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Genetic association between methionine sulfoxide reductase B1 polymorphisms and risk of noise-induced hearing loss

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ABSTRACT

Purpose: Hearing loss is the loss of hearing function in one or both ears. Noise-induced hearing loss (NIHL) is the second most prevalent type of sensorineural hearing loss, adversely affects the functional and social lives of workers in noisy environments. This study aimed to confirm the relationship between methionine sulfoxide reductase B1 (*MSRB1*) expression in the human auditory system and noise exposure by searching for related pathogenic single-nucleotide polymorphisms (SNPs).

Methodology: The study included 90 workers with NIHL and 90 with normal hearing. A hearing test was administered to each participant, and blood samples were collected from both groups to conduct genetic analyses. A comparison of the genomes of workers with NIHL and those with normal hearing was performed.

Results: The results showed that rs732510 was found in NIHL participants, while the other SNPs (rs2815304, rs11640479, and rs9934331) were found in both controls and NIHL subjects. Among the NIHL group, 44 participants had heterozygous mutants (TC), 30 had homozygous mutants (CC), and 13 had homozygous wild-type alleles (TT). The heterozygous mutant allele had a statistically significantly higher prevalence (48.9%) compared to the homozygous wild-type allele (14.4%) and homozygous mutant allele (33.3%) among patients with NIHL ($\chi 2[3] = 43.96$, $p < 0.05$).

Conclusion: This study is the first to report an association between rs732510 in MSRB1 and NIHL in the human auditory system. This finding paves the way for future research to discover more about the gene's role in the auditory function, suggesting it could be a promising biological biomarker associated with NIHL.

Keywords: NIHL, MSR, *MSRB1*, oxidative stress

INTRODUCTION

Hearing loss is the partial or total inability to hear in one or both ears, ranging from mild to profound. Approximately 5.3 % of people worldwide have hearing loss [1, 2], and this is caused by various factors such as infections, trauma, aging, genetic predisposition, or prolonged noise exposure [3]. Hearing loss can undoubtedly have a negative impact on a person's personal and professional life. Some critical aspects that are affected by hearing impairment are mental health and social and work lives, especially communication, in which hearing plays an essential role [4]. Regardless of the type of hearing loss, speech becomes harder to perceive and comprehend, leading to challenges in communication, learning, and overall development [4].

Noise-induced hearing loss (NIHL) ranks second in prevalence among sensorineural hearing losses, following presbycusis [5]. The National Institutes of Health reports that NIHL results from prolonged or intense exposure to loud sounds, particularly in occupational settings, leading to permanent damage to the inner ear [6]. According to the Occupational Safety and Health Administration (OSHA) [7], a hearing preservation action is established and required to be carried out by workers when noise levels, calculated during eight working hours or an 8-hour time-weighted average, are at or above 85 dBA. The principles of hearing protection aim to avoid the initiation of occupational hearing loss, conserve and maintain residual hearing, and provide workers with appropriate awareness and hearing conservation supplies [8]. NIHL is a consequence of multifactorial damage involving both environmental and genetic components. The severity of the condition varies among workers, implying that genetic susceptibility is essential to the acceleration of hearing impairment in a noise-exposure setting [9]

Oxidative stress is a significant biological mechanism that directly contributes to the pathophysiology of NIHL following noise exposure [10]. Oxidative stress is a disequilibrium in forming reactive oxygen species (ROS), which are normally managed endogenously by antioxidant processes [11]. ROS and reactive nitrogen species (RNS) contribute to sensorineural hearing loss by disrupting key cellular pathways,

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including mitochondrial function, calcium homeostasis, and DNA integrity, ultimately leading to outer hair cell death following noise exposure [12]. An imbalance between ROS/RNS and the inner ear's antioxidant defenses is critical in triggering apoptosis and necrosis, contributing to hair cell loss in NIHL and ischemia-reperfusion injury [10]. After noise exposure, aerobic respiration increases, and the mitochondria utilize more oxygen, resulting in higher levels of superoxide and other ROS; this has been shown to cause a transient restriction in cochlear blood flow [13]. When free radicals are sustained at low or moderate levels, they have many advantageous effects on the body and are biologically significant in several physiological systems. However, when the ROS/RNS ratio becomes imbalanced, oxidative stress induces many antioxidant enzymes and proteins. Therefore, the induction of the antioxidant system maintains the cell's reducing environment and prevents harm to numerous biomolecules [14].

Antioxidant defense enzymes are activated by changes in H_2O_2 or O_2 levels, producing or muting genes responsible for synthesizing defensive enzymes, transcription factors, and protein molecules [15]. An elevation in the catalytic effects of antioxidant enzymes following noise exposure has been confirmed, especially increased glutathione reductase, γ-glutamyl cysteine synthetase, and catalase levels [16].

In the cochlea, the high secretion of oxidized proteins can be triggered by oxidative stress, which can destroy the organ of corti and auditory fibers. Therefore, hearing loss is aggravated [17]. The methionine sulfoxide reductase (*MSR*) system includes two stereospecific enzymes, *MSRA* and methionine sulfoxide reductase (*MSRB*), which respectively reduce methionine-S-sulfoxide and methionine-R-sulfoxide–oxidized forms of methionine generated by ROS–back to methionine, helping preserve protein function and cellular redox balance [18]. *MSR* family members were variably localized and identified within the auditory system of 20 mice, specifically inside the cochlea and vestibule [19]. *MSRA* is localized in different cochlear structures involving the Reissner's membrane, spiral ligament, supporting cells, spiral ganglion, and spiral limbus. Methionine sulfoxide reductase B1 (*MSRB1*) has been reported to be present in spiral ganglia and hair cells, while Methionine sulfoxide reductase B2 (*MSRB2*) is expressed in the tectorial membrane, spiral ganglion, and stria vascularis. In the vestibular structures, *MSRA* and *MSRB1* were found in vestibular ganglia and sensory cells, whereas *MSRB2* was limited to the vestibular ganglion.

