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Investigation of the Frequency of 167delT Mutations of *Gap Junction Protein Beta 2 (GJB2)* Gene in the Deaf Population of Non-Syndromic West of Iran

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A B S T R A C T

Introduction: Deafness is one of the most common sensory-neural disorders, with 80% of hereditary deafness being non-syndromic. The aim of this study was to investigate the frequency of the 167delT mutation of the *gap junction protein beta 2* (*GJB2*) gene in the deaf population of non-syndromic West Iran.

Materials & Methods: Seventy individuals were included in the study, with 34 being deaf and 36 being healthy. The RFLP-PCR technique was used to detect the 167delT mutation.

Results: The frequency of the homozygous mutant 167delT was 5.7% (4 individuals, equivalent to 8 chromosomes), with the heterozygous carriers at 75.7% (53 individuals, equivalent to 106 chromosomes), and the homozygous genotype in healthy individuals was 18.6% (13 individuals, equivalent to 26 chromosomes). In deaf individuals, the frequency of the homozygous mutant 167delT was 5.7% (4 individuals, equivalent to 8 chromosomes), the heterozygous genotype was 39.6% (27 individuals, equivalent to 54 chromosomes), and the frequency of the wild-type homozygous genotype was 4.7% (3 individuals, equivalent to 6 chromosomes). In healthy individuals, there was zero mutant genotype, 36.1% heterozygous carriers (26 individuals, equivalent to 52 chromosomes), and 13.9% wild-type homozygous (10 individuals, equivalent to 20 chromosomes).

Conclusion: Gene mutations play a role in causing non-syndromic autosomal recessive deafness in our statistical population from Kermanshah Province. The *GJB2* 167delT mutant is responsible for 5.7% of ARNSHL deafness in the population of Kermanshah, which differs from the rates reported in other parts of Iran.

Keywords: non-syndromic deafness, 167delT, *GJB2 gene*, ARNSHL deafness

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Introduction

Hearing impairment is the most common sensory impairment, affecting approximately 1 in every 1000 babies born (1). Non-diagnosis or delayed diagnosis of hearing impairment can have a profound effect on language communication skills, cognitive development, and psychological development. Hearing impairment can be caused by environmental factors, genetic defects, or a combination of the two (1). Around half of all hearing disorders have a genetic background, with almost 1% of all human genes involved in the hearing process (2). Genetic disorders can be inherited in four ways: through obvious autosomal (DFNA), latent autosomal (DFNB), or latent sex-linked (DFNX) inheritance or through mutation in the mitochondrial gene (3).

Around 70% of genetic deafness is non-syndromic, meaning the deaf person has no other disorders. The most common form of severe hereditary hearing loss is autosomal recessive nonsyndromic hearing loss (ARNSHL), accounting for 85% of deafness cases (4). This form of deafness is caused by mutations in two genes located in the 1DFNB locus of chromosome 13q-11-12: the *gap junction protein beta 2* (*GJB2*) and *gap junction protein beta 6* (*GJB6*) genes (5). The *GJB2* gene encodes the connexin 26 protein, and its mutation spectrum varies significantly among different populations (6). The 35delG mutation is common in white populations, the 167delT mutation in Ashkenazi Jews, and the 35delC mutation in East Asian populations (7).

The 167delT mutation is the most commonly known mutation in the American Ashkenazi Jewish population, with a current frequency of 4% (2). Mutations in the *GJB2* gene sequence, such as the 167delT mutation, involve the deletion of the thymine base at position 167. This deletion causes the UAG stop codon to be created in the 81st amino acid of the *GJB2* coding gene sequence, resulting in a change in the molecular framework. The presence of the 167deIT mutation leads to the removal of the recognition site of the PstI cutting enzyme (8).

