were targeted using primers manufactured by Ribosite Biotechnology for advanced molecular biology, technical support, and customized services (Carthagenetics, Tunisia). The primer sequences were adopted from [16, 18], their genomic location was investigated and confirmed using an online database. Forward and reverse primers were used to amplify a 685-bp fragment of the wild-type allele, while an additional reverse primer (reverse deletion) was used for targeting a 460-bp fragment corresponding to the del(*GJB6*-D13S1830) mutation (Table 1) and (Figure 2). Regarding the del(*GJB6*-D13S1854) mutation, forward and reverse primers were used to amplify a 333-bp fragment representing the wild type allele, and another forward primer (forward deletion) and a reverse primer (reverse deletion) were used to target a 564-bp fragment corresponding to the del(*GJB6*-D13S1854) mutation (Table 1) and (Figure 2).

Table 1. The primer sequences of GJB6 Deletions

Primer	Deletion	Sequence
Forward primer	del(*GJB6*-D13S1830)	5'-TTTAGGGCATGATTGGGGTGATTT-3'
Reverse primer	del(*GJB6*-D13S1830)	5'-CACCATGCGTAGCCTTAACCATTTT-3'
Reverse deletion primer	del(*GJB6*-D13S1830)	5'-TCATCGGGGGTGTCAACAAACA-3'
Forward primer	del(*GJB6*-D13S1854)	5' -TTTAGGGCATGATTGGGGTGATTT-3'
Reverse primer	del(*GJB6*-D13S1854)	5'-CACCATGCGTAGCCTTAACCATTTT-3'
Forward deletion primer	del(*GJB6*-D13S1854)	5'-TCATAGTGAAGAACTCGATGCTGTTT-3'
Reverse deletion primer	del(*GJB6*-D13S1854)	5'-CAGCGGCTACCCTAGTTGTGGT-3'

PCR amplification for DNA mutation screening was executed using Hot Firepol Blend Master Mix (5x) with 10Mm $MgCl_2$, (Solis BioDyne, Estonia), following the manufacturer's guidelines. Thermal cycling was programmed, adapted from [18], were initiated by 95°C for 2min followed by 45 cycles consisting of denaturation at 94°C for 30sec, annealing at 62°C for 30sec, and extension at 72°C for 2min. A final extension step at 72°C for 7min was added. PCR products were visualized by 2 % agarose gel electrophoresis. From some families, 15 µl of two PCR products (one affected and one unaffected sample) were sent for sequencing using forward and/or reverse primers to confirm PCR results.

Sequencing and primer analysis

Bioinformatics tools were employed to validate primer binding sites and support sequencing analysis. The UCSC Genome Browser (University of California, Santa Cruz) and the National Center for Biotechnology Information (NCBI) databases were utilized to confirm the genomic locations and specificity of the primers. Furthermore, Chromas (v2.6.6) software (https://technelysium.com.au/wp/chromas/) was used to analyze sequencing chromatograms and to detect potential mutations within the sample sequences. Finally, the MultiAlin web tool (Multiple sequence alignment with hierarchical clustering; Corpet) was used to align the obtained sequences with reference sequences from public databases [31].

Ethical approval

This study was approved by the Ethics Committee at the National Center for Disease Control under reference number NBC: 002.H-25.43.

