Association of STAT3 Gene polymorphism with noise-induced hearing loss(NIHL) in the Chinese population

Dengfeng Gao
School of Public Health, Southeast University
Boshen Wang
School of Public Health, Southeast University
Dawei Sun
Southeast University
Ning Wang
Southeast University
Jiadi Guo
Nanjing Medical University
Suhao Zhang
Nantong University
Baoli Zhu (zhbl5888@sina.com)
Jiangsu Province Center for Disease Control and Prevention https://orcid.org/0000-0002-4345-0154

Research article

Keywords: Noise-induced hearing loss, STAT3, Smoking, Single nucleotide polymorphism, Association study

Posted Date: August 11th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-51818/v1

Abstract

Objectives: NIHL is a disease with irreversible damage caused by multi-factor interaction, and the STAT3 is an essential protein with signal transduction and transcription functions. This study is aimed to explore the effect of STAT3 polymorphism on individual susceptibility to NIHL individuals, and to further examine the interaction between lifestyles and NIHL in the dominant model .

Methods: This study was designed a case-control study and included 609 NIHL cases and 611 healthy hearing controls from the Jiangsu province of China. By collecting the lifestyle and demographic information of the study participants, genotyping the collected blood samples, and performing Real-time Quantitative PCR (qRT-PCR) to analyze and investigate the potential association between STAT3 SNPs and NIHL.

Results: Statistical analysis results show that individuals carrying the C allele of rs1053023 and rs1053005 were more likely to develop NIHL than individuals carrying the T allele (OR = 1.367, 95% CI = 1.148-1.628, *P* = 0.0001 and OR = 1.37, 95% CI = 1.147-1.636, *P* = 0.001). In the dominant model (TC/CC genotype and TT genotype), stratified analysis of lifestyle, we found that people who smoke regularly are more likely to suffer from NIHL than non-smokers or occasional smokers. Besides, the expression of STAT3 was higher in NIHL cases by real-time quantitative PCR.

Conclusion: Gene polymorphisms, rs1053023 and rs1053005, from STAT3, are associated with NIHL and C alleles of rs1053023 and rs1053005 within STAT3 may become biomarkers for workers exposed to noise.

Background

Noise can cause annoyance，in severe cases, and it will cause insomnia and hearing damage, which will have a considerable impact on people's normal life. For workers who have been exposed to noise for a long time, noise is inevitable, so Noise-induced hearing loss (NIHL) is also their most common occupational health hazard. A large number of studies have shown that NIHL is an irreversible hearing loss caused by the interaction of multiple risk factors such as environment(e.g.occupational noise exposure), genes and lifestyle(e.g. smoking and drinking) [1, 2]. Current studies have found that this hearing impairment manifested cochlear hair cell synaptic damage and hair cell apoptosis have irreversible properties ,but its occurrence can be reduced by effective protection[3]. In industrial countries, noise is the most important cause of hearing loss compared with other risk factors for hearing loss [4]. Also, NIHL has been a critical and leading occupational disease, just following age-related hearing loss (ARHL) worldwide[3]. A research report released by the World Health Organization estimates that nearly 500 million noise-exposed workers worldwide are at high risk of hearing loss [5, 6] .

The degree of noise-induced hearing impairment is affected by many factors. In addition to noise intensity, frequency, exposure time and the genetic susceptibility, the impairment, is also influenced by individual susceptibility, polymorphism individual susceptibility gene[7] . Therefore, exploring NIHL susceptibility genes and discovering NIHL susceptible people as early as possible, thereby reducing the incidence of NIHL, this screening method will have obvious benefits for the prevention of NIHL, whether for companies or individuals. Published and available research has identified many single nucleotide polymorphisms (SNPs)

of genes by interaction with other environmental factor associated with NIHL susceptibility. GSTT1, GSTM1, GSTP1[8], CAT[9], KCNQ4, KCNE1,[10, 11], MYH14[12], HSP70[13], SOD1 and SOD2[14, 15] are related to genetic predisposition of NIHL.

Previous research found that the significant mechanisms of NIHL are cell apoptosis and oxidative stress[16].Signal Transducer and Activator of Transcription 3 (STAT3) has both transmission and transcription functions[17]-[18]. STAT3, as a cytoplasmic transcription factor has many vital functions,such as regulating cell growth, differentiation and programmed death, and vascularization, etc[19-21]. Animals experiment show that STAT3 can be expressed on inner ear hair cells, and as a downstream molecule of Notch signal, involved in the inner ear hair cells during development [22]. Therefore, STAT3 may be included in the event of NIHL.Wilson.et al. have indicated the crucial role of JAK2/STAT3 signalling pathway on the development of NIHL[23]. However, there is no research on the correlation between the SNPs of STAT3 and NIHL. By inhibiting the function of JAK / STAT3 pathway, the oxidative stress and apoptosis of inner ear hair cells can be appropriately reduced;we speculated that SNPs in STAT3 might be related to the genetic susceptibility of NIHL. This study is the first report to explore the correlation of multiple SNP sites of STAT3 genes, lifestyle factors with NIHL. So this study is to pursue the potential relevance of the five SNP sites of STAT3(rs4796793, rs1053023, rs1053005, rs1053004, and rs3744483) and NIHL.

Materials And Methods

2.1 Subjects

The research subjects came from employees who are exposed to noise in automobile, energy, and cotton spinning factories in Jiangsu Province, China. Employees who had been working steadily for more than three years and exposed to > 85dB (A) noise formed our study cohort. They all participated in the physical examination and completed the noise-exposure survey by a face-to-face conversation with a professional questionnaire investigator. Through the questionnaire, we collect information as follows: (1) general demographic information (age, gender, etc.);(2) type of work and working-age; (3) lifestyle factors(smoking, drinking). The variables involved are defined as follows:: 1) smoking: “yes” means “smoking every day”, “ever” means “smoking occasionally”, and “never” means “never smoking”.; 2) drinking: “yes” representatives have daily alcohol intake, e.g. white wine ≥ 50 g, red wine ≥ 150 g, or beer ≥ 500 g, and last for one year or longer, “ever” means “drinking occasionally”, and “never” means “never drinking” .(4) overall health(the history of illness, medication history). This study uses case-control research, and the exclusion criteria included: a) Employees with ear deformities or diseases that may affect hearing thresholds (such as mumps, measles, otitis media, Meniere syndrome, ear canal stenosis, etc.); b) Workers who have taken or are taking ototoxic drugs (such as aspirin, quinolones, aminoglycosides, etc.); c) Workers with a family history of deafness and head trauma. At last, there are 1220 subjects entered the study. All research subjects signed an informed consent form, and the institutional review committee of the Jiangsu Center for Disease Control and Prevention approved the study.

