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ORIGINAL RESEARCH

Association Between Susceptibility to SSHL and Single Nucleotide Polymorphisms at the rs2228612 Locus of the DNMT1 Gene and the rs5570459 Locus of the GJB2 Gene

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ABSTRACT

Context • Sudden deafness (SSHL) belongs to the category of diseases causing neurological hearing loss with a sudden and unknown etiology. The pathogenesis and mechanism of SSHL aren't clear at present. Gene polymorphisms may be associated with increased or reduced risk of hearing impairment.

Objective • The study intended to investigate the association between susceptibility to SSHL and single nucleotide polymorphisms (SNPs) at the rs2228612 locus of the DNA methyltransferase (DNMT1) gene and at the rs5570459 locus of the gap junction protein Beta 2 (GJB2) gene, to provide a basis for the prevention and treatment of the SSHL.

Design • The research team performed a case-control study.

Setting • The study took place at Tangshan Gongren Hospital in Tangshan, China.

Participants • Participants were 200 SSHL patients admitted to the hospital between January 2020 and June 2022, the study group, and 200 people with normal hearing, the control group.

Outcome Measures • The research team: (1) performed the Hardy-Weinberg Balance Test to determine the frequency distribution of the data for the rs2228612 locus of the DNMT1 gene and for the RS5570459 locus of the GJB2 gene for the groups, (2) analyzed the relationships between the genotypes and SSHL susceptibility, (3) determined the relationship between gene frequencies and gender and the SSHL susceptibility of males and females with different genotypes, (4) determined the relationship between gene frequencies and smoking and the SSHL susceptibility of smokers and nonsmokers with different genotypes, and (5) determined the relationship between gene frequencies and drinking alcohol and the SSHL susceptibility of drinkers and nondrinkers with different genotypes.

Results • The numbers of participants in the study group with the CC genotype and the C allele at the rs2228612 locus of the DNMT1 gene were significantly lower than the numbers in the control group ($P < .05$). The CC and C alleles were significant protective factors against SSHL ($P < .05$).The numbers of participants in the study group with the GG genotype and the G allele at the rs5570459 locus of the GJB2 gene were significantly higher than the numbers in the control group ($P < .05$), and the GG genotype and the G allele significantly increased SSHL susceptibility ($P < .05$). The TC+CC genotype at the rs2228612 locus of the DNMT1 gene was a protective factor against SSHL in male and smoking participants ($P < .05$). The AG+GG genotype at the rs5570459 locus of the GJB2 gene increased the susceptibility of females, smokers, and drinkers to SSHL ($P < .05$).

Conclusions • The TC+CC genotypes at the rs2228612 locus of the DNMT1 gene were significant protective factors against SSHL. The SSHL susceptibility was higher in participants carrying the AG+GG genotype at the rs5570459 locus of the GJB2 gene. In addition, gender and drinking can affect SSHL susceptibility (*Altern Ther Health Med.* 2023;29(3):224-229).

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Sudden deafness (SSHL) is a common otorhinolaryngologic emergency. The disease belongs to the category of diseases causing neurological hearing loss with a sudden and unknown etiology and has a short onset time and rapid progression. Patients' hearing can decrease significantly within minutes, hours, or days, and tinnitus and vertigo of varying degrees can accompany the loss, seriously affecting patients' physical and mental health.[1,2]

In recent years, the incidence of SSHL has been on the rise, and it's affecting younger people. Therefore, the

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prevention and early treatment of SSHL are particularly important; however, the pathogenesis and mechanism of SSHL aren't clear at present.