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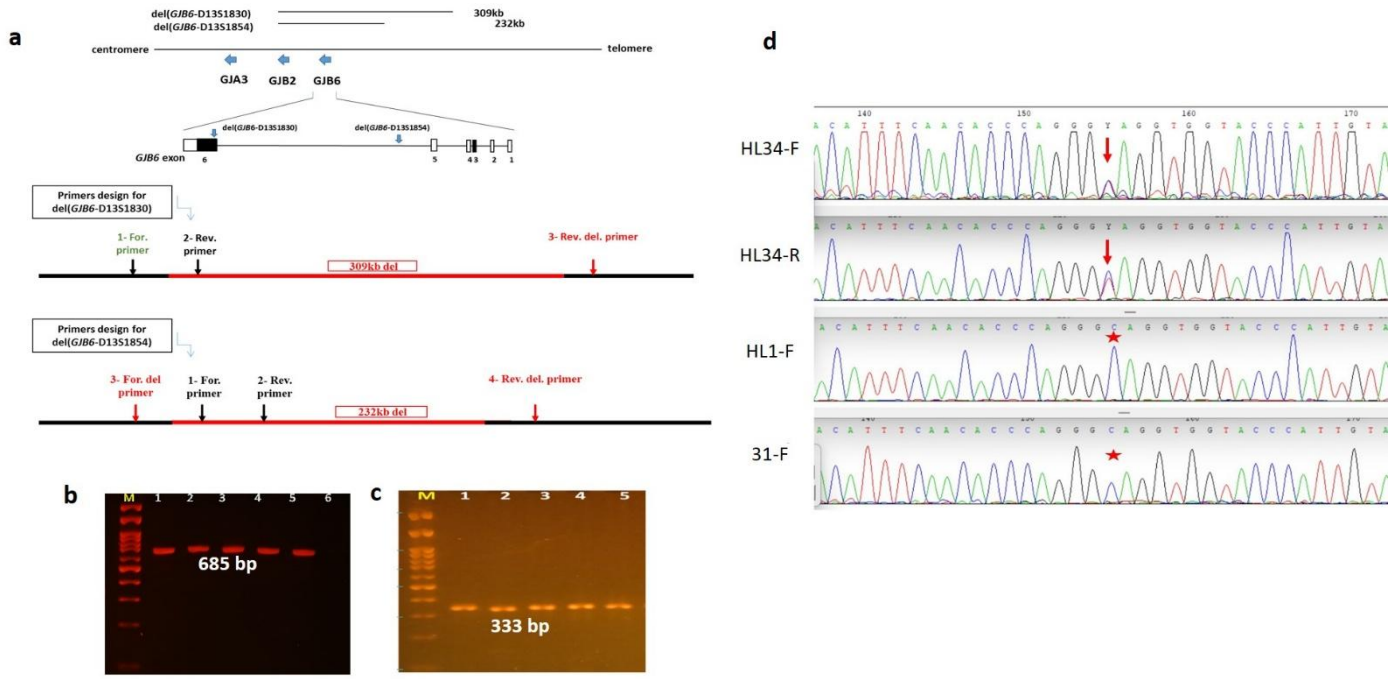

Figure 2. GJB6 gene deletions analysis. a) Schematic representation of the GJB6 gene deletions (del(GJB6-D13S1830) and del(GJB6-D13S1854)) and the locations of their respective primer sets. *The deletions span 309 kb and 232 kb, respectively. The positions of wild-type primers are shown in black, deletion-specific primers in red, and primers common to both designs in green.* ***b) Agarose gel electrophoresis image showing the results of Multiplex PCR for del(GJB6-D13S1830).*** *Lane M: 100bp DNA ladder. Lanes 1-5: DNA samples of affected and non-affected individuals, which show bands at 685bp that represent the WT fragment. Lane 6: negative control.* ***c) Agarose gel electrophoresis image showing the results of Multiplex PCR for del(GJB6-D13S1854).*** *Lane M: 100bp DNA ladder. Lanes 1-5: DNA samples of affected and non-affected individuals, which show bands at 333bp that represent the WT fragment.* ***d) Chromatogram analysis of the identified C/T variation in the GJB6 flanking region among patients and family members.*** *(HL34-F) Sequence chromatogram of the affected patient (HL34) showing a heterozygous C/T variation (indicated by a red arrow) using the forward primer. (HL34-R) Sequence chromatogram of the same patient confirming the heterozygous C/T variation (arrow) using the reverse primer. (HL1-F) Sequence chromatogram of a second patient without the variation, showing a homozygous wild-type C/C genotype at the corresponding position (indicated by a red star), sequenced with the forward primer. (31-F) Sequence chromatogram of the unaffected mother of patient HL34, who carries the variation, demonstrating a homozygous wild-type sequence at the corresponding nucleotide position (indicated by a red star), sequenced with the forward primer.*