The function of the *MSR* system in preventing hearing loss has only recently been studied. *MSRA* knockout mice showed a substantial reduction in spiral ganglion cells and fibrocystic class IV fibroblasts, suggesting that *MSRA* contributes to protection against cochlear hearing loss [17]. *MSRB1* expression was also enhanced in mice after noise exposure, implying that *MSRB1* may be involved in protecting the hearing function from oxidative stress [17].

Given the lack of human studies on *MSRB1*, this study investigates its polymorphisms in workers with and without NIHL to explore potential genetic susceptibility. This research is crucial as it bridges the gap between animal models and human applications, providing a deeper understanding of the genetic factors involved in NIHL. Using single-nucleotide polymorphism (SNP) genotyping analysis, we identified genetic variations impacting the gene function and expression. These findings are significant as they enhance the diagnosis of NIHL in patients, helping to reduce the severity of the illness and improve the quality of life. Additionally, the study provides insights into the role of *MSRB1* in preventing hearing defects, thereby contributing to better diagnostic and therapeutic strategies for NIHL. By elucidating the genetic mechanisms underlying NIHL, this study paves the way for targeted interventions and personalized medicine approaches in occupational health.

METHODOLOGY

Study Design and Participants

A cross-sectional design was adopted for this study. Previous literature suggests that the sample size for correlation studies should exceed 50 participants per group [20]. Thus, the sample was divided into two groups: workers with NIHL (n = 90) and workers with normal hearing (n = 90). Besides, since this research is exploratory, the selected sample size is adequate and acceptable [21]. All participants in the study were involved voluntarily, and informed consent forms were obtained prior to their involvement. This study followed the ethical guidelines set by the relevant institutional and national research committees, which aligned with the 1964 Helsinki Declaration and its subsequent amendments (approval number 2021/144/27).

Both groups of workers with NIHL and workers with normal hearing were recruited in the period between November 11, 2021, and June 7, 2023, from the following noisy workplaces in Jordan: The operation and maintenance unit at Jordan University of Science and Technology (JUST) in Irbid, the Engineering Workshops at JUST in Irbid, Al-Durra International Company in Irbid, the Jordanian Company of Mills in Irbid, Jordan Soils and Supply General Company of Irbid. After signing consent forms, each participant underwent a hearing evaluation and SNP genotyping. Then, auditory capability and genetic materials were compared between intact and impaired hearing workers.

Each participant provided information on key characteristics distinguishing the control and subject groups, along with demographic data. Demographic and work exposure information was collected by a qualified audiologist, assisted by a trained research assistant. The main variables analyzed included age, duration of noise exposure while using hearing protection, use of sound headphones for listening to music or watching videos, and average daily call time. Data collection methods were based on standardized protocols commonly used in occupational hearing research [22] and were reviewed by experts to ensure relevance and clarity (see **Appendix A**).

The inclusion criteria for the subjects were as follows: Participants aged 18-60 years, to ensure that the auditory system is fully matured and avoid confounding with age-related hearing loss, who have cumulative times of noise exposure in the workplace that are certainly harmful to their hearing (noise exposure time ≥ 8 h/day or 40 h/week, noise intensity ≥ 80 dBA for more than one year) [23]. Noise levels were quantified using a calibrated sound level meter in accordance with standard measurement protocols. The study in [7] states that continuous exposure exceeding 90 dBA over an 8-hour time-weighted average surpasses the permissible exposure limit and poses a significant risk for noise-induced

Table 1. Candidate SNPs and primer design

	Sequence	Length	GC (%)[a]	TM[b]
rs732510				
Forward primer	TGCCAGTCCAATCGCATCTC	20	55.0	54.7
Reverse primer	TGCAAAGGCGGTTTCACCTG	20	55.0	55.9
Probe	/6-FAM/ CCGGAGGTCATGCAGCCTTTCCGCGCCC/FQ/ZEN	28	71.4	70.1
rs2815304				
Forward primer	TGCCTGCAATCCCAGCTACTTG	22	54.5	57.2
Reverse primer	ATGGAGTCTTGCTCTGTCGTGC	22	54.5	57.0
Probe	/JOE/TGAGCTGAGATCGCGCCACTGCACAC/BHQ1	26	61.5	65.1
rs9934331				
Forward primer	ATCCACCAACCCAGGCAAAGAC	22	54.5	57.1
Reverse primer	TTCCTTCACGCTGCACACAC	20	55.0	55.7
Probe	/TEX615/ ACCCGCCACGGGAGGCGCTG/BHQ2	20	80.0	66.2
rs11640479				
Forward primer	AGCCAGGCTCTAGATCACTCAG	22	54.5	55.1
Reverse primer	CCACCACGCCAGGCTAATTTTC	22	54.5	56.5
Probe	/CY5/AGTGCCCAGGGATAGACCTTAAGAGTCTCTCCTGC/ RQ/TAO	36	55.6	66.5

Note. [a]Guanine-Cytocine content; [b]Temperature; GC (%): Percentage of Guanine-Cytosine content; & TM: Temperature

auditory damage. In addition, exposure levels of 85 dBA or higher require the implementation of a hearing conservation program to prevent potential auditory health effects [7]. Subjects who were exposed to a blast or head injuries within the month preceding the health checkup, had a family history of hearing issues, medical history related to hearing, fever, or other microbial infections (influenza, chicken pox, and hepatitis), previous intake of ototoxic medications, and any audiometric results suggestive of conductive deafness were excluded.