2.2 PTA and case definition

Before the audiometry , each individual was removed from the noise exposure environment for more than 16 hours following the relevant testing requirements according to the provisions of GBZ 49-2014 "Diagnostic Standards for Occupational Noise Deafness", pure tone audiometry (PTA) was performed by otolaryngologists in a room with sound insulation and a noise background of less than 25 dB. The audiometry study subjects binaural hearing thresholds at six frequencies (500, 1000, 2000, 3000, 4000, and 6000 Hz). All measurement results were corrected for age and gender according to standards. The case group was defined whose pure-tone hearing test binaural high-frequency (3000, 4000, 6000Hz) average hearing threshold ≥ 25 dB (A), otherwise as to the control group.

2.3 DNA extractions

EDTA anticoagulation tube was used to collect peripheral blood (> 2 mL) and store blood in a refrigerator at -80℃ before DNA extractions. The DNA extraction kit (Tiangen Biochemical, Beijing) was used to extract the peripheral blood DNA, and using an Implant P360 ultramicro spectrophotometer (Shanghai Boyi Biotechnology Co., Ltd., China) to detect the concentration and purity of the DNA.

2.4 SNP selection

Screen unlabeled SNPs according to the following methods: (1) minimum allele frequency (MAF)> 0.05; (2) the literature searched for frequently re-ported SNPS in previous studies; (3) linkage disequilibrium (LD) value r^2> 0.8. The selected data come from the 1000 Genomes Project (1000 Genomes Project, www.1000genomes.org) and the National Center for Biotechnology Information (NCBI) dB SNP database. Finally, the 5 SNPs (rs4796793, rs1053023, rs1053005, rs1053004, and rs3744483) of STAT3 were selected for the study.

2.5 Statistical analysis

SPSS 24.0 software (IBM, NYC, USA) was used for statistical analysis of all data. For normally-distributed continuous variables, the statistic "mean ± standard deviation (SD) " was used in the article and using Student's *t*-tests or one-way analysis of variance to analyze. The categorical variables were analyzed using the chi-square test and expressed as frequencies (percentage). The representativeness of the sample was tested by Hardy-Weinberg genetic balance . Estimating the Hardy–Weinberg equilibrium (HWE) use the chi-square test. After adjusting the risk factors of age, gender, smoking and drinking, analyze the correlation between the selected SNP and NIHL risk and between the different genotypes and NIHL risk and calculate the odds ratio (OR) and 95% confidence interval (95% CI). $P < 0.05$ means statistically significant and shown in bold.

Result

3.1 General characteristics

Table 1 shows the general symptoms, including: lifestyle features (age, sex, tobacco, and alcohol consumption habits) and occupational characteristics (working years of noise exposure, noise intensity, and high-frequency hearing threshold) of case group and the control group. No statistically significant

differences in demographic and lifestyle features between the two groups (P> 0.05) was found. However, the statistically significant(p<0.001) of the threshold between cases the control subjects was found, and the hearing threshold in case(37.50±12.39) was higher than control (15.58±4.76).

3.2 Linkage disequilibrium (LD)

The LD heatmap of these five SNPs (rs4796793, rs1053023, rs1053005, rs1053004, and rs3744483) were shown in Figure 1. By calculated correlation coefficient (r^2) for each pair of the five SNPs in 3´untranslated region, we found that SNP rs3744483, rs1053005 and rs1053023 present a state of complete LD (r^2 = 1, using 1000 genome phase 3 data).

3.3 Multivariate analysis of STAT3 tag SNPs with the risk of NIHL

The association between genetic models of STAT3 tag SNPs (rs4796793, rs1053023, rs1053005, rs1053004, and rs3744483) in 609 NIHL patients and 611 control groups with NIHL was examined and the results shown in Table 2. After adjusting for gender, age, smoking and drinking, the rs1053023 and rs1053005 codominant and dominant models were demonstrated to be significantly related to NIHL. In the dominant model, for loci rs1053023, individuals carrying the TT genotype were detected to be less likely to suffer from NIHL than those with TC / CC genotype (adjusted: OR=1.516, 95% CI = 1.201-1.913, P = 0.0001). Similarly, for loci rs1053005, relative to the TT genotype, research participants who were carrying the TC/CC genotype had a higher risk to be NIHL, the odds ratio (OR) is 1.509 (adjusted: 95% CI = 1.191-1.912, P = 0.001). Furthermore, the proportion of the C alleles of rs1053023 and rs1053005 in NIHL case group was obviously more than that of the control group (adjusted: OR=1.367, 95% CI =1.148-1.628, P =0.001; OR=1.370, 95% CI =1.147-1.636, P =0.002 respectively). This suggests that, among all study subjects, who carrying the T allele at the lower risk to affect NIHL than those carrying the C allele. Thus, We could speculate that allele T may be a protective factor for hearing loss.

3.4 Stratified analysis of rs1053023 and rs1053005 polymorphism and NIHL risk

We further examined the influence of rs1053023 and rs1053005 different genotypes about NIHL on a series of risk characteristics in the dominant model. Table 3 and Table 4 presents the consequences of the analysis. Compared with women in gender stratification, the NIHL risk of the TC / CC combination genotype of rs1053023 (adjusted: OR= 1.545, 95% CI = 1.215-1.964, P = 0.001) in men is 1.545 times than the TT genotype. Analogously, the NIHL risk of TC / CC genotype of rs1053005 (adjusted: OR= 1.531, 95% CI = 1.199-1.955, P = 0.002) in men is 1.531 times than that of TT genotype. In addition ,for loci rs1053023, when smoking and drinking were stratified, the individual carrying the TC/CC genotype relatively prone to NIHL disease in the regular smoking group (OR = 1.595, 95% CI = 1.163-2.187, P = 0.008), the never-drinking group (OR = 1.467, 95% CI = 1.076-2.002, P = 0.019) and the group drinking occasionally(OR = 10.55, 95% CI = 3.803-29.287, P <0.001). After adjustment for gender, age, within the strata of less than ten years noise exposure, subjects with TC / CC genotype were is more likely to develop into NIHL (OR = 2.448, 95% CI = 1.498-3.998, P = 0.001). Similar data features were found in the noise exposure group over 20 years (OR = 1.678, 95% CI = 1.151-2.447, P = 0.031). A similar statistical analysis result was found for loci rs1053005,

which is that individuals carrying TC / CC genotypes were likely to be NIHL when they regular smoking, or never drinking, or the year of noise exposure was less than 10(or more than 20).