Results

The two *GJB6* deletions del(*GJB6*-D13S1830) and del(*GJB6*-D13S1854) were studied in 38 NSHL Libyan patients by PCR and sequencing. Based on the data collected from the participant questionnaires, statistical analysis results revealed the proportion of individuals affected by NSHL in each family, as presented in Tables 2 and 3. Notably, a family history of consanguinity was reported in Family III. Among the 38 individuals, the average age was 34 years, including 19 (50%) females and 19 (50%) males.

Table 2. Number and percentage of affected (NSHL) and non-affected individuals in each family

Family (no)	Affected (NSHL) (n)	Affected (%)	Non-Affected (n)	Non-affected (%)	Total
Family I	9	45%	11	55%	20
Family II	8	57.1%	6	42.9%	14
Family III	16	32%	34	68%	50
Family IV	3	15%	17	85%	20

PCR and gel electrophoresis results

Consistent results were obtained from both traditional PCR and multiplex PCR reactions targeting (*GJB6*-D13S1830) and (*GJB6*-D13S1854) deletions. The wild type allele of the first deletion (*GJB6*-D13S1830) was successfully detected as a 685bp band, while no amplification was observed at 460bp corresponding to the del(*GJB6*-D13S1830), indicating the absence of this deletion in the tested samples (Figure 2b).

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The PCR for the second deletion, *GJB6*-D13S1854, was designed to detect the deleted band at 564bp and the wild type at 333bp (Figure 2c). The wild type allele of the *GJB6*-D13S1854 was successfully detected as a 333bp band, while no amplification was observed at 564bp, indicating the absence of this deletion in the tested samples (Figure 2c).

Table 3. Questionnaire data of affected individuals with NSHL from each family

Sample (no)	Degree of HL	Age	Gender	Marital status	Pre-lingual	Consanguinity	Affected siblings (no)	Affected children (no)
1	Profound	42	F	Married	Pre-lingual	Cousin	6	NO
5	Sever profound	57	M	Married	Pre-lingual	Cousin	6	NO
7	Profound	36	M	Married	Pre-lingual	No	No	1
8	Profound	29	F	Married	Pre-lingual	Cousin	3	1
9	Profound	23	M	Single	Pre-lingual	Cousin	3	/
11	Sever	4	M	Single	Pre-lingual	No	No	/
13	Profound	55	F	Married	Pre-lingual	No	4	3
14	Sever	17	F	Single	Pre-lingual	No	2	/
17	Sever	59	M	Married	Pre-lingual	No	2	3
18	Profound	37	F	Married	Pre-lingual	No	1	2
24	Sever	11	M	Single	Pre-lingual	No	1	/
25	Profound	42	M	Married	Pre-lingual	No	4	2
26	Profound	55	F	Married	Pre-lingual	Cousin	6	NO
28	Profound	23	M	Single	Pre-lingual	Cousin	3	/
34	Profound	5	M	Single	Pre-lingual	No	3	/
37	Profound	47	M	Married	Pre-lingual	cousin	2	NO
38	Profound	39	F	Married	Pre-lingual	cousin	6	NO

DNA sequencing

DNA sequencing was performed on four selected samples (out of 38) to validate the gel electrophoresis findings for both *GJB6* deletions. Sequencing of affected (samples HL34 and HL1) and non-affected (samples 4 and 31) individuals, using combinations of wild-type and deletion-specific primers for both del(*GJB6*-D13S1830) and del(*GJB6*-D13S1854), consistently showed no evidence of either deletion. These results confirmed the absence of both GJB6 deletions in all sequenced samples.