The hypothesis exist in clinical practice about the pathogenesis of SSHL, such as viral infections, immune-mediated mechanisms, and abnormal cell stress responses in the cochlea that can cause a blood-circulation disorder and metabolic dysfunction in patients' inner ears, resulting in sensory epithelial cell damage in the inner ear, and eventually, deafness. [3,4] Drouet et al found that the pathological basis of SSHL might be a deficiency in inner-ear function caused by blood circulation disorders.[5]

Therefore, the study of the internal etiology of SSHL to prevent the occurrence of sudden deafness is of great significance. Li et al believed that SSHL may be related to genetics and a cochlear viral infection.[6] Previous population studies found that people's hearing thresholds could shift to different degrees.[7] The differences could be large, suggesting that individual differences existed in susceptibility to SSHL that might be related to differences in gene polymorphisms in different populations.[8] Guo et al found that multiple gene polymorphisms were associated with increased or reduced risk of hearing impairment.[9]

DNA Methyltransferase (DNMT)

DNMT is the catalyst of methylation and is mainly involved in its maintenance. Li et al found that a DNMT1 gene mutation could cause neurodegenerative disease, accompanied by dementia and sensorineural hearing loss.[6]

DNMT1 plays an important role in chromosome reconstruction and gene expression, while a DNMT1 gene mutation can reduce the activity of methyltransferase and impair its ability to bind chromatin in the G2 phase, resulting in local hypermethylation and leading to sensory hearing loss or genetic sensory disorders.[10] Seker et al found that mutation of the genotypes at the rs2228612 locus of the DNMT1 gene can be a protective factor for males and smokers against SSHL.[11]

Gap Junction Protein Beta 2 (GJB2)

Fukunaga et al proposed that the gene encoding GJB2, which encodes the connexin 26 (CX26) protein, is expressed at a high level in the cochlea, plays an important role in cellular-gap-junction communication, and has an association with SSHL susceptibility.[12]

The GJB2 gene is located on human chromosome 13Q11-12. Azadegan-Dehkordi et al found that a GJB2 gene mutation can be the main, recessive, genetic cause of comprehensive hearing loss but that significant differences existed among different populations.[13] Those researchers found that the rs5570459 locus of the GJB2 gene was located downstream of the mutation site, in the exon region of GJB2.

Al-Janabi et al found that changes in the polymorphic loci in the exon region can lead to the reduction in or disappearance of the biological functions of original coding products, thus affecting hearing.[14] In addition, the connexin that the GJB2 gene encodes is an important channel for electrolytes and metabolites in cell transformation, among which CX26 protein is highly expressed in the cochlea.[13]

Neagu et al found that mutation of the GJB2 gene can lead to abnormal hearing conduction, resulting in hearing impairment.[15] The rs5570459 locus of the GJB2 gene is in the inner ring of the mRNA stem loop structure of the GJB2 gene. Kim et al found that an A>G mutation at the rs5570459 locus, in a population with the G allele, can change the mRNA sequence, structure, and stability; downregulate the GJB2 level; and then affect electrolytes, metabolites and other conversion channels, thus affecting normal hearing.[16]

Current Study

The current study intended to investigate the association between susceptibility to SSHL and single nucleotide polymorphisms (SNPs) at the rs2228612 locus of the DNMT1 gene and at the rs5570459 locus of the GJB2 gene, to provide a basis for the prevention and treatment of the SSHL.

METHODS

Participants

The research team performed a a case-control study. The study took place at Tangshan Gongren Hospital in Tangshan, China. Potential participants were SSHL patients admitted to the hospital between January 2020 and June 2022, the study group, and 200 people with normal hearing, the control group. The SSHL patients involved in the study were all patients admitted to our department; and those with normal hearing were volunteers recruited from the community by our research team. All those included in the study were informed about the study.

The diagnostic criteria for SSHL are as follows[17]: sudden onset of sensorineural hearing loss of unknown origin within 72 hours, with hearing loss of ≥20dBHL at two adjacent frequencies at least.

Potential participants were included in the study group if they: (1) met the diagnostic criteria of SSHL and (2) were aged 30-50 years.

Potential participants were excluded from the study group if they: (1) had a history of diabetes, hypertension, hyperlipidemia, stroke, autoimmune diseases, or infections; (2) had otitis media and acoustic nerve tumors; (3) had family with a history of deafness; (4) had senile hearing decline.

Potential participants were included in the control group if they: (1) were aged 30-50 years;(2) Agreed to participate in this study

Potential participants were excluded from the control group if they: (1) Previously had SSHL;(2) had family with a history of deafness;(3) Previously diagnosed with coronary artery disease, hypertension, diabetes, stroke, etc.(4) refused to have blood drawn .