3.5 Comparison of changes in high-frequency hearing thresholds of different genotypes for rs1053023 and rs1053005.

The results of the comparison of high-frequency hearing threshold shift of rs1053023 and rs1053005 genotypes in all noise-exposed workers were shown in Figure 2. The CC genotype bars were substantially higher than those of TC (P = 0.039) and TT (P= 0.024). Besides, subjects with rs1053005 TT genotype demonstrated a significantly lower hearing threshold shift when compared with subjects carrying CC and TC genotype (P = 0.046 and P = 0.033).

3.6 Quantitative Real-time PCR

Figure 3 shows the expression level of STAT3 in the population by Quantitative Real-time PCR(qRT-PCR) experiments. We can notice that STAT3 was highly expressed in the case group than the control group. The primer information used in qRT-PCR experiments was shown in Table 5.

Discussion

The most common sensory organ defect in the world is hearing loss [24]. Hearing can be impaired directly by high-intensity and long-term noise exposure, eventually leading to NIHL[25]. The number of people with hearing disability is rising year by year, with 42,000 in 1985, 120 million in 1995, 250million in 2001, and up to 360 million in 2011, speculated by the World Health Organization (WHO)[26]. NIHL, a multifactoral disease, has been the focus of research in recent years, especially in the study of the association with genetic susceptibility. Population epidemiological studies have shown that after noise exposure, 50% of the variation in hearing loss can be explained by genetic factors[27, 28].

Single-nucleotide polymorphism (SNP) has become the third generation of polymorphism markers due to the characteristics of the high density of genetic markers, high stability, and secure automation of typing tests. There are four theories about the mechanism of NIHL: oxidative stress theory, Ca^{2+} overload theory, immune inflammation theory, and vascular theory. Previous studies have found that KCNQ4[29], HSP70[30], FOXO3[31], Notch[32] are associated with NIHL. Most of the physiological processes involved in these genes belong to only one of these four theories. However, the STAT3 gene is involved in not only oxidative stress but also immune inflammation[33]. Therefore, to study the potential relationship between STAT3 gene polymorphism and NIHL provides a basis for the future research on the specific molecular mechanism of hearing loss, and may offer a scientific basis for the future prevention of NIHL.

STAT3 is an essential signalling protein. It was improved that STAT3 exerts a vital role in a series of reactions in which extracellular signals are transmitted into the nucleus and promote transcription reactions [23]. An animal experiment shows that STAT3 affects the occurrence and development of NIHL. In this noise-exposed mouse experiment, the mice were processed using JSI-124 before the noise exposure, a JAK2/STAT3 signal path-specific inhibitor, which allowed a significant recovery of hearing sensitivity after two weeks of exposure

to noise[23]. This indicates that STAT3 may be as a negative regulatory factor in the development of NIHL in the animal model. But the relationship between the SNP in STAT3 and NIHL need to be illuminated.

The five common SNPs of STAT3 (rs4796793、rs1053004, rs1053005,rs1053023 and rs3744483) were selected in our study. Our results showed that the rs1053023 and rs1053005 loci of STAT3 have statistical significance with NIHL. After adjusting for factors other than genotype such as age, gender, smoking and drinking, individuals carrying the C allele were more susceptible to NIHL than those carrying the T allele in the rs1053023 (OR = 1.367, 95% CI = 1.148- 1.628, *P* = 0.0001) and rs1053005 (OR = 1.37, 95% CI = 1.147-1.636, *P* = 0.001) through Logistic regression analysis. In addition, the correlation analysis of different genotypes and a series of risk factors was conducted, and observed the results of that regardless of the locus rs1053023 or rs1053005, the risk of NIHL carrying TC / CC combination genotype is higher than that of the TT genotype (rs1053023, OR = 1.545, 95% CI = 1.215-1.964, *P* = 0.001 and rs1053005, OR = 1.531, 95% CI = 1.199-1.955, *P* = 0.002). When stratifying the working years exposed to noise, it is obvious that in the group with a noise exposure period of fewer than ten years or more than 20 years, compared with people with TT genotypes, those with TC / CC genotypes Subjects had a higher risk to NIHL (adjusted: rs1053023, *P* = 0.001, *P* =0.031, respectively;rs1053005, *P* = 0.003, *P* =0.027). However, there was no statistical significance between the distribution of genotypes and the years of noise exposure within 10~20 years. The reason for this result may be related to complex regulatory functions of STAT3, which may appear as early promotion and late inhibition in immunomodulation[34].

In the dominant model, in analyzing the impact of smoking and NIHL, we found that people who smoke regularly are more likely to suffer from NIHL than non-smokers or occasional smokers. This coincides with the results of the Mofateh et al.'s study[35]. Using the same manner to analyze the relationship between drinking and NIHL, we found that not drinking but has a higher risk of NIHL, which consistent with of the Upile et al.'s found (hearing loss can be induced by alcohol even eliminating the effects of noise)[36].

The result of real-time quantitative PCR shows that STAT3 expression is higher in NIHL cases group than the control group. This confirmed that some SNPs of STAT3 might be potentially associated with NIHL. Our study is an association study showing that the C allele of the STAT3 gene increases the risk of NIHL in the Chinese population. Being consistent with the previous experiment, the results of this study show that there is a superimposed effect between the risk factors and SNPs.The interaction between drinking, smoking, and rs1053023/ rs1053005 had an association with NIHL.

At present, the mechanism of NIHL's occurrence is not precise. But many scientists agree that, when the workers are exposed to heavy noise, DNA damage of cochlear hair cells severely affect the development of NIHL [16]. When an individual was exposed to noise, the inner ear tissue can be damaged severely. An important potential cause of that is the production of reactive oxygen[16, 37, 38]. Many scientists have also proposed that apoptosis and necrosis of inner ear cells caused by metabolites or oxidative stress, as well as structural damage directly affecting the structure of the cochlea, maybe the most likely pathogenesis of NIHL[16, 39, 40].

After engaged a multitude of growth factors and cytokines, STAT3 can lead to diverse biological outcomes, such as cell growth, differentiation, and survival、and has a relatively meaningful impact on oxidative stress-

mediated tissue injury. More and more experiment research has indicated that the JAK2/STAT3 signalling pathway can effectively regulate damage due to oxidative stress, and the hydrogen peroxide-induced cell death can be effectively reduced through the activity of JAK2/STAT3 signal pathway[41, 42]. In animal models, STAT3 tyrosine 705 phosphorylation can significantly increase due to noise exposure[23]. Inhibition of JAK2 / STAT3 signalling can reduce noise exposure-induced phosphorylation of STAT3 and the expression of STAT3 target genes. The suppression of the JAK2/STAT3 pathway improves the recovery of loud sound exposure[23]. The pathogenic mechanism of NIHL may be explained by further study of STAT3 in depth.