An additional inclusion criterion for the normal hearing group developed from scientific observation and previous relevant research designs included and is as follows: having bilateral hearing thresholds that do not exceed 25 dB (HL) at the audiometric test frequencies of 500, 1,000, 2,000, 3,000, 4,000, and 6,000 Hz. Furthermore, they must match the NIHL group in

(1) type of work and labor sites,

(2) age (±3 years), and

(3) occupational noise exposure time (±1 year) [24].

Hearing Evaluation

All recruited workers' hearing abilities were evaluated using otoscopic examination, tympanometry, and pure tone audiometry. Otoscopy is a routine clinical procedure used to inspect the ear structures, specifically the external auditory canal, tympanic membrane, and middle ear. The examination was performed using an Otoscope (LuxaScope Auris LED 2.5 V). The clinical significance of the otoscopic examination in the current research was to exclude any conductive hearing loss caused by cerumen impaction, tympanic membrane perforation, or any otitis media infection. The second basic hearing assessment test was performed using tympanometry. Tympanometry is a test that primarily helps to diagnose and assess the middle ear function. This was accomplished by Interacoustics AA222, a hybrid device that involves both an audiometer and a tympanometer; it is manufactured by Interacoustics, a company based in Denmark.

Pure-tone audiometry was conducted in accordance with ANSI S3.6-2018 standards for audiometric equipment and ISO 8253-1:2010 protocols for threshold testing [25, 26], ensuring reliable and consistent measurement of hearing sensitivity. As a behavioral assessment, pure-tone audiometry determines the quietest sounds an individual can hear across a range of frequencies [27]. According to the "diagnosis of occupational noise deafness" (GBZ 49-2014) criteria, binaural hearing thresholds were assessed at frequencies 500, 1,000, 2,000, 3,000, 4,000, and 6,000 Hz [28]. In this study, workers whose bilateral averages of high frequencies (3,000, 4,000, and 6,000 Hz) are greater than 25 dB (HL), in addition to workers whose unilateral averages of high frequencies (3,000, 4,000, and 6,000 Hz) are greater than 20 dB (HL) are considered to have NIHL [29], while those with thresholds less than 25 dB (HL) are defined as normal hearing workers.

Single Nucleotide Polymorphisms and Primers

Candidate SNPs for *MSRB1* were selected from NCBI-SNP (http://www.ncbi.nlm.nih.gov/snp/) according to a related optimization setting of linkage disequilibrium (LD) ≥ 80% and minor allele frequency ≥ 0.05. The reference SNP (rs) numbers for the SNPs of interest were rs732510, rs2815304, rs9934331, and rs11640479. Optimal primers were designed and ordered based on the selection results using the Thermo Fisher OligoPerfect™ Primer Designer (https://www.thermofisher.com/us/en/home/life-science/oligonucleotides-primers-probes-genes/custom-dna-oligos/oligo-design-tools.html) as described in **Table 1**. The Thermo Scientific Reviewer primer design software was used to optimize the primers for efficient amplification of the selected SNPs. The software evaluates primer characteristics such as melting temperature (Tm), GC content, and primer specificity, ensuring reliable and specific binding to the target sequences.

DNA Extraction

A certified phlebotomist collected 2 mL of whole blood from each participant following IRB-approved protocols. Blood samples were drawn into K_3EDTA-coated vacuum tubes (AFCO, Jordan; REF FV01022, LOT 110205), ensuring the correct blood-to-anticoagulant ratio. Samples were then stored at −20 °C until DNA extraction. Genomic DNA was extracted from blood samples using the G-spin™ total DNA extraction mini kit (iNtRON Biotechnology, South Korea). A volume of 200 µL of whole blood was pipetted into a 1.5 mL microcentrifuge tube, followed by the addition of 20 µL of proteinase K and 5 µL of RNase A. The solution was added to a sample tube and gently mixed. Then, 200 µL of binding buffer (buffer BL) was mixed thoroughly into the upper sample tube.

The mixture was placed at room temperature for 2 minutes. Next, the lysate was incubated at 56 °C for 10 minutes. The 1.5 mL tube was briefly centrifuged to detach drops from the inside of the lid. Subsequently, 200 µL of absolute ethanol was added to the lysate and mixed by pulse vortex. After mixing, the 1.5 mL tube was briefly centrifuged to detach drops from the inside of the lid. Later, the mixture was carefully applied to the spin column (2 mL) and centrifuged at 15,111 × g for a minute.

The filtrate was discarded, and the spin column was placed in a new 2 mL collection tube. Subsequently, 700 µl of buffer WA (buffer WB) was added to the spin column without wetting the rim and centrifuged for a minute at 15,111 × g. The flow-through was discarded, and the collection tube was reused. This step was repeated twice to ensure the drying of the membrane.