Participants were informed about the study and signed informed consent forms. The hospital's ethics committee approved the study's protocols.

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Table 1. Hardy-Weinberg Balance Test of DNMT1 and GJB2 Genes in the Research and Control Groups (N = 400). a vs b indicates that comparation between Actual Value and Expected Value in Research Group. c vs d indicates that comparation between Actual Value and Expected Value in Contron Group

	Research Group n = 200		Control Group n = 200		a vs b		c vs d	
Genotype	Actual Value n (%)	Expected Value n (%)	Actual Value n (%)	Expected Value n (%)	χ^2	*P* value	χ^2	*P* value
DNMT1 RS2228612					.543	.461	.067	.967
TT	90 (45.00)	88 (44.00)	66 (33.00)	64 (32.00)				
TC	78 (39.00)	76 (38.00)	80 (40.00)	80 (40.00)				
CC	32 (16.00)	36 (18.00)	54 (27.00)	56 (28.00)				
GJB2 RS5570459					.127	.722	.450	.502
AA	125 (62.50)	124 (62.00)	155 (77.50)	152 (76.00)				
AG	51 (25.50)	53 (26.50)	36 (18.00)	37 (18.50)				
GG	24 (12.00)	23 (11.50)	9 (4.50)	11 (5.50)				

a, indicating that Actual Value in Research Group
b, indicating that Expected Value in Research Group
c, indicating that Actual Value in Control group
d, indicating that Expected Value in Control group

Abbreviations: DNMT1, DNA methyltransferase; GJB2, gap junction protein beta 2.

Procedures

The research team: (1) collected 2 ml of peripheral venous blood from all participants, (2) extracted the genomic DNA of cells, and (3) amplified the product sequences using polymerase chain reaction (PCR) to detect the genotypes at the RS2228612 locus of the DNMT1 gene and at the RS5570459 locus of the GJB2 gene.

Primer sequences. Shanghai Shenggong Bioengineering (Shanghai, China) synthesized the primers. The primer sequences at the RS2228612 locus of the DNMT1 gene were: (1) upstream, 5 '-AAA AGT GAGACC TTT ACC TTT TCA T-3' and (2) downstream, 5 '-AGGCCC GAA GAA AAA GAA CC-3'. The PCR primer sequences at the RS55704559 locus of the GJB2 gene were: (1) upstream, 5 '-TGC AAC CAT TTG AAA CCC CTG-3' and (2) downstream, 5 '-TTA GGG GAG CAG AGC TCC AT-3'.

PCR. The PCR reaction system was 20 μl , purchased from Thermo Fisher Scientific(Shanghai ,China) The reaction conditions were: (1) 95°C for 5 min; (2) 94°C for 20 s; (3) 60°C for 30 s; (4) 72°C for 30 s for 30 cycles; and (5) 72°C for 5 min. The research team stored the final products at 4°C, and after completion of the PCR reaction, sequenced the amplified products.

Single nucleotide polymorphisms (SNPs). It mainly refers to the DNA sequence diversity caused by the variation of a single nucleotide at the genome level, which is numerous and rich in polymorphism. Theoretically, each SNP site can have four different forms of variation (substitution (conversion or reversal), insertion or deletion), but in practice only two occur, namely conversion and reversal, with a ratio of 2:1. SNPs occur most frequently in CG sequences, and most are C to T conversions, because C in CG is often methylated and becomes thymine after spontaneous deamination.

Outcome measures. The research team: (1) performed the Hardy-Weinberg Balance Test[18] to determine the frequency distribution of the data for the rs2228612 locus of the DNMT1 gene and for the RS5570459 locus of the GJB2 gene for the groups, (2) analyzed the relationships between the genotypes and SSHL susceptibility, (3) determined the relationship between gene frequencies and gender and the SSHL susceptibility of males and females with different genotypes, (4) determined the relationship between gene frequencies and smoking and the SSHL susceptibility of smokers and nonsmokers with different genotypes, and (5) determined the relationship between gene frequencies and drinking alcohol and the SSHL susceptibility of drinkers and nondrinkers with different genotypes.