Detection of a novel heterozygous C/T Variation

In addition to confirming the absence of both GJB6 deletions, sequencing of the same four samples revealed a heterozygous C/T substitution within the del(*GJB6*-D13S1830) amplified region (chr13:20222771–20223455, GRCh38/hg38). In the context of familial NSHL, this C/T variant was detected in one affected individual from Family IV (patient HL34, male), and was confirmed in both forward and reverse sequencing reads (Figure 4d), supporting its authenticity as a true genetic variant rather than a sequencing artifact. This substitution was absent in the second affected patient (HL1) (Figure 4d) as well as in the unaffected individual (4). Moreover, the variant was not observed in the unaffected mother of patient 31 (Family IV), whose sequencing chromatogram revealed a homozygous wild-type genotype (Figure 4d). Notably, this variant has not been reported in commonly used public SNP databases, suggesting it may represent a rare or potentially novel mutation.

Discussion

According to the Homepage of Hereditary Hearing Loss, a total of 156 genes associated with NSHL have been identified to date [11]. More than 50% of families with ARNSHL present mutations in the DFNB1 locus. Based on the literature, the prevalence of mutations in the *GJB2* and *GJB6* genes in individuals with HL has been determined in different populations [16, 17, 19]. A large deletion involving the 5′ noncoding region of *GJB6* that extended into the coding region of the gene was reported by del Castillo *et al.* 2003 and other groups [16, 32, 33]. The current study, therefore, aimed to evaluate the role of two common large deletions

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in the *GJB6* gene, specifically del(*GJB6*-D13S1830) and del(*GJB6*-D13S1854), in patients with ARNSHL from the selected Libyan families. The results of the present study revealed that none of the probands in the cohort carried either del(*GJB6*-D13S1830) or del(*GJB6*-D13S1854) deletions, indicating that *GJB6* deletions are not a contributing factor to HL in the studied patients from Libyan families.

This finding aligns with previous studies conducted in various neighboring and regional populations, including those from Tunisia, Morocco, Egypt, Qatar, Iran, India, Jordan, Turkey, and Cyprus, where the absence of these deletions has also been consistently reported [20-23, 34, 35].

The findings of this study contrast with findings reported by Almontashiri *et al.* (2018), who identified the del(*GJB6*-D13S1830) mutation in a homozygous state in one of 33 Saudi patients. Similarly, Zaidieh *et al.* (2015) reported that del(*GJB6*-D13S1830) occurred in homozygosity in just one proband (2.43%), while the del(*GJB6*-D13S1854) mutation was absent in 41 Syrian participants. In contrast, Al-Achkar *et al.* (2017) found a compound heterozygous genotype of del(*GJB6*-D13S1854)/c.IVS1+1G>A in five Syrian families. Additionally, this mutation was combined with the c.35delG mutation in two families, and in four families, it was observed in a heterozygous state (del(*GJB6*-D13S1854)/unknown). However, del(GJB6-D13S1830) was not detected in the patients studied by Al-Achkar *et al.* (2017). Furthermore, Del Castillo *et al.* (2005) identified a 232 kb deletion del(*GJB6*-D13S1854) in trans in 12 of 47 unrelated affected Spanish individuals, which accounted for 25.5% of the affected *GJB2* heterozygotes.

It has been noted in previous studies that when the (*GJB6*-D13S1830) and (*GJB6*-D13S1854) deletions are present in trans with a *GJB2* mutation, they can explain HL in 67% of their deaf individual who are heterozygotes *for the GJB2* mutation. Although this deletion was previously reported as a 342 kb deletion, the current estimate for del(*GJB6*-D13S1830) is 309 kb [17]. According to reports, the percentage of deaf people who have this deletion varies from as high as 15% in Southern France to as low as 1.4% in Italy and Belgium, and between 9% and 5% in Spain, the United Kingdom, and other countries. [36]. Some other studies reported less common or nonexistent conditions in Eastern Europe and other countries [37, 38]. Other deaf *GJB2* heterozygotes, primarily in Spain and the United Kingdom, were later found to have a second, smaller deletion in the 5 untranslated region of *GJB6* del(*GJB6*-D13S1854) [17]. The observed pattern strongly suggests that *GJB6* deletions are geographically and ethnically restricted, likely due to a founder effect in Western European populations, particularly Spain and France, where these mutations have been reported at significant frequencies [16, 39]. In the present study, both *GJB6* deletions were absent in all analyzed Libyan families, despite their varied geographic origins (Al-Jabal Al-Garbi, Aljosh, and Tripoli). Although the precise ancestral backgrounds of these families could not be confirmed genetically, historical information suggests that they represent different ethnic groups, including Amazigh and Arab lineages. Taken together, these findings indicate that these deletions are likely absent in both ethnic populations.