The flow-through and the collection tube were wholly discarded. The spin column was placed into a new 1.5 mL tube, and 100 µL of DNA column elution buffer (Buffer CE) was added directly onto the membrane. It was incubated for a minute at room temperature, and finally, it was centrifuged for a minute at 15,111 × g to elute.

Multiplex Real-Time Polymerase Chain Reaction

Polymerase chain reaction (PCR) is a powerful technique that specifically amplifies a certain extracted DNA segment in vitro [30]. Multiplex PCR was used because it has the potential to amplify more than one target sequence, typically by running a reaction that contains more than one pair of primers. A thin-walled PCR tube was placed on ice, and the PCR master mix was prepared by mixing water, buffer, dNTPs, primers, and dreamTaq DNA polymerase. Samples were vortexed at 1,000 rpm for 1 minute, followed by a brief spin-down for 30 seconds. Real-time PCR was performed using 40 thermal cycles, which included an initial denaturation at 95 °C for 2 minutes, followed by denaturation at 95 °C for 30 seconds, annealing at 60 °C for 30 seconds, and extension as specified by the manufacturer's protocol.

Conventional Polymerase Chain Reaction

The PCR reaction mix consisted of AmpliTaq Gold 360, which included DNA polymerase, $MgCl_2$, a stabilizing buffer, an enhancer for GC-rich sequences, and dNTPs for strand synthesis. Additional components were added to the mix, including forward and reverse primers, the DNA template, and nuclease-free water to complete the final reaction volume. Thermal cycling was carried out under the following conditions: one cycle of initial denaturation at 95 °C for 3 minutes, followed by 25 cycles of denaturation at 95 °C for 30 seconds, annealing at 60 °C for 30 seconds, and extension at 72 °C for 1 minute. This was succeeded by one cycle of final extension at 72 °C for 15 minutes.

DNA Gel Electrophoresis

Preparation of agarose gel

Tris-acetate-EDTA buffer (1X) was prepared by adding 20 mL of 50X TAE buffer to 980 mL of distilled water. A 0.6% agarose gel was prepared using 0.30 g of ultra-pure agarose dissolved in 50 mL of 1X TAE buffer. The solution was microwaved for approximately one minute, or longer if undissolved, then swirled after the addition of 1 µL of ethidium bromide. The agarose solution was poured into a gel tray with approximate dimensions of 8.3 cm (L) × 3 cm (W), placed inside a gel cassette. A comb was inserted at the top of the gel, which was left to solidify for 30 minutes.

Agarose gel electrophoresis was performed following real-time PCR amplification to confirm the presence of the SNP that was observed only in the NIHL group. A 1 kb DNA ladder, covering a range suitable for fragments between 1 kb and 20 kb, was used to estimate the size of the PCR products. The gel was run at 90 V, corresponding to an electric field of approximately 1.084 mV/cm.

Due to the carcinogenic nature of ethidium bromide, all steps involving the dye were carried out with strict safety precautions. Nitrile gloves, a lab coat, and safety goggles were worn throughout, and all ethidium bromide-contaminated materials, including pipette tips, were discarded in designated biohazard bins.

Running gel electrophoresis

When the gel solidified, the comb was removed by pulling it straight up. Then, the gel was confirmed to be in the correct orientation, with the negative/black electrode above the wells to facilitate the DNA running toward the positive/red electrode. The samples were prepared by adding 6X loading buffer to each. After that, 5 µL of DNA was combined with 1 µL of 6X loading buffer, and then 5 µL of 1 kb DNA ladder was loaded into one gel lane. The samples were loaded into wells, and the lid was placed on the cassette. Next, the red and black wires were connected to the matching red and black electrodes on the cassette, and the gel ran at 90V for 30-50 minutes. Finally, the gel tray was removed and imaged with ultraviolet light.

ExoSAP-IT™ Express PCR Product Cleanup

PCR product cleanup is necessary to remove excess amounts of dNTPs, enzymes, buffers, and DNA. This is done using the Exonuclease I–Shrimp Alkaline Phosphatase–Inactivation Technology (ExoSAP-IT™) Express reagent, a product developed by Thermo Fisher Scientific in the United States. A total of 5 µL of PCR reaction product was mixed with 2 µL of ExoSAP-IT™ Express reagent, resulting in a final reaction volume of 7 µL. The mixture was gently vortexed and then centrifuged briefly (approximately 5 seconds) to ensure the contents were collected at the bottom of the tube. The reaction was carried out in a thermal cycler with a heated lid, starting with incubation at 37 °C for 4 minutes to degrade excess nucleotides and primers. It was then heated to 80 °C for 1 minute to inactivate the ExoSAP-IT™ Express reagent, which had been stored at 4 °C. Following incubation, the samples were immediately placed on ice.

Sanger Sequencing

PCR products were purified and analyzed using Sanger sequencing at Biotrust Laboratories in Irbid, Jordan. A BigDye Terminator aliquot, composed of 4 µL BigDye RR mixture and 2 µL BigDye buffer, was taken out from the freezer and allowed to thaw on an ice rack. Then, 2 µL of the sequencing primer was added to each tube. Further, 2 µL of purified PCR product (3 ng) was added. The tubes were transferred to the thermocycler to start the sequencing.