Statistical Analysis

The research team performed the statistical analysis using SPSS 21.0 software (IBM, Armonk, NY, USA). The team: (1) performed the Hardy-Weinberg genetic equilibrium test to obtain the distribution of p30articipants' genotypes for the DNMT1 and GJB2 genes in the study and control groups; (2) used the χ^2 test for the count data, expressed as numbers and percentages (%); (3) used the *t* test for two, independent-samples, measurement data, expressed as means ± standard deviations (SDs); and (4) analyzed the associations between the DNMT1 and GJB2 genotypes using multifactor logistic regression to determine the alleles and the susceptibility of participants with them to SSHL. $P < .05$ indicated statistically significant differences.

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Table 2. Comparison of SNP Genotypes of DNMT1 and GJB2 Between the two Groups (N = 400)

Genotype	Research Group n = 200 n (%)	Control Group n = 200 n (%)	χ^2	Between Groups *P* value	OR (95% CI)	Effects Against SSHL *P* value
DNMT1 RS2228612						
Genotype			9.346	.009[a]		
TT	90 (45.00)	66 (33.00)			1.00	
TC	78 (39.00)	80 (40.00)			0.803 (0.564 to 1.142)	.222
CC	32 (16.00)	54 (27.00)			0.421 (0.212-0.836)	.014[b]
Allele			10.914	.001[a]		
T	258 (64.50)	212 (53.00)			1.00	
C	142 (35.50)	188 (47.00)			0.651 (0.448 to 0.944)	0.024[b]
GJB2 RS5570459						
Genotype			12.619	.002[a]		
AA	125 (62.50)	155 (77.50)			1.00	
AG	51 (25.50)	36 (18.00)			1.350 (0.930 to 1.959)	.115
GG	24 (12.00)	9 (4.50)			3.387 (1.271 to 9.025)	.015[b]
Allele			16.365	<.001[a]		
A	301 (75.25)	346 (86.50)			1.00	
G	99 (24.75)	54 (13.50)			1.822 (1.316 to 2.523)	<.001[b]

[a]$P < .005$, indicating that the numbers of participants in the study group carrying the CC genotype and C allele at the RS2228612 locus of the DNMT1 gene were significantly lower and the numbers carrying the GG genotype and the G allele at the RS5570459 locus of the GJB2 gene were significantly higher than the numbers in the control group
[b]$P < .005$, indicating that the CC genotype and the C allele at the RS2228612 locus of the DNMT1 gene were significant protective factors against SSHL and that the GG genotype and the G allele at the RS5570459 locus of the GJB2 gene significantly increased SSHL susceptibility

Abbreviations: DNMT1, DNA methyltransferase; GJB2, gap junction protein beta 2; SNP, single nucleotide polymorphism.

RESULTS

Participants

The study included and analyzed the data of 400 participants. The study group included 200 participants, 100 males and 100 females with an average age of 41.16 ± 7.52 years and a range from 30 to 50 (data not included). Of them, 28 participants smoked, and 31 drank alcohol (data not included). The control group included 200 participants, 100 males and 100 females with an average age of 40.91 ± 6.96 years and a range from 30 to 50 years old (data not included). Of them, 30 participants smoked. and 30 drank alcohol (data not included). No significant differences existed in participants' demographic characteristics between the groups ($P > .05$).

Hardy-Weinberg Balance Test

Table 1 shows that the frequency distribution of the data for the rs2228612 locus of the DNMT1 gene and for the RS5570459 locus of the GJB2 gene for the groups was in line with the Hardy-Weinberg equilibrium test ($P > .05$).

SNP Genotypes

Table 2 shows that the numbers of participants in the study group carrying the CC genotype and the C allele at the RS2228612 locus of the DNMT1 gene were significantly lower than the numbers in the control group, with $P = .009$ and $P = .001$, respectively. The CC genotype (OR = 0.421, $P = .014$) and the C allele (OR = 0.651, $P = .024$) were significant protective factors against SSHL.