This study is helpful to the development of NIHL's gene chip. Although this study is a relatively complete correlation study based on a large sample population, it also has some shortcomings. First, the subjects of this study come from just a few noise-exposed textile and automobile manufacturing enterprises from China, not all noise-exposed enterprises. Therefore, the research results cannot be generalized to all individuals. Furthermore, the noise exposure characteristics of different enterprises and the operation schedule cannot be the same, so the degree of hearing loss of their employees may also differ.

Conclusion

In conclusions, the rs1053023 and rs1053005 polymorphism were found to be associated with NIHL and smoking, and drinking also affect NIHL's susceptibility to some extent. Our results suggest that the rs1053023 and rs1053005 C alleles of STAT3 may play a pivotal role in the occurrence of NIHL and maybe a susceptibility biomarker of NIHL for Chinese workers. These results provide a theoretical basis for NIHL prevention and provide data support for future NIHL genetic testing.

Declarations

Acknowledgements

The authors would like to acknowledge all the participants and institutions.

Authors' contributions

GD and WB performed the statistical analysis for this study and wrote themanuscript. WB designed the study and revised the manuscript. GJ,ZS and WN were responsible for quality control of the project. GD and SD conducted the study design, carried out the experiment. ZB was responsible for data collection. All authors approved the final manuscript.

Funding

This work was supported by the Jiangsu Province's Outstanding Medical AcademicLeader program under Grant (number CXTDA2017029).

Availability of data and materials

Please contact author for data requests.

Ethics approval and consent to participate

All research subjects signed an informed consent form, and the institutional review committee of the Jiangsu Center for Disease Control and Prevention approved the study.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Author details

Dengfeng Gao[1,2]*, E-mail :gafgaodengfeng@163.com;

Boshen Wang[1,2]*, E-mail : doudouwbs@126.com;

Dawei Sun[1,2], E-mail : jssundawe@sina.com;

Ning Wang[1,2], E-mail : 18795857113@126.com;

Jiadi Guo[3], E-mail : guojiadi@163.com;

Suhao Zhang[4], E-mail : zsh1994420@163.com;

Baoli Zhu[1,2#],E-mail:zhubaoli1965@126.com.

[1] School of Public Health, Southeast University, Nanjing, Jiangsu Province, China.

[2] Institute of Occupational Disease Prevention, Jiangsu Provincial Center for Disease Prevention and Control, Nanjing, Jiangsu Province, China.

[3] School of Public Health, Nanjing Medical University, Nanjing, Jiangsu Province, China.

[4] School of Public Health, Nantong University, Nantong, Jiangsu, China.

* These authors contributed equally to this article

#Correspondence should be addressed to Baoli Zhu

Abbreviations

STAT3:Signal Transducer and Activator of Transcription 3; NIHL:Noise-induced hearing loss; qRT-PCR:Real-time Quantitative PCR; SNPs: Single nucleotide polymorphisms; PTA:Pure tone audiometry; MAF:Minimum

allele frequency; LD:Linkage disequilibrium; NCBI:National Center for Biotechnology Information; SD:Ttandard deviation; HWE:Hardy–Weinberg equilibrium; OR:Odds ratio; WHO:World Health Organization

References

1. Zhang XH, Ni YQ, Liu Y, Zhang L, Zhang MB, Fang XY, Yang ZP, Wang Q, Li H, Xia YY, et al. Screening of noise-induced hearing loss (NIHL)-associated SNPs and the assessment of its genetic susceptibility. Environ Health. 2019;18:8.
2. Carlsson PI, Van Laer L, Borg E, Bondeson ML, Thys M, Fransen E, Van Camp G. The influence of genetic variation in oxidative stress genes on human noise susceptibility. Hear Res. 2005;202(1–2):87–96.
3. Konings A, Van Laer L, Van Camp G. Genetic Studies on Noise-Induced Hearing Loss: A Review. Ear Hear. 2009;30(2):151–9.
4. Kwak SH, Nam GS, Bae SH, Jung J. Effect of Specific Retinoic Acid Receptor Agonists on Noise-Induced Hearing Loss. *International journal of environmental research and public health* 2019, 16(18).
5. Loukzadeh Z, Sani HE, Sheikhha MH, Ratki FM. Association of GST gene polymorphism and noise-induced hearing loss Running title: GST gene polymorphism and NIHL. Aims Public Health. 2019;6(4):546–53.
6. Wang B, Ding E, Shen H, Wang J, Sun K, Chen S, Han L, Zhang H, Zhu B, Xu M. Association of TagSNP in lncRNA HOTAIR with susceptibility to noise-induced hearing loss in a Chinese population. Hear Res. 2017;347:41–6.
7. Yang JH, Zhang JY, Wang XM, Wang CY, Chen JC, Qian Y, Duan ZX. Identification of functional tag single nucleotide polmorphisms within the entire CAT gene and their clinical relevance in patients with noise-induced hearing loss. Int J Clin Exp Pathol. 2015;8(3):2852–63.
8. Lin CY, Wu JL, Shih TS, Tsai PJ, Sun YM, Guo YL. Glutathione S-transferase M1, T1, and P1 polymorphisms as susceptibility factors for noise-induced temporary threshold shift. Hearing research. 2009;257(1–2):8–15.
9. Konings A, Van Laer L, Pawelczyk M, Carlsson PI, Bondeson ML, Rajkowska E, Dudarewicz A, Vandeveldel A, Fransen E, Huyghe J, et al. Association between variations in CAT and noise-induced hearing loss in two independent noise-exposed populations. Hum Mol Genet. 2007;16(15):1872–83.
10. Ishikawa K, Naito T, Nishio SY, Iwasa YI, Nakamura KI, Usami S, Ichimura K. A Japanese family showing high-frequency hearing loss with KCNQ4 and TECTA mutations. Acta Oto-Laryngol. 2014;134(6):557–63.
11. Pawelczyk M, Van Laer L, Fransen E, Rajkowska E, Konings A, Carlsson PI, Borg E, Van Camp G, Sliwinska-Kowalska M. Analysis of Gene Polymorphisms Associated with K + Ion Circulation in the Inner Ear of Patients Susceptible and Resistant to Noise-induced Hearing Loss. Ann Hum Genet. 2009;73:411–21.
12. Konings A, Van Laer L, Wiktorek-Smagur A, Rajkowska E, Pawelczyk M, Carlsson PI, Bondeson ML, Dudarewicz A, Vandevelde A, Fransen E, et al. Candidate Gene Association Study for Noise-induced Hearing Loss in Two Independent Noise-exposed Populations. Ann Hum Genet. 2009;73:215–24.