The tubes were placed in the thermocycler to initiate the sequencing reaction, starting with an initial denaturation at 95 °C for 1 minute. This was followed by 25 cycles, each consisting of denaturation at 96 °C for 10 seconds, annealing at 50 °C for 5 seconds, and extension at 60 °C for 4 minutes. The program concluded with a final hold at 4 °C. This process was essential

Table 2. Characteristics of the participants

Variable/ category	Control (n = 90)	NIHL (n = 90)[a]	p
Age, year	42 ± 9.69	46 ± 9.55	0.070
Gender			
Male	67 (74.4%)	80 (88.9%)	0.140
Female	23 (25.6%)	10 (11.1%)	

Note. [a]Noise-induced hearing loss; N: Number of subjects; mean ± standard error (SE); %: Percentage of subjects; & *the statistical significance level was set at 0.05

Table 3. Audiological and demographic characteristics of NIHL subjects

Variable/category	NIHL
Geographic distribution of the participants in Jordan	
Irbid	81 (90%)
Amman	2 (2%)
Jarash	2 (2%)
Ajloon	3 (3%)
Mafraq	2 (2%)
Age of onset (years)	46 ± 9.55
Types of hearing loss	
Sensorineural	87 (97%)
Conductive	0 (0%)
Mixed	3(3%)
Laterality	
Bilateral	52 (58%)
Unilateral	38 (42%)
Use hearing protection	
Yes	15 (17%)
No	75 (83%)

Note. Mean ± standard error (SE); %: Percentage of subjects; & *the statistical significance level was set at 0.05

for incorporating labeled terminators and preparing the DNA for analysis. After cycle sequencing, the reaction plate was centrifuged. Cleanup was then performed by adding 45 µL of stabilization additive matrix™ solution and 10 µL of cross-contamination terminator (XTerminator™) solution to each well. Then, the plate was vortexed for 2 minutes to ensure thorough mixing of the reagents. Finally, the reaction plate was placed in the applied biosystems SeqStudio genetic analyzer (capillary electrophoresis-based) for sequencing, which was done in Enzyme Center Labs in Jordan/Irbid.

Statistical Analysis

Statistical analysis was conducted using the statistical package for the social sciences, version 21. Comparison tests between the control and experimental groups were selected based on data distribution. Continuous variables were represented as median ± standard deviation. The Mann-Whitney U test, a nonparametric test used to compare differences between two independent groups, was applied to assess age and gender differences between NIHL and control subjects. The Chi-square test was used to evaluate associations between significant SNPs and NIHL-related variables such as hearing protection use, hearing loss severity, laterality, age, age of onset, noise exposure, gender, and non-occupational noise exposure (e.g., hobbies). Fisher's exact test, appropriate for small sample sizes, was employed to assess correlations between allele types and categorical variables. Cochran's Q test, used to determine differences in proportions across related groups, was applied to identify the most prevalent genotypes among NIHL patients. All statistical tests were two-tailed, and significance was set at $p < 0.05$. The dataset had no missing data, allowing for complete analysis across all variables.

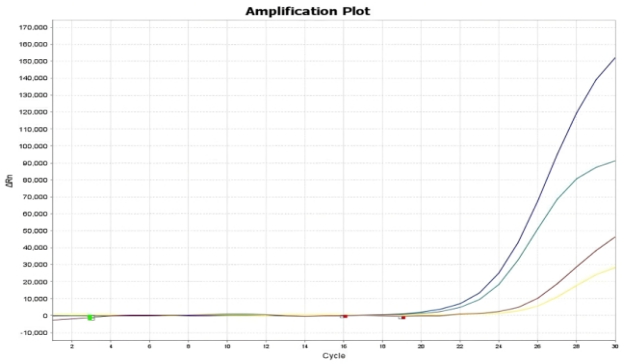

Figure 1. Real-time PCR amplification plot for rs732510, rs2815304, rs11640479, and rs9934331 in NIHL subjects (Source: Authors' own elaboration)

RESULTS

The study included 180 participants, selected through simple random sampling from workers employed in various high-noise occupational environments. Following audiological evaluation, participants were classified into two groups based on their hearing status: control subjects with normal hearing (n = 90) and subjects with NIHL (n = 90). This post-evaluation grouping reflects an observational case–control design. All participants were Jordanians aged between 20 and 60 years. As shown in **Table 2**, there were no significant differences in age or gender between the two groups ($p > 0.05$).

Tests of normality were conducted using the Kolmogorov-Smirnov and Shapiro-Wilk tests, along with visual inspection of the dataset, including histograms with fitted standard curves and Q-Q plots. The results indicated that all dependent variables were non-normally distributed, with p-values below 0.05 in both tests. Therefore, non-parametric analyses were applied to explore the collected data, as presented in **Table 3**. Participants with normal and impaired auditory function were subjected to occupational noise exposure ranging from 89.9 to 100.9 dBA for durations of up to 8 hours per day, across five to six working days per week.

Frequency of rs2815304, rs732510, rs11640479, and rs9934331 Assigned to *MSRB1*

Amplification results for rs732510 were obtained exclusively from subjects in the NIHL group (**Figure 1**), with no amplification products observed in the control group (**Figure 2**).

Amplification results for rs732510 were obtained only from subjects in the NIHL group, as shown in **Figure 1**, while no amplification products were observed in the control group, as shown in **Figure 2**.

Frequency of rs732510 of *MSRB1*

The R**s**732510 SNP was amplified in all 90 samples from the NIHL group, which represent all lanes with a size of 220-bp DNA molecule, as described in **Figure 3**.