Many studies indicate that consanguineous marriages are risk factors for an increased number of children with deafness and/or muteness in a population [40-44]. In the same line, the results of this study showed that Family III has a positive history of consanguinity; the percentage of affected NSHL was 32% in this family. During sequencing analysis of the deletion del(*GJB6*-D13S1830), a heterozygous C/T variation was identified in one NSHL patient sample (Male, HL34 in Family IV). This variant was obtained from both forward and reverse strands, supporting its authenticity. It was absent in the second patient and in two sequenced controls, and it is not reported in public single-nucleotide polymorphism (SNP) databases, suggesting that it is most likely a rare or novel variant. Notably, the variation was not detected in the unaffected mother of the patient sample (HL31 in Family IV), whose sequence showed a homozygous wild-type genotype. The variant occurred in only one affected person, and no co-segregation data are available to determine whether the variant is likely pathogenic or merely a benign polymorphism. Therefore, the clinical significance of this variation remains uncertain, and further investigation is needed to determine its potential contribution to NSHL.

It is worth noting that, during the period when the present study was conducted in the laboratory, another research on *GJB2* (*c.35delG*) of the same samples of this study was being carried out. The yet unpublished results of that study indicated the presence of *GJB2* (*c.35delG)* deletion in 2 (50%) of the participating families. The other 2 (50%) families were negative for the *GJB2* (c.35delG) and for the deletions of this study. These results indicated that those 2 (50%) families may have been affected by another mutation. Notably, the C/T variant identified in this study was detected in Family IV, which does not carry the common *GJB2* (c.35delG) mutation. The detection of the C/T variant in a family free of the *GJB2* (c.35delG) mutation raises the possibility that this variant may represent an alternative genetic cause of HL in this family.

It is interesting to note that although *GJB6* deletions and *GJB2* mutations together are known to generate digenic inheritance in some populations [45]. Our findings do not suggest a similar contribution in our cohort. The limited significance of *GJB6* in our context was further supported by the fact that none of the patients who were heterozygous for *GJB2* mutations displayed signs of concomitant *GJB6* deletions. This

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implies that substantial deletions in the nearby *GJB6* gene are unlikely to account for the high percentage of monoallelic *GJB2* instances in our group; instead, other genetic pathways need to be considered.

Conclusion

The findings of the current study indicate the absence of del(*GJB6*-D13S1830) and del(*GJB6*-D13S1854) deletions in some families in the Northwest Libyan population. These results suggest that loci other than *GJB6* may contribute to the pathogenesis of ARNSHL. This may indicate that the full spectrum of genes involved in NSHL in the Libyan population is not yet fully elucidated. The prevalence of *GJB6* mutations, particularly deletions such as del(*GJB6*-D13S1830) and del(*GJB6*-D13S1854), varies widely across different populations. While *GJB2* mutations, especially c.35delG, remain the primary genetic cause of ARNSHL in many regions, *GJB6* deletions seem to play a more significant role in certain populations, such as in Spain and among European Jews. The international findings suggest that while these mutations are of global concern, their frequency and impact are highly context-dependent, influenced by geographic and ethnic factors. Importantly, the heterozygous C/T variation identified in the current study represents a preliminary result that may suggest the presence of a novel or rare variant contributing to HL in at least one family without a *GJB2* mutation. Further research involving larger and more diverse cohorts is essential to confirm these findings and to better characterize the genetic landscape of NSHL in the Libyan population.