The most dangerous genes that cause deafness in different regions include *TMC1TPRSS3, CDH23, OTOF, MYO15A, SLC26A4, GJB6, GJB2, DFNA5, COL11A2, ACTG1, TRIOBATP2B2, MYO1A, LHFPL5, KCNQ4, GJB3, EYA4, ESPN, DFNB5, PCDH15, MYO7A, MYO6, OMT1, TECTA, STRS*, and *TMIE* genes. Of course, the number of affected genes is much greater, but the mentioned genes have a significant contribution to the occurrence of deafness. These genes are usually not associated with autosomal dominant deafness (9 - 21).

Hearing loss associated with the *GJB2* mutation is an autosomal recessive genetic disorder (3). This means that the mutation only causes hearing loss in individuals who have inherited two copies of the mutated gene, one from each parent. A person with one mutated allele and one wild-type allele is considered a carrier, but they are not deaf (10).

The *GJB6* gene encodes the connexin 30 protein. Disruption of its production leads to a percentage of deafness. In the *GJB6* gene, three large deletions (Del(*GJB6*)) and a deletion of 920 kilobases have been reported as homozygous or heterozygous. When combined with the *GJB2* gene, it causes deafness. The mutation involves the deletion of a 309 kb fragment from the telomeric region of the *GJB6* gene and a 232 kb fragment. Additionally, in the 920 kb deletion, eight genes are deleted, including the *CRYLI, MPHOSPH8, PCPCI, ZMYM5, ZMYM2, GJB6*, and *GJB2* genes (14). The contribution of *GJB2* and *GJB6* genes to causing deafness is much higher than other genes (15). In the case of the *GJB6* gene, the deletion frequency is higher, with the average frequency of *GJB2* mutations being approximately 16% in Iran (16). Rapid diagnosis of deafness is crucial for a child's speech and social skills development, leading to a better quality of life and improved use of hearing aids, including cochlear implants. The purpose of the current study is to investigate the prevalence of

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the 167delT mutation of the *GJB2* gene and determine the percentage of this mutation in relation to the occurrence of deafness in a number of individuals in the cities of Kermanshah province.

Materials and methods

Study Design

This research was conducted as a case-control study aimed at evaluating genetic factors in individuals with hearing impairments. The design compares a group of patients exhibiting non-syndromic hearing loss with a healthy control group to identify specific genetic mutations, specifically focusing on the *GJB2* gene.

Setting and Participants

The study was carried out at the welfare center of Kermanshah province, Iran, where 34 deaf and hard-of-hearing individuals were referred for genetic counseling. These patients were compared against a control group of 36 healthy individuals. The total participant pool included 70 unrelated men and women, ranging in age from 3 to 62 years. Clinical status for the patient group was confirmed by a specialist doctor based on audiometric tests and criteria for non-syndromic hearing loss with autosomal recessive inheritance. Conversely, all healthy controls were required to have no family history of hearing loss and completely normal audiometric results.

Sample Size

The total sample size for this study consisted of 70 participants. This was divided into 36 healthy controls (approximately 51.4%) and 34 patients (approximately 48.6%). While the specific statistical formula used to determine this sample size (e.g., power analysis or G*Power) was not explicitly mentioned in the provided text, the distribution suggests an nearly equal 1:1 ratio between cases and controls.

Measurements & Validity and Reliability

The primary tool for gathering demographic data was a pre-designed questionnaire and the construction of a family tree for each patient. The demographic variables collected included age, gender, and family history of deafness. Clinical data was collected through audiometric testing conducted by specialists to verify the hearing threshold of all participants. For molecular data collection, PCR-RFLP (Polymerase Chain Reaction-Restriction Fragment Length Polymorphism) was utilized. Genomic DNA was extracted from 10 mL of venous blood using the phenol-chloroform method. The reliability of the molecular tool was ensured by using primers from reputable literature (Castro, 2013) and verifying DNA quality through nanodrop and 1.5% agarose gel electrophoresis. Genotypes were interpreted by observing specific band patterns on a 3% agarose gel after digestion with the PstI enzyme: wild-type (112/91/69 bp), 167delT mutation (91/181 bp), or heterozygous carrier (69/112/91/181 bp).