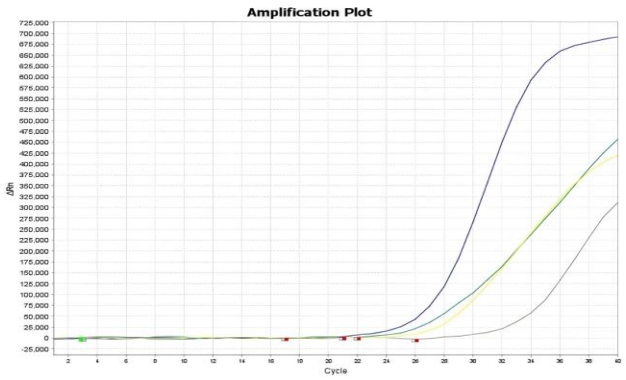

Figure 2. Real-time PCR amplification plot for rs732510, rs2815304, rs11640479, and rs9934331 in control subjects (Source: Authors' own elaboration)

Figure 3. Agarose gel electrophoresis of PCR products demonstrating the rs732510 of NIHL subjects (Source: Authors' own elaboration)

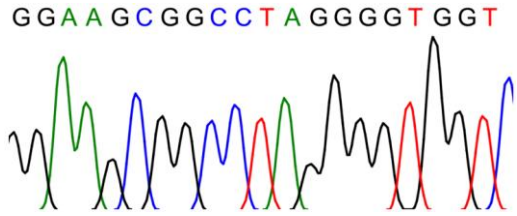

Figure 4. Chromatogram of the homozygous wild-type allele (Source: Authors' own elaboration)

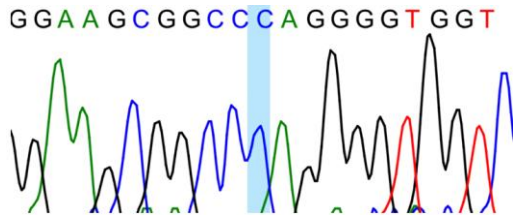

Figure 5. Chromatogram of the homozygous mutant allele (Source: Authors' own elaboration)

However, no PCR product was seen in the empty lane because water was used as a negative control.

Sanger's Sequencing

The rs732510 PCR results were confirmed by Sanger sequencing and analyzed using FinchTV software. Of the 90 individuals in the NIHL group, 13 (14.4%) were homozygous wild-type (TT) (**Figure 4**), 30 (33.3%) homozygous mutant (CC) (**Figure 5**), and 44 (48.9%) heterozygous (TC) (**Figure 6**).

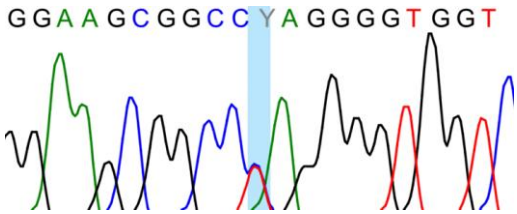

Figure 6. Chromatogram of the heterozygous mutant allele (Source: Authors' own elaboration)

Relationship between rs732510 Variance and Study Variables

Table 4 reveals that there was no statistically significant association between the genotypes of rs732510 variance and the study variables, such as hearing severity ($p = 0.54$), hearing protection ($p = 0.26$), noise exposure ($p = 0.11$), age ($p = 0.80$), age onset ($p = 0.42$), gender ($p = 1.00$), and hobbies of non-work activities ($p = 0.73$). These findings suggest that the study variables did not predict variation in rs732510 among patients with NIHL.

Cochran's Q test measured a statistically significant difference in the proportion of types of rs732510 variance genotypes that occurred among patients with NIHL, $\chi2(3) = 43.96$, $p < 0.05$. It is noted from **Table 5** that the heterozygous mutant has a statistically significantly higher occurrence compared to homozygous wild-type and homozygous mutant among patients with NIHL.

DISCUSSION

This study examined the association between *MSRB1* expression in the human auditory system and exposure to loud sounds by identifying pathological SNPs within the *MSRB1* gene that may contribute to NIHL. Moreover, it focused on determining the interaction effects between the associated SNPs and NIHL. Genotype frequencies of rs2815304, rs11640479, and rs9934331 were assessed using real-time PCR as part of the methodological framework. However, the incidence of rs732510 was only detected in the NIHL group, indicating a possible relationship between this variation and noise overexposure. This study presents the first evidence of a potential association between rs732510 and NIHL in humans.

The Role of Genetic Mutations in The Susceptibility to Noise-Induced Hearing Loss

NIHL is a preventable auditory condition caused by exposure to damaging sound levels. Noise exposure is estimated to contribute to approximately 30% of hearing loss cases [31], with prevalence in industrial populations ranging from 37% to 60% [32].

NIHL is a complex, multifactorial disorder influenced by environmental and genetic factors [5]. According to the American Speech-Language-Hearing Association, hearing loss is classified by severity, ranging from slight to profound [33]. In this study, participants exhibited sensorineural hearing loss with varying degrees of severity: slight (23.3%), mild (41.1%), moderate (23.3%), severe (8.9%), and profound (3.3%).