13. Yang M, Tan H, Yang QL, Wang F, Yao HL, Wei QY, Tanguay RM, Wu TC. Association of hsp70 polymorphisms with risk of noise-induced hearing loss in Chinese automobile workers. Cell Stress Chaperones. 2006;11(3):233–9.
14. Liu YM, Li XD, Guo X, Liu B, Lin AH, Rao SQ. Association between polymorphisms in SOD1 and noise-induced hearing loss in Chinese workers. Acta Oto-Laryngol. 2010;130(4):477–86.
15. Wang SL, Yu LG, Liu RP, Zhu WZ, Gao WM, Xue LP, Jiang X, Zhang YH, Yi D, Chen D, et al. Gene-Gene Interaction of GJB2, SOD2, and CAT on Occupational Noise-induced Hearing Loss in Chinese Han Population. Biomed Environ Sci. 2014;27(12):965–8.
16. Henderson D, Bielefeld EC, Harris KC, Hu BH. The role of oxidative stress in noise-induced hearing loss. Ear Hear. 2006;27(1):1–19.
17. Yu H, Lee H, Herrmann A, Buettner R, Jove R. Revisiting STAT3 signalling in cancer: new and unexpected biological functions. Nature reviews Cancer. 2014;14(11):736–46.
18. Sinden D, Badgett M, Fry J, Jones T, Palmen R, Sheng X, Simmons A, Matunis E, Wawersik M. Jak-STAT regulation of cyst stem cell development in the Drosophila testis. Dev Biol. 2012;372(1):5–16.
19. Mali SB. Review of STAT3 (Signal Transducers and Activators of Transcription) in head and neck cancer. Oral Oncol. 2015;51(6):565–9.
20. Fathi N, Rashidi G, Khodadadi A, Shahi S, Sharifi S. STAT3 and apoptosis challenges in cancer. Int J Biol Macromol. 2018;117:993–1001.
21. Siddiquee KAZ, Turkson J. STAT3 as a target for inducing apoptosis in solid and hematological tumors. Cell Res. 2008;18(2):254–67.
22. Hertzano R, Montcouquiol M, Rashi-Elkeles S, Elkon R, Yucel R, Frankel WN, Rechavi G, Moroy T, Friedman TB, Kelley MW, et al. Transcription profiling of inner ears from Pou4f3(ddl/ddl) identifies Gfi1 as a target of the Pou4f3 deafness gene. Hum Mol Genet. 2004;13(18):2143–53.
23. Wilson T, Omelchenko I, Foster S, Zhang Y, Shi X, Nuttall AL. JAK2/STAT3 inhibition attenuates noise-induced hearing loss. PloS one. 2014;9(9):e108276.
24. Wu YY, Ni JT, Qi MJ, Cao CJ, Shao YX, Xu LW, Ma HY, Yang L. Associations of genetic variation in CASP3 gene with noise-induced hearing loss in a Chinese population: a case-control study. Environ Health. 2017;16:8.
25. Adelman C, Weinberger JM, Kriksunov L, Sohmer H. Effects of furosemide on the hearing loss induced by impulse noise. *Journal of Occupational Medicine and Toxicology* 2011, 6.
26. Olusanya BO, Neumann KJ, Saunders JE. The global burden of disabling hearing impairment: a call to action. Bull World Health Organ. 2014;92(5):367–73.
27. Abreu-Silva RS, Rincon D, Horimoto A, Sguillar AP, Ricardo LAC, Kimura L, Batissoco AC, Auricchio M, Otto PA, Mingroni-Netto RC. The search of a genetic basis for noise-induced hearing loss (NIHL). Ann Hum Biol. 2011;38(2):210–8.
28. Sliwinska-Kowalska M, Davis A. Noise-induced hearing loss. Noise Health. 2012;14(61):274–80.
29. Guo H, Ding E, Sheng R, Cheng J, Cai W, Guo J, Wang N, Zhang H, Zhu B. Genetic variation in KCNQ4 gene is associated with susceptibility to noise-induced hearing loss in a Chinese population. Environmental toxicology pharmacology. 2018;63:55–9.

30. Lei S, Huang L, Liu YQ, Xu LW, Wang DH, Yang L. Association between polymorphisms of heat shock protein 70 genes and noise-induced hearing loss: A meta-analysis. PloS one. 2017;12(11):16.
31. Guo H, Ding E, Bai Y, Zhang H, Shen H, Wang J, Song X, Cai W, Guo J, Zhu B. Association of genetic variations in FOXO3 gene with susceptibility to noise induced hearing loss in a Chinese population. PloS one. 2017;12(12):e0189186.
32. Ding E, Liu J, Shen H, Gong W, Zhang H, Song H, Zhu B. Notch polymorphisms associated with sensitivity of noise induced hearing loss among Chinese textile factory workers. BMC medical genetics. 2018;19(1):168.
33. Yu S, Zhao G, Han F, Liang W, Jiao Y, Li Z, Li L. Muscone relieves inflammatory pain by inhibiting microglial activation-mediated inflammatory response via abrogation of the NOX4/JAK2-STAT3 pathway and NLRP3 inflammasome. Int Immunopharmacol. 2020;82:106355.
34. Yu H, Pardoll D, Jove R. STATs in cancer inflammation and immunity: a leading role for STAT3. Nature reviews Cancer. 2009;9(11):798–809.
35. Mofateh M, Karimi Q, Hosseini MH, Sharif-Zadeh GR. Effect of smoking on hearing loss in refractory's factory male worker with occupational noise exposure in Iran. J Pak Med Assoc. 2017;67(4):605–8.
36. Upile T, Sipaul F, Jerjes W, Singh S, Nouraei SAR, El Maaytah M, Andrews P, Graham J, Hopper C, Wright A. The acute effects of alcohol on auditory thresholds. BMC Ear Nose Throat Disord. 2007;7:4.
37. Ohlemiller KK, Wright JS, Dugan LL. Early elevation of cochlear reactive oxygen species following noise exposure. Audiology Neuro-Otology. 1999;4(5):229–36.
38. Evans P, Halliwell B: Free radicals and hearing - Cause, consequence, and criteria. In: *Ototoxicity: Basic Science and Clinical Applications.* Edited by Henderson D, Salvi RJ, Quaranta A, McFadden SL, Burkard RF, vol. 884; 1999: 19–40.
39. Le Prell CG, Dolan DF, Schacht J, Miller JM, Lomax MI, Altschuler RA. Pathways for protection from noise induced hearing loss. Noise Health. 2003;5(20):1–17.
40. Le Prell CG, Yamashita D, Minami SB, Yamasoba T, Miller JM. Mechanisms of noise-induced hearing loss indicate multiple methods of prevention. Hear Res. 2007;226(1–2):22–43.
41. Duan WX, Yang Y, Yi W, Yan JJ, Liang ZX, Wang N, Li Y, Chen WS, Yu SQ, Jin ZX, et al: New Role of JAK2/STAT3 Signaling in Endothelial Cell Oxidative Stress Injury and Protective Effect of Melatonin. *PLoS One* 2013, 8(3).
42. Yu HM, Zhi JL, Cui Y, Tang EH, Sun SN, Feng JQ, Chen PX. Role of the JAK-STAT pathway in protection of hydrogen peroxide preconditioning against apoptosis induced by oxidative stress in PC12 cells. Apoptosis. 2006;11(6):931–41.