Ethical consideration

The study was conducted in strict accordance with ethical standards, receiving formal approval from the Research Committee of the Sanandaj Branch, Islamic Azad University (Ethical Committee Code: IR.IAU.SDJ.L.8244.P). All protocols involving human subjects complied with the Declaration of Helsinki as mandated by the Iranian Ministry of Health and Medical Education. Informed consent was obtained from all patients or their guardians, who signed a pre-designed consent form after the study's objectives were explained. Furthermore, all personal information remained strictly confidential.

Statistical and Data Analysis

The results obtained were entered into the SPSS 19. The significance of polymorphisms and deafness was then analyzed at a 5% significance level. Various statistical tests, including Fisher's chi-square, were used to investigate and determine significance.

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Results

In numerous studies, the role and contribution of the *GJB2* gene in the occurrence of deafness has been significant. It is associated with the highest number of genetic congenital deafness diseases with an autosomal recessive inheritance pattern. This study focused on 34 deaf and 36 normal individuals without any history of the disease from Kermanshah and Kurdistan provinces.

Analysis of the PCR product of the *GJB2* gene in gel electrophoresis

According to the results of this study, the frequency of homozygous mutations in the *GJB2* gene was 5.7%, heterozygous genotypes were 75.7%, and homozygous genotypes in healthy individuals were 18.6%. Primers were used to amplify the *GJB2* gene, and a 422 base pair fragment should have been observed when the primer pairs were joined (Figure 1).

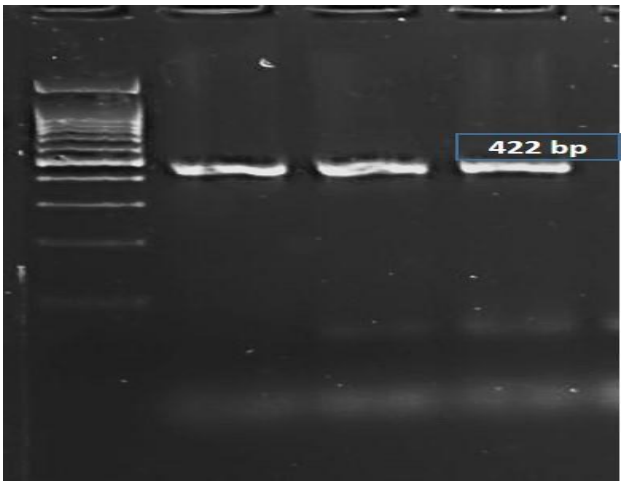
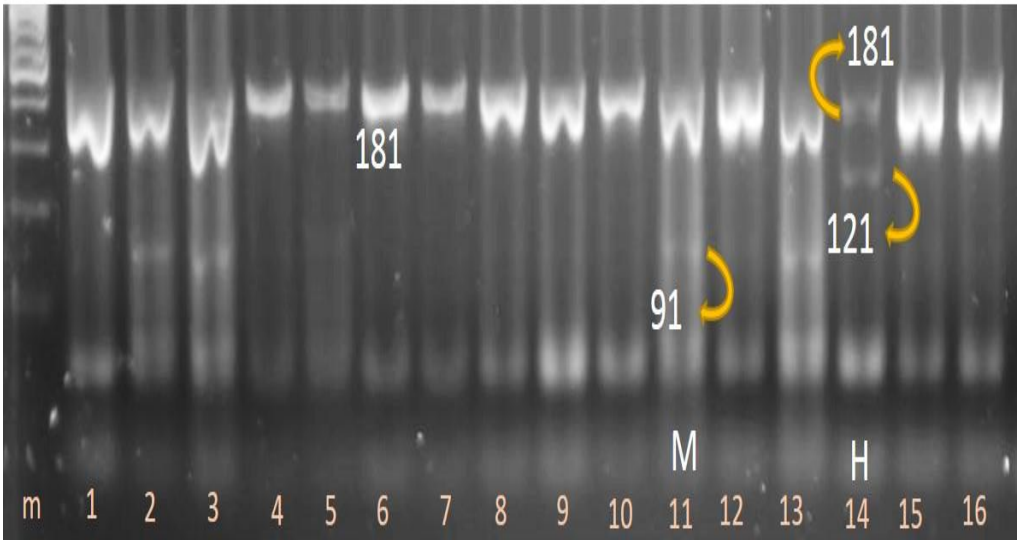


Figure 1. The results of electrophoresis of the PCR product with a length of 422 bp (next to the 100 bp marker).