Table 4. Results of Fisher's exact test to measure the correlations between the type of rs732510 alleles and study variables

Variable	Categories of variable	Total (N = 90)	Homozygous mutant (N = 30)	Heterozygous mutant (N = 44)	Homozygous wild-type (N = 13)	Noise (N = 3)	p
Hearing severity	Slight. N (%)	21 (23.3%)	4 (13.3%)	15 (34.1%)	1 (7.7%)	1 (33.3%)	0.54
	Mild. N (%)	37 (41.1%)	15 (50.0%)	13 (29.5%)	7 (53.8%)	2 (66.7%)	
	Moderate. N (%)	21 (23.3%)	7 (23.3%)	10 (22.7%)	4 (30.8%)	0 (0.0%)	
	Severe. N (%)	8 (8.9%)	3 (10.0%)	4 (9.1%)	1 (7.7%)	0 (0.0%)	
	Profound. N (%)	3 (3.3%)	1 (3.3%)	2 (4.5%)	0 (0.0%)	0 (0.0%)	
Noise exposure/hours	< 8 hours. N (%)	81 (8.9%)	6 (20.0%)	2 (4.5%)	0 (0.0%)	0 (0.0%)	0.11
	8 hours & more. N (%)	82 (91.0%)	24 (80.0%)	42 (95.5%)	13 (100%)	3 (100%)	
Age	Less than 30 years. N (%)	8 (8.9%)	3 (10.0%)	4 (9.1%)	1 (7.7%)	0 (0.0%)	0.80
	30-49 years. N (%)	52 (57.8%)	16 (53.3%)	27 (61.4%)	6 (46.2%)	3 (100%)	
	50 years & more. N (%)	30 (33.3%)	11 (36.7%)	13 (29.5%)	6 (46.2%)	0 (0.0%)	
Age onset	Less than 30 years. N (%)	13 (14.4%)	6 (20.0%)	5 (11.4%)	1 (7.7%)	1 (33.3%)	0.42
	30-49 years. N (%)	63 (70.0%)	19 (63.3%)	34 (77.3%)	8 (61.5%)	2 (66.7%)	
	50 years & more. N (%)	14 (15.6%)	5 (16.7%)	5 (11.4%)	4 (30.8%)	0 (0%)	
Laterality	Unilateral. N (%)	38 (42.2%)	16 (53.3%)	17 (38.6%)	4 (30.8%)	1 (33.3%)	0.47
	Bilateral. N (%)	52 (57.8%)	14 (46.7%)	27 (61.4%)	9 (69.2%)	2 (66.7%)	
Gender	Male. N (%)	80 (88.9%)	26 (86.7%)	39 (88.6%)	12 (92.3%)	3 (100%)	1.00
	Female. N (%)	10 (11.1%)	4 (13.3%)	5 (11.4%)	1 (7.7%)	0 (0.0%)	
Hearing protection	Yes. N (%)	15 (16.7%)	3 (10.0%)	7 (15.9%)	4 (30.8%)	1 (33.3%)	0.26
	No. N (%)	75 (83.3%)	27 (90.0%)	37 (84.1%)	9 (69.2%)	2 (66.7%)	
Hobbies of non-work activities	No. N (%)	76	27 (90.0%)	36 (81.8%)	10 (76.9%)	3 (100%)	0.73
	Metalworking. N (%)	7	2 (6.7%)	3 (6.8%)	2 (15.4%)	0 (0.0%)	
	Loud music. N (%)	4	1 (3.3%)	1 (2.3%)	2 (15.4%)	0 (0.0%)	
	Hunting. N (%)	4	0 (0.0%)	3 (6.8%)	1 (7.7%)	0 (0.0%)	
	Wood cutting. N (%)	1	0 (0.0%)	1 (2.3%)	0 (0.0%)	0 (0.0%)	

Note. N: Number of subjects; %: Percentage of subjects; & *the statistical significance level was set at 0.05

Table 5. Cochran's Q-test to define the more visible alleles among the rs732510 SNP

Alleles	N (%)	Cochran's Q	p
Homozygous mutant (CC)	30 (33.3%)	43.96	< 0.01**
Heterozygous mutant (TC)	44 (48.9%)		
Homozygous wild (TT)	13 (14.4%)		
Noise	(3.3%)		

Note. N: Number of subjects; %: Percentage of subjects; & *the statistical significance level was set at 0.05

Bilateral mild hearing loss was observed in the majority of participants. Despite similar occupational noise exposure levels, the variation in hearing loss severity among participants suggests that genetic susceptibility plays a significant role. This phenotypic variability underscores the importance of genetic factors in determining individual vulnerability to NIHL [34, 35].

The Associations Between Noise-Induced Hearing and Genetic Variations

Recent studies increasingly support oxidative stress as a key contributor to the pathogenesis of acquired sensorineural hearing loss, including NIHL [36-38]. The cochlea is a highly metabolically active organ, and noise overexposure leads to increased production of free radicals and excessive accumulation of ROS. When the antioxidant defense system is overwhelmed, these oxidative imbalances can cause damage to cochlear structures [39]. Given this mechanism, numerous studies have investigated genes involved in the oxidative stress response and their association with NIHL susceptibility. Genetic variants in oxidative stress-related genes such as *NOX3*, *SOD2*, *PON2*, *CAT*, and *GSTM1* have been linked to NIHL in various populations, including American, Italian, Swedish, and Polish cohorts [34, 40].