Author contributions

Mansur E. Shmela; designed the genetic study, performed bioinformatic analyses, interpreted the results, and wrote and revised the manuscript, secured funding, and supervised the research group. Hajer M. Almabrouk; recruited patients, collected clinical data, performed molecular genetic experiments, interpreted the results, and wrote the manuscript. Fahima A. Alnagar; designed the genetic study, interpreted the results, wrote and revised the manuscript, secured funding and supervised the research group. Mufida O. Alwakel; recruited patients and acquired clinical data. Mohamed M. Said; revised the manuscript. Najwa A. Benfayed, Alamin M. Zaid, and Mohamed M. Albendag; assisted with molecular genetic experiments. All authors have read and approved the final version of the manuscript.

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Conflicts of interest

The authors declare no conflict of interest in relation to the publication of this work.

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REFERENCES

1. Petit C, Levilliers J, Hardelin JP. Molecular genetics of hearing loss. Annu Rev Genet. 2001;35:589-646.
2. WHO. deafness and hearing loss. World Health Organization. 2022.
3. Wang J, Sung V, Carew P, Burt RA, Liu M, Wang Y, *et al.* Prevalence of childhood hearing loss and secular trends: a systematic review and meta-analysis. Academic pediatrics. 2019;19(5):504-14.
4. Morton CC, Nance WE. Newborn hearing screening--a silent revolution. The New England journal of medicine. 2006;354(20):2151-64.
5. Toriello HV, Smith SD. Hereditary Hearing Loss and Its Syndromes. Third Edition ed 2013.
6. Carpena NT, Lee MY. Genetic Hearing Loss and Gene Therapy. Genomics Inform. 2018;16(4):e20.
7. Shearer AE, Hildebrand MS, Schaefer AM, Smith RJ. Genetic hearing loss overview. GeneReviews®[Internet]. 2023.
8. Morton N. Genetic epidemiology of hearing impairment. Annals of the New York Academy of Sciences. 1991;630(1):16-31.
9. Jagger DJ, Forge A. Connexins and gap junctions in the inner ear--it's not just about K^+ recycling. Cell Tissue Res. 2015;360(3):633-44.
10. Yum SW, Zhang J, Scherer SS. Dominant connexin26 mutants associated with human hearing loss have trans-dominant effects on connexin30. Neurobiology of disease. 2010;38(2):226-36.
11. Walls WD AH, Smith RJH. . hereditary hearing loss.org. Hereditary Hearing Loss Homepage2025 [updated 2/19/25.
12. Petersen M, Willems P. Non-syndromic, autosomal-recessive deafness. Clinical genetics. 2006;69(5):371-92.

Received*: 20-12-2025 -* ***Accepted****: 17-02-2026 -* ***Published****: 23-02-2026*