Results from Pst I enzymatic cleavage

The results of Pst I enzymatic cleavage showed that cutting the 5-CTGCA/G-3 sequence with this enzyme produced different bands. TT homozygotes (healthy individuals) had bands at 69, 91, and 112, while CC homozygotes (mutated individuals) had bands at 91 and 181. Individuals with TC heterozygotes had bands at 69, 91, 112, and 181 (Figure 2).



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Figure 2. Cut pattern with Pst I in some study samples on agarose gel next to the 100 bp marker. Sample 11 indicates a homozygous person (CC), most samples indicate a homozygous person (TT) and sample 14 indicates a heterozygous carriers (CT) at this position.

Data analysis and statistical analysis

Results showed a significant difference in genotype distribution was observed between patients and healthy controls, demonstrating an association between the del*GJB2* T167 variant and hearing impairment (χ2 7.7, p 0.02). Carriers of at least one mutant allele (h ct or m cc) showed higher odds of being affected compared with non carriers, with an odds ratio of 3.97 (95% CI: 0.99–16.0). but no significant difference in allelic frequency (2.1 X2, 0.14). When individual genotypes were evaluated, heterozygous carriers exhibited an increased risk relative to wild-type individuals (OR 3.46, 95% CI: 0.88–13.6), and the homozygous mutant genotype demonstrated the strongest association (OR 10.77, 95% CI: 0.47–247.7). In contrast, allele frequencies did not differ significantly between groups (χ^2 2.1, p 0.14), and the allelic odds ratio (t vs. c) was not statistically significant (OR 0.54, 95% CI: 0.28–1.04). (Table 1, figure 3 and 4). According to the results obtained from this research, among all participants, the frequency of homozygous wild type (w-tt) 167delT was 18.58% (13 individuals, equivalent to 26 chromosomes). The heterozygous carriers (h-ct) were 75.71% (53 individuals, equivalent to 106 chromosomes), and homozygous mutants (m-cc) were 5.71% (4 individuals, equivalent to 8 chromosomes). Furthermore, in deaf individuals, the frequency of the wild-type homozygous genotype was 8.82% (4 individuals, equivalent to 8 chromosomes). The heterozygous carriers were 79.41% (27 individuals, equivalent to 54 chromosomes), and the homozygous mutant *GJB2* 167delT was 11.77% (4 individuals, equivalent to 8 chromosomes). In healthy individuals, the frequency was 27.78% for the wild-type homozygous genotype (10 individuals, equivalent to 20 chromosomes), 72.22% for heterozygous carriers (26 individuals, equivalent to 52 chromosomes), and zero for the mutant genotype.

Table 1. Genotype and allele frequencies of del*GJB2* T167 in patients and controls, with odds ratios (OR) and 95% confidence intervals (CI).

del*GJB2* T167	Participate N=70(%)	Patients N=34 (%)	Controls N=36 (%)	X^2	P-Value	OR (95% CI)
w-tt	13(18.58)	4 (8.82)	10(27.78)	7.737	0.021	Reference
h-ct	53(75.71)	27 (79.41)	26 (72.22)			3.46 (0.88–13.6)
m-cc	4(5.71)	4 (11.77)	0 (0)			10.77 (0.47–247.7)
Carriers (ct + cc)	**3.97 (0.99–16.0)**	—	—	57	31	26
Alleles						OR (95% CI)
t	79(56.43)	33(48.53)	46(63.89)	2.1	0.145	Reference
c	61 (43.57)	35(51.47)	26(36.11)			0.54 (0.28–1.04)

w:wild type, h:hetrozygote, m:mutant

Hardy–Weinberg equilibrium analysis supported the reliability of the observed genotype distributions, with no deviation detected in patients (p 0.034) or controls (p 0.067). Overall, these findings indicate that the del*GJB2* T167 variant, particularly in its mutant forms, is associated with increased susceptibility to hearing impairment, and this association appears independent of age and gender (Table 2).