Among the oxidative stress-related genes, *MSRA* and *MSRB* represent a subgroup of oxidative stress genes responsible for reducing methionine-S-sulfoxide and methionine-R-sulfoxide, respectively. *MSRA* and *MSRB1* are transcriptionally active in cochlear tissues [19]. *MSRA* has been implicated in attenuating noise-induced cochlear damage in the mouse model, whereas *MSRB1* is hypothesized to exert a similar protective effect within the auditory system, based on preliminary findings from the same study [17].

In addition to oxidative stress genes, several other genetic associations with NIHL risk have been reported. Sequence variants such as rs1104085, rs3777781, rs212769, rs666026, and rs2521768 have been linked to genes including *PCDH15*, *EYA4*, *GRHL2*, and *DFNA5*, respectively [41, 42]. Variants in *GSTM1*, *CAT*, *CDH23*, *KCNE1*, *HSP70*, and *OGG1* have also been identified as NIHL-related in prior research [42-45]. While *MSRB1* has not been widely studied in human hearing, a U.S.-based study found a significant association between rs732510, located in *MSRB1*, and colorectal cancer [46, 47]. In contrast, other *MSRB1*-related SNPs–rs2815304, rs9934331, and rs11640479–have not been reported as pathogenic in the literature.

Our results indicated no significant association between the *MSRB1* variants rs9934331, rs11640479, and rs2815304 and NIHL, as these SNPs were present in both the control and NIHL groups. Conversely, rs732510 was exclusively detected in the NIHL group, providing the first evidence of a possible link between this variant and auditory dysfunction. These findings suggest that genetic variation in MSRB1 may influence individual susceptibility to NIHL following noise exposure.

The Nature of rs732510 Variance Induced by NIHL

Among the 90 participants diagnosed with NIHL, 13 (14.4%) exhibited the homozygous wild-type genotype (TT), 30 (33.3%) had the homozygous mutant genotype (CC), and 44 (48.9%) presented with the heterozygous genotype (TC). A notably higher frequency of the heterozygous genotype (TC) was observed compared to both TT and CC genotypes within the NIHL group. To our knowledge, this is the first study to report

an association between the *MSRB1* rs732510 polymorphism and NIHL in humans.

Despite offering new insights into MSRB1 polymorphisms and NIHL susceptibility, the study's findings require cautious interpretation due to specific limitations. First is the historical information about noise exposure and protection, which was collected based on participants' self-reports. The second limitation is the lack of previous genetic studies on *MSRB1* variants associated with hearing loss, resulting in primer design challenges. Because of the budget limitations, only four candidate SNPs have undergone testing. Finally, the gender imbalance in the study sample reflects the male-dominated nature of Jordan's industrial labor force. According to the Jordanian Chamber of Industry, women comprise only 35% of the workforce in the industrial sector [48].

CONCLUSION

This study identifies rs732510 as a potentially novel SNP associated with NIHL, based on its presence exclusively in NIHL subjects and absence in controls. In contrast, the other three SNPs (rs2815304, rs11640479, and rs9934331) showed no association, as they appeared in both groups. The heterozygous genotype (TC) of rs732510 exhibited a significantly higher frequency among individuals with NIHL compared to both the homozygous wild-type (TT) and homozygous mutant (CC) genotypes. These findings lay the groundwork for further investigations into *MSRB1*'s role in auditory function. Given its potential as a biological biomarker, rs732510 could help improve NIHL diagnosis and prevention, thereby reducing disease severity and enhancing quality of life.

Author notes: Hanin Alwaqfi and Safa Alqudah contributed equally to this work and share first authorship.

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APPENDIX A: NOISE EXPOSURE QUESTIONNAIRE

Name:

Gender:

Phone number:

Marital status:

Educational level:

Address:

Profession:

1. When did you first notice your hearing loss?
2. Was the onset of hearing loss: □ Sudden □ Gradual
3. What kind(s) of hearing problems are you having:
   a. Difficulty hearing on the phone
   b. Difficulty hearing spoken communication in a one-on-one conversation
   c. Difficulty understanding spoken communication in the presence of surrounding noise
   d. Ringing in the ears
   e. Other – Please explain
4. Hearing symptoms were in: □ Right ear □ Left ear □ Both ears
5. While employed, did your hearing interfere with your work: □ No □ Yes, Please explain
6. Do you use any type of hearing protection during work? □ Yes □ No
7. What type of hearing protection do you use: _____
   How often:_____
8. Type of machinery or equipment used: _____
9. Exposure to noise: (hours/shift): _____
10. Have you been examined by an audiologist in the past for hearing loss: □ Yes–discuss the results □ No
11. Have you had any illness that affected your ears or hearing?: □ No □ Yes-indicate name of illness:
    a. Mumps
    b. Depression
    c. Fracture of the skull bone
    d. Tuberculosis
    e. Hypertension
    f. Parkinson's disease
    g. Multiple sclerosis
    h. Diabetes
    i. Meningitis
    j. Allergies
12. Have you ever had a head injury? □ No □ Yes–describe the injury below
13. Have you had any illness involving a high fever? □ No □Yes–indicate when and name of illness
14. Have any members of your family suffered hearing loss or deafness?
    □ No □Yes-specify relationship (mother, father, uncle, etc.):
15. Do you have any hobbies or non-work activities that involve loud noise?
    a. Listening to loud music
    b. Metal working
    c. Wood cutting
    d. Hunting
    e. Other–please specify
16. Have you taken or do you take any medication on a regular basis? If yes, please list the medication and the reason you are taking it.