Tables

Table 1. Demographic characteristics and clinical feature.

		case	control		
Variables		n=609	n=611	x^2/t	P
Sex					
	male	572	575	0.018*	0.892
	female	37	36		
Smoking					
	yes	346	316	3.753*	0.153
	ever	26	35		
	never	236	259		
Drinking					
	yes	215	194	4.936*	0.085
	erer	57	80		
	never	336	336		
Age		40.27±7.440	40.04±8.046	0.529**	0.597
Work time with noise		18.44±8.66	17.53±8.84	1.810**	0.071
Expose level with noise (dB)		87.15±7.73	88.07±7.72	-1.839**	0.066
threshold		37.50±12.39	15.58±4.76	36.511**	**0.000**

* Two-sided χ^2 test; ** Students' t-test.

The bold in this table means P value < 0.05.

Table2. Distribution of three polymorphisms and the association with NIHL

Genetic models	Genotypes		case	control		P[a]	Adjusted OR (95% CI)[b]
			n=609	n=611			
rs4796793		n=590	%	n=585	%		
Codominant	CC	220	37.3	250	42.7		1.00（Ref.）
	CG	287	48.6	267	45.6	0.111	1.239(0.966-1.589)
	GG	83	14.1	68	11.6	0.081	1.498(1.022-2.195)
Dominant	CC	220	37.3	250	42.7		1.00（Ref.）
	CG/GG	370	62.7	335	57.3	0.057	1.280（1.012-1.621）
Recessive	CC/CG	507	85.9	517	88.4		1.00（Ref.）
	GG	83	14.1	68	11.6	0.211	1.341（0.944-1.906）
Alleles	C	727	61.6	767	65.6		1.00（Ref.）
	G	453	38.4	403	34.4	0.047	1.218（1.028-1.444）
rs1053023		n=592		n=597			
Codominant	TT	242	40.9	299	50.1		1.00（Ref.）
	TC	287	48.5	252	42.2	**0.005**	1.484（1.163-1.894）
	CC	63	10.6	46	7.7	**0.013**	1.883（1.226-2.894）
Dominant	TT	242	40.9	299	50.1		1.00（Ref.）
	TC/CC	350	59.1	298	49.9	**0.001**	1.516（1.201-1.913）
Recessive	TT/TC	529	89.4	551	92.3		1.00（Ref.）
	CC	63	10.6	46	7.7	0.079	1.466（0.981-2.190）
Alleles	T	771	65.1	850	71.2		1.00 (Ref.)
	C	413	34.9	344	28.8	**0.001**	1.367

							1.148-1.628
rs1053005		n=577		n=575			
Codominant	TT	236	40.9	287	49.9		1.00 (Ref.)
	TC	279	48.4	244	42.4	**0.008**	1.470 1.148-1.883
	CC	62	10.7	44	7.7	**0.012**	1.940 1.251-3.009
Dominant	TT	236	40.9	287	49.9		1.00 (Ref.)
	TC/CC	341	59.1	288	50.1	**0.002**	1.509 1.191-1.912
Recessive	TT/TC	515	89.3	531	92.3		1.00 (Ref.)
	CC	62	10.7	44	7.7	0.069	1.501 0.998-2.259
Alleles	T	751	65.1	818	71.1		1.00 (Ref.)
	C	403	34.9	332	28.9	**0.002**	1.370 1.147-1.636
rs1053004		n=607		n=601			
Codominant	AA	222	36.6	245	40.8		1.00 (Ref.)
	AG	303	49.9	287	47.8	0.218	1.205 0.942-1.540
	GG	82	13.5	69	11.5	0.148	1.426 0.978-2.081
Dominant	AA	222	36.6	245	40.8		1.00 (Ref.)
	AG/GG	385	63.4	356	59.2	0.135	1.230 0.973-1.554
Recessive	AA/AG	525	86.5	532	88.5		1.00 (Ref.)
	GG	82	13.5	69	11.5	0.287	1.246 0.882-1.759
Alleles	A	747	61.5	777	64.6		1.00 (Ref.)
	G	467	38.5	425	35.4	0.113	1.174 0.994-

							1.387）
rs3744483		n=609		n=711			
Codominant	TT	250	41.1	307	43.2		1.00 (Ref.)
	TC	291	47.8	357	50.2	0.993	1.470 （1.155-1.870）
	CC	68	11.2	47	6.6	**0.005**	1.982 （1.301-3.021）
Dominant	TT	250	41.1	307	43.2		1.00 (Ref.)
	TC/CC	359	58.9	404	56.8	0.435	1.520 （1.208-1.913）
Recessive	TT/TC	541	88.8	664	93.4		1.00 (Ref.)
	CC	68	11.2	47	6.6	**0.003**	1.554 （1.048-2.302）
Alleles	T	791	64.9	971	68.3		1.00 (Ref.)
	C	427	35.1	451	31.7	0.069	1.387 （1.167-1.648）

The bold in this table means P value < 0.05.

a: Two-sided χ^2 test.

b: Adjusted for age, sex, smoking, drinking in logistic regression model.

Table3. Association between rs1053023 and NIHL in a dominant model.