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Table 2. HWE results with OR and 95% CI.

	Tt(%)	Ct(%)	Cc(%)	T(%)	C(%)	P-Value	OR (95% CI)
Participate	13(18.58)	53(75.71)	4(5.71)	79(56.43)	61 (43.57)	0.01	3.97 (0.99–16.0)
Patients	4 (8.82)	27 (79.41)	4 (11.77)	33(48.53)	35(51.47)	0.034	
Controls	10(27.78)	26 (72.22)	0 (0)	46(63.89)	26(36.11)	0.067	

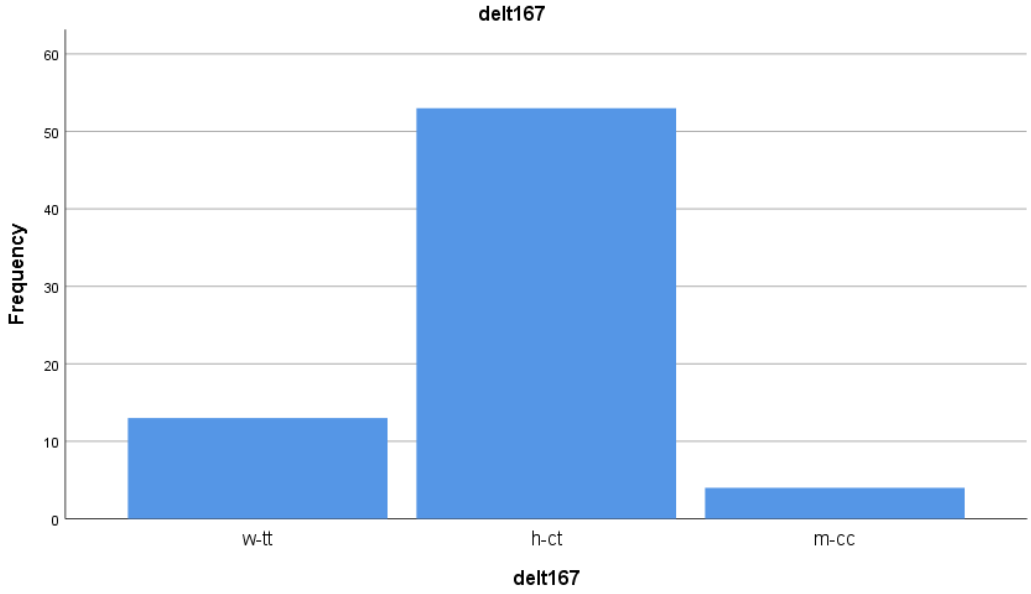


Figure 3. The distribution of genotypes in the studied population for the del*GJB2* T167