No significant correlation existed between the TT and TC genotypes and SSHL occurrence ($P > .05$).The numbers of participants in the study group carrying the GG genotype and the G allele at the RS5570459 locus of the GJB2 gene were significantly higher than the numbers in the control group, with $P = .002$ and $P < .001$, respectively. The GG genotype (OR = 3.387, $P = 0.015$) and the G allele (OR = 1.822, $P < .001$) significantly increased SSHL susceptibility ($P < .05$). No significant correlation existed between the AA and AG genotypes and the occurrence of SSHL ($P > .05$).

Gene Frequencies and Gender

Table 3 shows that the number of male participants in the study group carrying the TC+CC genotype at the rs2228612 locus of the DNMT1 gene was significantly higher than the number of female participants in the group ($P < .001$). The TC+CC genotype was a significant protective factor against SSHL (OR = 0.507, $P = .029$). The number of male participants in the study group carrying the AG+GG genotype at the RS5570459 locus of the GJB2 gene was significantly higher than the number of female participants in the group ($P = .002$). The AG+GG genotype in male significantly increased susceptibility to SSHL (OR = 3.320, $P < .001$).

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Table 3. Comparison of DNMT1 and GJB2 Gene Frequencies Between Men and Women in the Study Group (n = 200)

Genotype	Males n=100 n (%)	Females n = 100 n (%)	χ^2	Between Groups *P* value	OR (95% CI)	Effects Against SSHL *P* value
DNMT1 RS2228612			23.354	<.001[a]		
TT	28 (28.00)	62 (62.00)			1.00	
TC+CC	72 (72.00)	38 (38.00)			0.507 (0.276 to 0.930)	.029[b]
GJB2 RS5570459			9.408	.002[a]		
AA	52 (52.00)	73 (73.00)			1.00	
AG+GG	48 (48.00)	27 (27.00)			3.320 (1.672 to 6.593)	<.001[b]

[a]*P* < .005, indicating that the number of male participants in the study group carrying the TC+CC genotype at the RS2228612 locus of the DNMT1 gene and the number carrying the AG+GG genotype at the RS5570459 locus of the GJB2 gene was significantly higher than the number of female participants
[b]*P* < .005, indicating that the TC+CC genotype at the RS2228612 locus of the DNMT1 gene was a significant protective factor against SSHL and that the AG+GG genotype at the RS5570459 locus of the GJB2 gene significantly increased SSHL susceptibility

Abbreviations: DNMT1, DNA methyltransferase; GJB2, gap junction protein beta 2

Table 4. Comparison of DNMT1 and GJB2 Gene Frequencies Between Smokers and Nonsmokers in the Study Group (n=200)

Genotype	Smoking Group n = 28 n (%)	Nonsmoking Group N=172 N (%)	χ^2	Between Groups *P* valuee	OR (95% CI)	Effects Against SSHL *P* value
DNMT1 RS2228612			15.464	<.001[a]		
TT	3 (10.71)	87 (50.58)			1.00	
TC+CC	25 (89.29)	85 (49.42)			0.368 (0.162 to 0.838)	0.018[b]
GJB2 RS5570459			0.044	.833		
AA	18 (64.29)	107 (62.21)			1.00	
AG+GG	10 (35.71)	65 (37.79)			1.419 (0.788 to 2.555)	0.244

[a]*P* < .005, indicating that the number of participants in the study group carrying the TC+CC genotype at the RS2228612 locus of the DNMT1 gene who smoked was significantly higher than the number who didn't smoke
[b]*P* < .005, indicating that the TC+CC genotype at the RS2228612 locus of the DNMT1 gene was a significant protective factor against SSHL

Abbreviations: DNMT1, DNA methyltransferase; GJB2, gap junction protein beta 2.

Gene Frequencies and Smoking

Table 4 shows that the number of participants carrying the TC+CC genotype at the rs2228612 locus of the DNMT1 gene was significantly higher among participants who smoked than those who didn't smoke (*P* < .001). The TC+CC genotype was a significant protective factor against SSHL (OR = 0.368, *P* = .018). No significant difference existed between smokers and nonsmokers in the number of participants carrying the AG+GG genotype at the rs5570459 locus of the GJB2 gene (*P* > .05), and no significant correlation existed between that genotype and SSHL susceptibility (OR = 1.419, *P* > .05).