Variables	TT(case/control)		TC/CC(case/control)		P[a]	Adjusted OR(95% CI)[b]
	n	%	n	%		
Age						
≤40	110/142	18.9/24.4	178/153	30.5/26.2	0.015	1.548 (1.107-2.166)
>40	132/157	21.8/25.9	172/145	28.4/23.9	0.0035	1.492 (1.078-2.065)
Sex						
male	225/281	20.1/25.1	332/281	29.7/25.1	**0.001**	1.545 (1.215-1.964)
female	17/18	24.3/25.7	18/17	25.7/24.3	0.811	1.133 (0.428-2.996)
Smoking						
yes	142/161	22.1/25.0	195/145	30.3/22.6	**0.008**	1.595 (1.163-2.187)
ever	6/17	10.2/28.8	18/18	30.5/30.5	0.068	3.080 (0.925-10.295)
never	94/121	19.4/24.9	136/134	28.0/27.6	0.145	1.312 (0.913-1.886)
Drinking						
yes	105/97	26.8/24.7	100/90	25.5/23.0	0.897	1.010 (0.676-1.509)
ever	5/40	3.6/29.2	52/40	38.0/29.2	**0.000**	10.55 (3.803-29.287)
never	132/162	20.1/24.6	197/167	29.9/25.4	**0.019**	1.467 (1.076-2.002)
Work time with noise						
≤10	41/78	13.3/25.3	102/87	33.1/28.2	**0.001**	2.448 (1.498-3.998)

10< ≤20	98/107	24.0/26.2	101/102	24.8/25.0	0.694	1.043 (0.703-1.549)
>20	103/114	21.8/24.1	147/109	31.1/23.0	**0.031**	1.678 (1.151-2.447)
Expose level with noise (dB)						
≤87	125/86	27.6/19.0	137/105	30.2/23.2	0.572	1.955 (1.436-2.662)
>87	117/128	24.8/27.2	107/119	22.7/25.3	0.929	0.966 (0.670-1.393)

The bold in this table means P value < 0.05.

a: Two-sided χ^2 test..

b: Adjusted for age, sex, smoking, drinking in logistic regression model.

Table4. Association between rs1053005 and NIHL in a dominant model.

Variables	TT(case/control)		TC/CC(case/control)		P[a]	Adjusted OR(95% CI)[b]
	n	%	n	%		
Age						
≤40	108/134	19.0/23.6	176/151	30.9/26.5	**0.030**	1.499 (1.066-2.106)
>40	128/153	22.0/26.2	165/137	28.3/23.5	**0.028**	1.526 (1.095-2.125)
Sex						
male	218/269	20.2/24.9	322/272	29.8/25.2	**0.002**	1.531 (1.199-1.955)
female	18/18	25.4/25.4	19/16	26.8/22.5	0.718	1.131 (0.434-2.948)
Smoking						
yes	138/156	22.2/25.0	186/143	29.9/23.0	**0.017**	1.545 (1.121-2.129)
ever	6/17	10.0/28.3	19/18	31.7/30.0	0.054	3.414 (1.031-11.305)
never	92/114	19.7/24.4	135/126	28.9/27.0	0.129	1.341 (0.926-1.942)
Drinking						
yes	102/93	27.1/24.7	95/86	25.3/22.9	0.972	0.995 (0.662-1.497)
ever	5/39	3.6/28.5	52/41	38.0/29.9	**0.000**	10.13 (3.645-28.177)
never	129/155	20.3/24.3	193/160	30.3/25.1	**0.020**	1.468 (1.070-2.014)
Work time with noise						
≤10	40/73	13.2/24.2	100/89	33.1/29.5	**0.003**	2.244 (1.367-3.684)

10< ≤20	95/105	23.8/26.3	100/99	25.1/24.8	0.583	1.088 (0.729-1.623)
>20	101/109	22.4/24.2	141/100	31.3/22.2	**0.027**	1.761 (1.194-2.599)
Expose level with noise (dB)						
≤87	124/80	28.4/18.3	134/98	30.7/22.5	0.521	0.883 (0.601-1.299)
>87	112/123	24.8/27.3	101/115	22.4/25.5	0.848	0.921 (0.631-1.344)

The bold in this table means P value < 0.05.

a: Two-sided χ^2 test.

b: Adjusted for age, sex, smoking, drinking in logistic regression model.

Table 5. Primers used for real-time quantitative PCR experiments

Primer sequence	Sequence (5′ 3′)
Primer	AACTTGGTCTTCAGGTATGGG
	CCTGGTGTCTCCACTGGTCTA

Figures

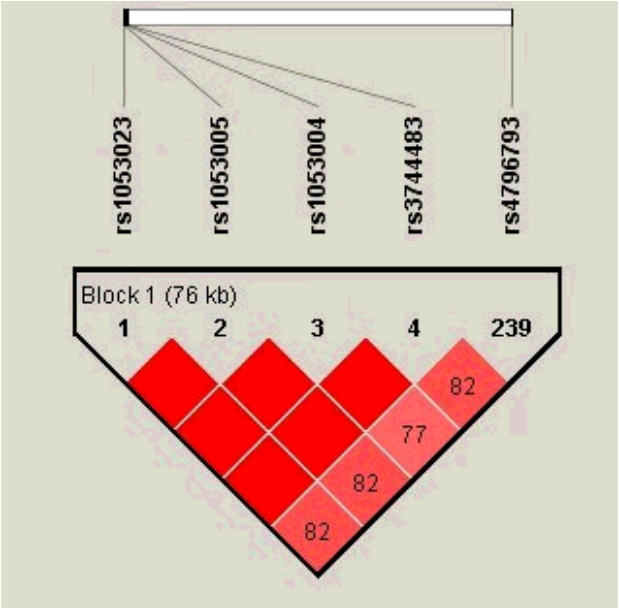

Figure 1

Linkage disequilibrium pattern of the five SNPs.