13. Ouyang XM, Yan D, Yuan HJ, Pu D, Du LL, Han DY, *et al.* The genetic bases for non-syndromic hearing loss among Chinese. Journal of human genetics. 2009;54(3):131-40.
14. Teubner B, Michel V, Pesch J, Lautermann J, Cohen-Salmon M, Söhl G, *et al.* Connexin30 (Gjb6)-deficiency causes severe hearing impairment and lack of endocochlear potential. Hum Mol Genet. 2003;12(1):13-21.
15. Buonfiglio P, Bruque CD, Luce L, Giliberto F, Lotersztein V, Menazzi S, *et al.* GJB 2 and GJB 6 genetic variant curation in an Argentinean non-syndromic hearing-impaired cohort. Genes. 2020;11(10):1233.
16. Del Castillo I, Moreno-Pelayo MA, Del Castillo FJ, Brownstein Z, Marlin S, Adina Q, *et al.* Prevalence and evolutionary origins of the del (GJB6-D13S1830) mutation in the DFNB1 locus in hearing-impaired subjects: a multicenter study. The American Journal of Human Genetics. 2003;73(6):1452-8.
17. Del Castillo FJ, Rodríguez-Ballesteros M, Alvarez A, Hutchin T, Leonardi E, De Oliveira CA, *et al.* A novel deletion involving the connexin-30 gene, del(GJB6-d13s1854), found in trans with mutations in the GJB2 gene (connexin-26) in subjects with DFNB1 non-syndromic hearing impairment. J Med Genet. 2005;42(7):588-94.
18. Zaidieh T, Habbal W, Monem F. Screening of GJB6 gene large deletions among Syrians with congenital hearing impairment. Genetic Testing and Molecular Biomarkers. 2015;19(7):405-7.
19. Al-Achkar W, Al-Halabi B, Ali B, Moassass F. First report of prevalence c.IVS1+1G>A and del (GJB6-13S1854) mutations in Syrian families with non-syndromic sensorineural hearing loss. International Journal of Pediatric Otorhinolaryngology. 2017;92:82-7.
20. Gazzaz B, Weil D, Raïs L, Akhyat O, Azeddoug H, Nadifi S. Autosomal recessive and sporadic deafness in Morocco: High frequency of the 35delG GJB2 mutation and absence of the 342-kb GJB6 variant. Hearing Research. 2005;210(1):80-4.
21. Esmaeili M, Bonyadi M, Nejadkazem M. Common mutation analysis of GJB2 and GJB6 genes in affected families with autosomal recessive non-syndromic hearing loss from Iran: Simultaneous detection of two common mutations (35delG/del(GJB6-D13S1830)) in the DFNB1-related deafness. International Journal of Pediatric Otorhinolaryngology. 2007;71(6):869-73.
22. Khalifa Alkowari M, Girotto G, Abdulhadi K, Dipresa S, Siam R, Najjar N, *et al.* GJB2 and GJB6 genes and the A1555G mitochondrial mutation are only minor causes of nonsyndromic hearing loss in the Qatari population. International journal of audiology. 2012;51(3):181-5.
23. Elbagoury NM, Soliman HN, Mohammed OS, Ghorab EA, Elruby MO, El-bassyouni HT, *et al.* Mutation analysis of the GJB2 and GJB6 genes in Egyptian patients with autosomal recessive sensorineural nonsyndromic hearing loss. Middle East Journal of Medical Genetics. 2014;3(1):11-5.
24. Talbi S, Bonnet C, Boudjenah F, Mansouri MT, Petit C, Khodja FA. The spectrum of GJB2 gene mutations in Algerian families with nonsyndromic hearing loss from Sahara and Kabylie regions. International Journal of Pediatric Otorhinolaryngology. 2019;124:157-60.
25. Almontashiri NAM, Alswaid A, Oza A, Al-Mazrou KA, Elrehim O, Tayoun AA, *et al.* Recurrent variants in OTOF are significant contributors to prelingual nonsydromic hearing loss in Saudi patients. Genetics in Medicine. 2018;20(5):536-44.
26. Pandya A, Arnos KS, Xia XJ, Welch KO, Blanton SH, Friedman TB, *et al.* Frequency and distribution of GJB2 (connexin 26) and GJB6 (connexin 30) mutations in a large North American repository of deaf probands. Genetics in Medicine. 2003;5(4):295-303.
27. Feldmann D, Denoyelle F, Chauvin P, Garabédian EN, Couderc R, Odent S, *et al.* Large deletion of the GJB6 gene in deaf patients heterozygous for the GJB2 gene mutation: genotypic and phenotypic analysis. American Journal of Medical Genetics Part A. 2004;127(3):263-7.
28. Dalamón V, Béhèran A, Diamante F, Pallares N, Diamante V, Elgoyhen AB. Prevalence of GJB2 mutations and the del(GJB6-D13S1830) in Argentinean non-syndromic deaf patients. Hearing Research. 2005;207(1):43-9.
29. Utrera R, Ridaura V, Rodriguez Y, Rojas MJ, Mago L, Angeli S, *et al.* Detection of the 35delG/GJB2 and del (GJB6-D13S1830) mutations in Venezuelan patients with autosomal recessive nonsyndromic hearing loss. Genetic Testing. 2007;11(4):347-52.
30. Primignani P, Trotta L, Castorina P, Lalatta F, Sironi F, Radaelli C, *et al.* Analysis of the GJB2 and GJB6 genes in Italian patients with nonsyndromic hearing loss: frequencies, novel mutations, genotypes, and degree of hearing loss. Genet Test Mol Biomarkers. 2009;13(2):209-17.
31. Corpet F. Multiple sequence alignment with hierarchical clustering. Nucleic acids research. 1988;16(22):10881-90.
32. Pallares-Ruiz N, Blanchet P, Mondain M, Claustres M, Roux A-F. A large deletion including most of GJB6 in recessive non syndromic deafness: a digenic effect? European Journal of Human Genetics. 2002;10(1):72-6.
33. Lerer I, Sagi M, Ben-Neriah Z, Wang T, Levi H, Abeliovich D. A deletion mutation in GJB6 cooperating with a GJB2 mutation in trans in non-syndromic deafness: a novel founder mutation in Ashkenazi Jews. Human mutation. 2001;18(5):460-.
34. Trabelsi M, Bahri W, Habibi M, Zainine R, Maazoul F, Ghazi B, *et al.* GJB2 and GJB6 screening in Tunisian patients with autosomal recessive deafness. International Journal of Pediatric Otorhinolaryngology. 2013;77(5):714-6.
35. Bhalla S, Sharma R, Khandelwal G, Panda NK, Khullar M. Absence of GJB6 mutations in Indian patients with non-syndromic hearing loss. International Journal of Pediatric Otorhinolaryngology. 2011;75(3):356-9.
36. Marlin S, Feldmann D, Blons H, Loundon N, Rouillon I, Albert S, *et al.* GJB2 and GJB6 mutations: genotypic and phenotypic correlations in a large cohort of hearing-impaired patients. Archives of Otolaryngology–Head & Neck Surgery. 2005;131(6):481-7.