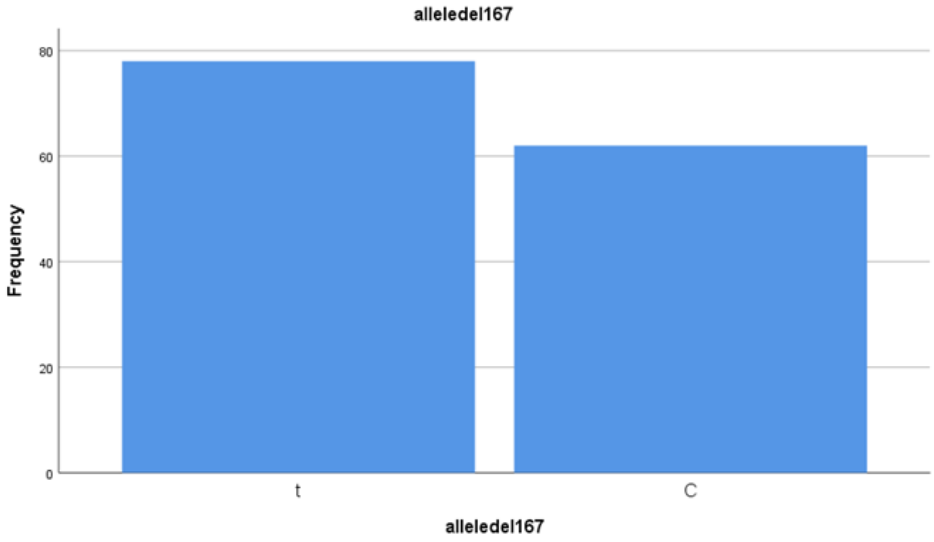


Figure 4. The distribution of Alleles in the studied population for the del*GJB2* T167

Stratification by gender revealed no significant differences in genotype (χ^2 0.49, p 0.46) or allele frequencies (χ^2 0.65, p 0.41). The allelic odds ratios were 0.10 (95% CI: 0.01–1.09) for females and 0.57 (95% CI: 0.02–15.0) for males, indicating that

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gender does not modify the association between the variant and disease status (Table 3).

Table 3. Frequency of genotypes in healthy and patient groups by gender, with OR and 95% CI.

groups			del*GJB2* T167			Total	p- value	OR (95% CI)
			w-tt	h-ct	m-cc			
Healthy controls	sex	female	7	15		22	0.460	3.97 (0.99–16.0)
		male	3	11		14		
	Total		10	26		36		
patients	sex	female	1	5	3	9		
		male	2	22	1	25		
	Total		3	27	4	34		
Total	sex	female	8	20	3	31		
		male	5	33	1	39		
	Total		13	53	4	70		

Age based analyses similarly showed no significant relationship between genotype (χ^2 2.54, p 0.638) or allele frequencies (χ^2 6.37, p 0.173) and age group (Table 4).

Table 4. Frequency of genotypes in healthy and patient groups based on age, with overall odds ratio.

groups			del*GJB2* T167			Total	p- value	OR (95% CI)
			w-tt	h-ct	m-cc			
Healthy controls	age	<10	0	2		2	0.638	3.97 (0.99–16.0)
		11-20	1	4		5		
		21-35	4	10		14		
		36-50	2	7		9		
		>51	3	3		6		
	Total		10	26		36		
patients	age	<10	0	3	0	3		
		11-20	0	2	1	3		
		21-35	1	14	0	15		
		36-50	2	8	2	12		
		>51	0	0	1	1		
	Total		3	27	4	34		
Total	age	<10	0	5	0	5		
		11-20	1	6	1	8		
		21-35	5	24	0	29		
		36-50	4	15	2	21		
		>51	3	3	1	7		
	Total		13	53	4	70		

Additionally, there is no significant relationship between allele frequency and age in the two groups of healthy individuals and patients (X^2 6.37, p-value 0.173) (Table 5).

Table 5. Allele frequencies between healthy and patient groups based on age, with overall odds ratio.

group	alleledel167	Total	p- value	OR (95% CI)

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			t	c			
healthy	age	<10	2	0	2	0.173	0.21 (0.02–2.02)
		11-20	4	1	5		
		21-35	14	0	14		
		36-50	9	0	9		
		>51	6	0	6		
	Total		35	1	36		
patients	age	<10	3	0	3		
		11-20	2	1	3		
		21-35	15	0	15		
		36-50	10	2	12		
		>51	0	1	1		
	Total		30	4	34		
Total	age	<10	5	0	5		
		11-20	6	2	8		
		21-35	29	0	29		
		36-50	19	2	21		
		>51	6	1	7		
	Total		65	5	70		

There is no significant difference in allele frequency or gender between the healthy and patient groups (X^2 0.65, p-value=0.41) (Table 6).