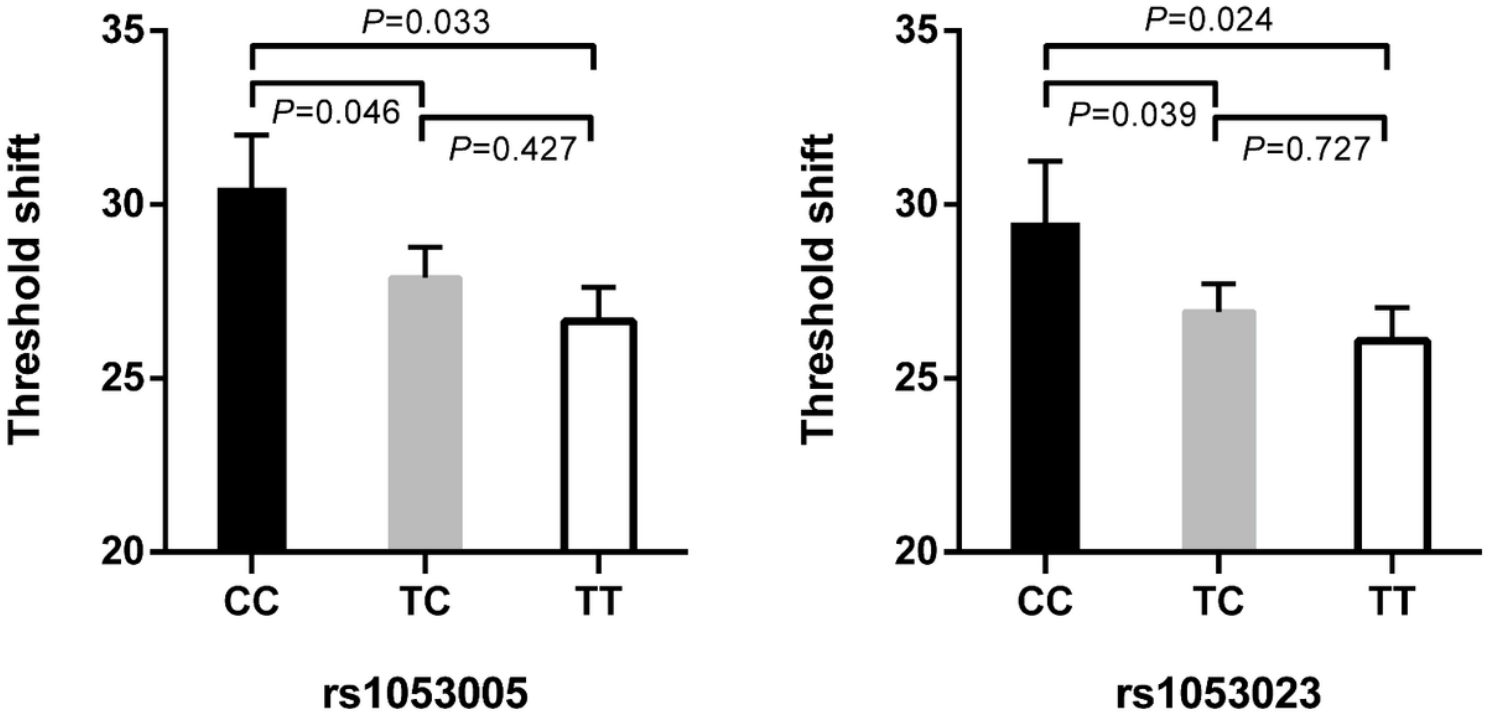

Figure 2

Comparison of high-frequency hearing threshold shift of rs1053005 and rs1053023 genotypes in all subjects. Data are presented as mean ± SE and analyzed by ANOVA.

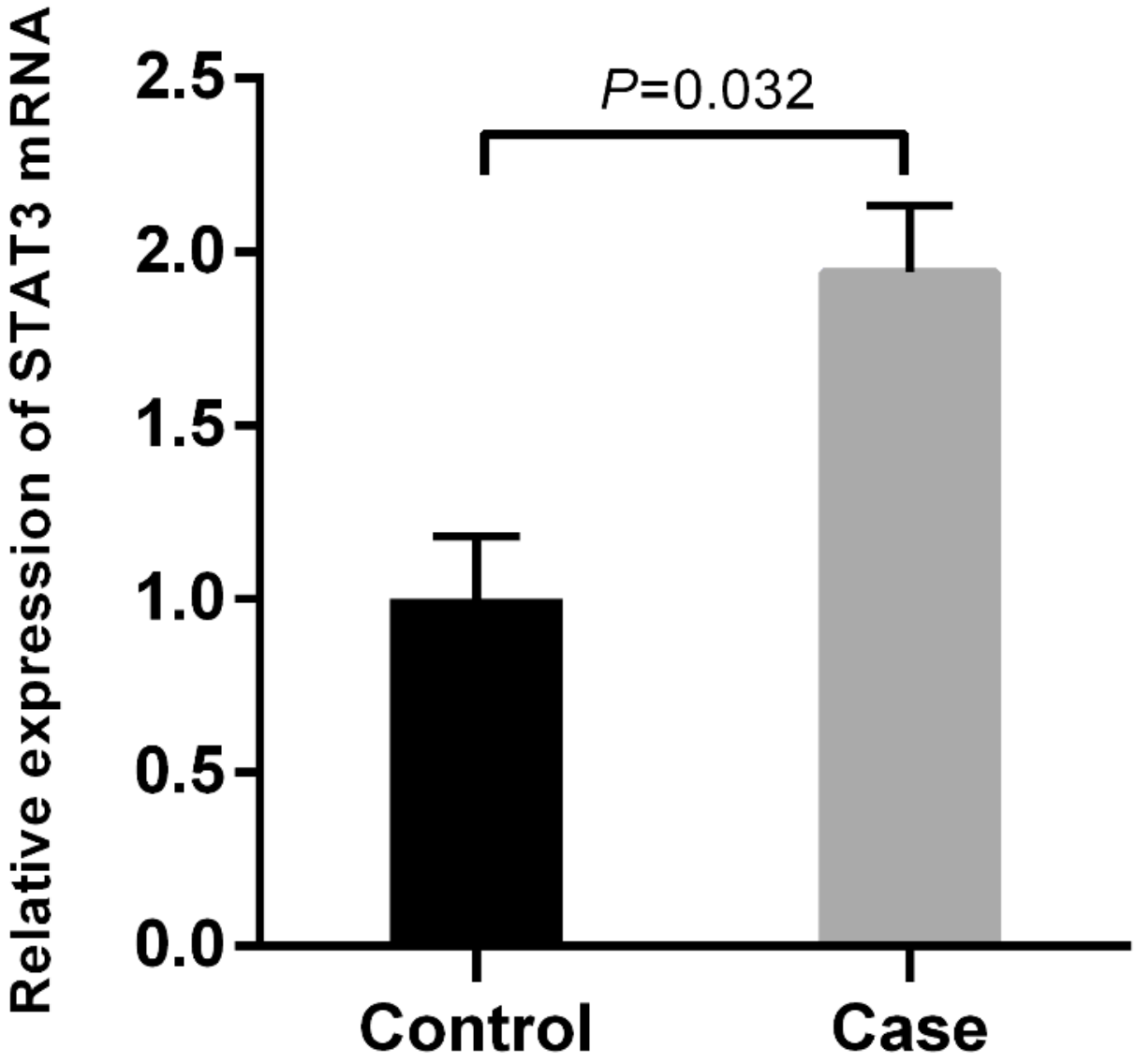

Figure 3

Quantitative Real-time PCR experiment of STAT3 in selected population.