Gene Frequencies and Drinking Alcohol

Table 5 shows that no significant differences existed between participants who drank and those who didn't drink alcohol in the number of participants carrying the TC+CC genotype at the rs2228612 locus of the DNMT1 gene (*P* > .05), and no significant correlation existed between drinking habits and SSHL occurrence (OR = 0.152, *P* > .05). The number of participants carrying the AG+GG genotype at the RS5570459 locus of the GJB2 gene was significantly higher among drinkers than nondrinkers (*P* < .05). The AG+GG genotype increased SSHL susceptibility in drinkers (OR = 0.2.460, *P* < .05).

DISCUSSION

The current study found that the CC genotype and the C allele at the rs2228612 locus of DNMT1 gene were significant protective factors of SSHL. DNMT1's effects on the DNA methylation state and the ability of the CC gene and C allele at the rs2228612 locus of DNMT1 gene to promote the expression of DNMT1 and increase the activity of methyl transferase may explain that finding.

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Table 5. Comparison of DNMT1 and GJB2 Gene Frequencies Between Drinkers and Nondrinkers in the Study Group (n=200)

Genotype	Alcohol Consumption n = 31 n (%)	No Alcohol Consumption n = 169 n (%)	χ^2	Between Groups *P* value	OR (95% CI)	Effects Against SSHL *P* value
DNMT1 RS2228612			0.000	0.984		
TT	14 (45.16)	76 (44.97)			1.00	
TC+CC	17 (54.84)	93 (55.03)			0.512 (0.088 to 2.986)	0.457
GJB2 RS5570459			29.138	<.001[a]		
AA	6 (19.35)	119 (70.41)			1.00	
AG+GG	25 (80.65)	50 (29.59)			2.460 (1.080 to 5.062)	0.033[b]

[a]*P* < .005, indicating that the number of participants in the study group carrying the AG+GG genotype at the RS5570459 locus of the GJB2 gene who drank alcohol was significantly lower than the number who didn't drink
[b]*P* < .005, indicating that the AG+GG genotype at the RS5570459 locus of the GJB2 gene significantly increased SSHL susceptibility

Abbreviations: DNMT1, DNA methyltransferase; GJB2, gap junction protein beta 2.

In addition, the current study also found that the TC+CC genotype at the rs2228612 locus of the DNMT1 gene was a protective factor for males and smokers against SSHL, which is a finding similar to that of Seker et al's study.[11] However, researchers need to further study the association between the SNP at the RS2228612 locus of the DNMT1 gene and SSHL susceptibility by expanding sample size and controlling variables.

The current study also found that carrying the GG genotype and the G allele at the rs5570459 locus of the GJB2 gene could increase susceptibility to SSHL, similar to the findings of other studies that these factors can impact hearing ability.[13-15] In addition, the current study also found that the AG+GG genotype at the rs5570459 locus of the GJB2 gene could increase the susceptibility of SSHL for men while the susceptibility of SSHL in men and people without a drinking history was lower, suggesting that the occurrence of SSHL may be the result of the joint action of genes and environmental factors.

The current study had some limitations. First, the occurrence of SSHL is the result of the interaction of multiple genes, environment, heredity, and other factors, and the risk of SSHL can't be determined by a single gene. Second, some subjective and objective bias in the sample selection of the study may have occurred, which might have affected the study's results to some extent, and the study's representativeness is relatively limited. Therefore, to further discuss the relationship between the DNMT1 gene, GJB2 gene, SNP, and SSHL susceptibility, it's necessary to expand the sample range and to perform a multifactorial, stratified experiment to verify the current results.

CONCLUSIONS

The TC+CC genotypes at the rs2228612 locus of the DNMT1 gene were significant protective factors against SSHL. The SSHL susceptibility was higher in participants carrying the AG+GG genotype at the rs5570459 locus of the GJB2 gene. In addition, gender, and drinking can affect SSHL susceptibility.

AUTHORS' DISCLOSURE STATEMENT

The authors have no potential conflicts of interest to report relevant to this article.

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