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37. Adhikary B, Ghosh S, Paul S, Bankura B, Pattanayak AK, Biswas S, *et al.* Spectrum and frequency of GJB2, GJB6 and SLC26A4 gene mutations among nonsyndromic hearing loss patients in eastern part of India. Gene. 2015;573(2):239-45.
38. Frei K, Lucas T, Ramsebner R, Schöfer C, Baumgartner W-D, Weipoltshammer K, *et al.* A novel connexin 26 mutation associated with autosomal recessive sensorineural deafness. Audiology and Neurotology. 2004;9(1):47-50.
39. Seeman P, Bendova O, Rašková D, Malikova M, Groh D, Kabelka Z. Double heterozygosity with mutations involving both the GJB2 and GJB6 genes is a possible, but very rare, cause of congenital deafness in the Czech population. Annals of Human Genetics. 2005;69(1):9-14.
40. Sajjad M, Khattak A, Bunn J, Mackenzie I. Causes of childhood deafness in Pukhtoonkhwa Province of Pakistan and the role of consanguinity. The Journal of Laryngology & Otology. 2008;122(10):1057-63.
41. Al Khabori M, Patton MA. Consanguinity and deafness in Omani children. International journal of audiology. 2008;47(1):30-3.
42. Yoong S, Feltbower R, Spencer N, McKinney P. Families affected by deafness: hospital services uptake in a multiethnic population. Archives of Disease in Childhood. 2005;90(5):454-9.
43. Ben Arab S, Masmoudi S, Beltaief N, Hachicha S, Ayadi H. Consanguinity and endogamy in Northern Tunisia and its impact on non-syndromic deafness. Genetic Epidemiology: The Official Publication of the International Genetic Epidemiology Society. 2004;27(1):74-9.
44. Ali Bozrayda S. Consanguinity and problems of hearing and speech among Libyan Population in Benghazi. 2020.
45. del Castillo FJ, del Castillo I. The DFNB1 subtype of autosomal recessive non-syndromic hearing impairment. Frontiers in bioscience (Landmark edition). 2011;16(9):3252-74.

Received*: 20-12-2025 -* ***Accepted****: 17-02-2026 -* ***Published****: 23-02-2026*