Table 6. Allele frequency compared between patients and healthy individuals by gender, with odds ratios.

group			alleledel167		Total	p-value	OR (95% CI)
			T	C			
healthy	sex	female	21	1	22	0.418	0.10 (0.01–1.09)
		male	14	0	14		0.57 (0.02–15.0)
	Total		35	1	36		
patient	sex	female	6	3	9		
		male	24	1	25		
	Total		30	4	34		
Total	sex	female	27	4	31		
		male	38	1	39		
	Total		65	5	70		0.21 (0.02–2.02)

Discussion

Findings from this study revealed a statistically significant difference in genotype frequency between the two groups, but no significant difference in allelic frequency. At first glance, this may seem contradictory, but genotype distribution tests (chi-square) compare the full pattern of genotypes between groups, being sensitive to differences in heterozygote versus homozygote frequencies, not just overall allele counts. In our results, there were more heterozygotes but fewer homozygotes, so the genotype test may detect significance. On the other hand, allele distribution tests collapse heterozygotes into counts of alleles, only detecting differences in overall allele frequency, not in how alleles are paired. If allele frequencies are similar between groups, but the way they combine into genotypes differs, the allele test shows no difference. Key reasons for the discrepancy lie in HWE. If one group deviates from HWE (e.g., excess heterozygotes), genotype tests

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will show significance even if allele frequencies are unchanged. Additionally, there are statistical power differences. Genotype tests use more degrees of freedom (df = 2 for 3 genotypes), while allele tests use fewer (df = 1). Depending on sample size and distribution, one test may detect differences that the other misses. In terms of biological interpretation, sometimes the risk is associated with genotype structure (dominant/recessive effects, heterozygote advantage/disadvantage) rather than allele frequency itself. So, genotype tests capture structural differences in how alleles combine, while allele tests only capture overall frequency differences (12-21).

Deafness is a sensory disability that affects millions of individuals worldwide (1). Deaf individuals face numerous challenges and dangers in their daily lives. Unfortunately, due to the heterogeneity of this condition, accurate genetic counseling cannot be provided to affected families to prevent other children from developing it. Currently, over 95 loci and 42 genes responsible for non-syndromic autosomal recessive deafness have been identified, although some genes remain unidentified for certain loci (15). Furthermore, the frequency of mutations in these genes varies across different populations. Therefore, it is crucial to study and identify the most common genes involved in each population in order to provide more effective solutions for deaf families. Numerous studies have highlighted the significant role and contribution of the *GJB2* gene in the development of deafness, particularly in cases of genetic congenital deafness with an autosomal recessive inheritance pattern (15).

To date, over 100 mutations have been reported in the *GJB2* gene (11). For example, in Pakistan, 50% of non-syndromic deaf individuals have been found to have mutations in the *GJB2* gene (12). In a study conducted in Turkey, the frequency of gene mutation in *GJB2* was reported to be around 25% (13). In a study conducted on 210 families from Tehran and Tabriz, it was discovered that 25.2% of these individuals had mutations in the *GJB2* gene. These mutations included W24X, R143W, 167delT, T8M 35delG, 136X, 235delC, V27I+E114G, E47XdelE120, R32H, V37I, and R184P. Additionally, 13.3% of the families were homozygous mutant 35delG (17).

In another study conducted in Sistan and Baluchistan, 100 families from two Baloch tribes were examined to investigate non-syndromic deafness with recessive inheritance on the *GJB2* gene. The mutations studied included W24X, 167delT, R127H, M93I, and K112I. The W24X mutation had the highest frequency at 9% (11). In 2009, an experiment was conducted in Colombia to investigate non-syndromic recessive deafness disorder. The study examined the *GJB2* gene sequence in 112 patients and found that the most common mutations were S199F and 35delG. The 167delT mutation had a frequency of 0.4% (18).

In a separate study conducted in Gilan and Khorasan provinces, 199 families and 260 patients were assessed for mutations in the *GJB2* gene. The mutations evaluated were M34T, R127H, W77X, 235delC, 35delG, delE120, L90P, and V27I+E114G mutations. The most common mutation, found in 78.2% of cases, was 35delG(19). The distribution pattern of the *GJB2* gene mutation in Iran shows a decrease in frequency from north to south and from east to west (19). The 167delT mutation has a frequency of 7.5%, which is similar to the findings of the aforementioned research.

Our finding that *GJB2* 167delT accounts for 5.7% of ARNSHL in Kermanshah fits into a broader pattern: Iran shows notable regional differences in which *GJB2* variants contribute to hearing loss, as well as in their overall frequency. Multiple studies report that while *GJB2* is a major locus for ARNSHL in Iran, the dominant variants and their proportions vary by ethnicity and province. One reason why Kermanshah's 167delT rate may differ from other Iranian regions is due to founder effects and micro-population structure. Small, relatively endogamous communities can carry and amplify specific variants

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(like 167delT), leading to local enrichment even when the national prevalence is dominated by 35delG. Additionally, ethnic stratification and migration history play important roles. Western Iran hosts diverse groups with distinct ancestries; historical gene flow and isolation shape local mutation spectra. Variable rates of consanguinity across provinces can increase homozygosity of locally prevalent mutations, affecting observed genotype distributions without necessarily shifting national allele frequencies (21).

Furthermore, methodological factors and differences in sampling (families versus sporadic cases), ascertainment, and the testing panel (targeted versus full sequencing) can lead to inflation or undercounting of certain variants regionally. Iran is a country with diverse ethnicities, and family marriages are common. As a result, genes associated with genetic deafness are spread among different ethnic groups in Iran (19). Compared to other countries, the contribution of *GJB2* to deafness in the Iranian population is low, particularly in Kermanshah. Therefore, it is important to identify common locations for autosomal recessive deafness in the Iranian population (19).

Limitation and Strengths

Unfortunately, there are many challenges in collecting samples and taking blood from individuals with the disease, such as their unwillingness to undergo these procedures. This problem leads to a small and limited sample size in the statistical population. Other limitations include the lack of deaf centers with sufficient equipment for sample collection at the provincial level. The relatively small sample size in this study limits the statistical power to detect subtle genotype–phenotype associations and increases the likelihood that observed differences may be due to chance. This constraint also reduces the precision of prevalence estimates and restricts our ability to perform meaningful subgroup analyses. Therefore, caution should be exercised in generalizing these findings to the broader population, and larger, multi-center studies are warranted.

Conclusion

As a result, considering the 5.7% prevalence of the 167delT mutation of the GJB2 gene and the significant difference between the genotype frequency of individuals in the two healthy and diseased groups, it indicates that the 167delT mutation of the GJB2 gene plays a role in the occurrence of deafness in our statistical population.

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CRediT authorship contribution statement

Conceptualization, Methodology, Validation, Formal Analysis, Investigation, Resources, Software, Data Curation, Writing–Original Draft Preparation, Writing–Review & Editing, Visualization, Supervision, Project Administration: PB, MK, FK.

Declaration of Generative AI and AI-assisted technologies in the writing process

This work was written and prepared by the authors independently, without the use of any outside writing agency. All ideas and opinions expressed here are those of the writers.

Conflict of interest

The authors declared no potential conflicts of interest with respect to the research or publication of this article.

Ethical considerations

The study was conducted in strict accordance with ethical standards, receiving formal approval from the Research Committee of the Sanandaj Branch, Islamic Azad University (IR.IAU.SDJ.L.8244.P). All protocols involving human subjects complied with the Declaration of Helsinki as mandated by the Iranian Ministry of Health and Medical Education. Informed consent was obtained from all patients or

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their guardians, who signed a pre-designed consent form after the study's objectives were explained. Furthermore, all personal information remained strictly confidential.

Data availability statement

Upon reasonable request, the data supporting the results of this research may be obtained from the corresponding author.

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