Plasma Metabolomic Profiling in Workers With Noise-induced Hearing Loss: A Pilot Study

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Abstract

Noise-induced hearing loss (NIHL) remains a leading occupational related disease and is a serious public health problem. Hence, the identification of potential biomarkers for NIHL prevention and diagnosis has become an urgent work. To discover potential metabolic biomarkers of NIHL, plasma metabolomics analysis among 62 NIHL patients and 62 controls was performed using ultrahigh performance liquid chromatography-mass spectrometry (UPLC/MS). Orthogonal partial least square-discriminant analysis (OPLS-DA) model was applied to distinguish metabolite profile alterations in plasma samples between the two groups. The alterations in autophagy pathway were in accordance with previous published studies, therefore, three autophagy-related genes (PI3K, AKT and ATG5) were selected and mRNA levels were detected by RT-qPCR analysis in peripheral white blood cells (WBCs) samples. Compared to the control group, 20 identified plasma metabolites were significantly altered in NIHL patients. Meanwhile, a total of seven metabolic pathways were enriched, including glycerophospholipid metabolism, glycosylphosphatidylinositol (GPI)-anchor biosynthesis, autophagy, choline metabolism, alpha-linolenic acid metabolism and linoleic acid metabolism, and retrograde endocannabinoid signaling pathway. Furthermore, the results indicated that the mRNA levels of three autophagy-related genes (PI3K, AKT and ATG5) were significantly decreased in NIHL cases compared with controls. Taken together, our current study firstly provides evidence that the identified aberrantly altered metabolites might be potential biomarkers of NIHL for noise-exposed workers. In addition, autophagy pathway may be involved in the occurrence and development of NIHL.

Introduction

Occupational noise, a common harmful factor that seriously affects the health of workers in the field of occupational health. Noise-induced hearing loss (NIHL) is one of the worst adverse health effect induced by occupational noise exposure in workplaces (Masterson et al. 2016). The World Health Organization (WHO) has reported that about ten percent of the world's population is exposed to high level of noise and is at risk of developing NIHL (Basner et al. 2014). Nowadays, NIHL is an urgent health problem and has a strong impact on social economy and human health. According to previous study, NIHL is one of the leading occupational related disease in China, accounting for approximately one-sixth of the annual increase of occupational disease (Miao et al. 2019). Although a number of studies were conducted, the specific pathogenesis underlying NIHL has still not been entirely illustrated. Therefore, it is of great significance to search new and potential biomarkers and further explore the mechanism underlying NIHL.

Metabolites in human body fluids represent the end products of metabolic pathways and reflect the final consequences of organisms in response to environmental stimulation and disease stress (Huang et al. 2018). More importantly, these endogenous metabolic changes can indicate the direct biological response to internal and external stressors, such as environmental exposure, disease and nutritional imbalances (Wang et al. 2017). Now, metabolomics has become a powerful and effective platform to identify extensive biomolecule closely correlated with environmental factors and health effect (Chen et al. 2019, Huang et al. 2018). Thus, identification of metabolic biomarkers could help to better understand the possible molecular mechanism of adverse health effects induced by harmful exposure factors and may further contribute to high-risk individuals' identification (Floegel et al. 2013).

It has been reported that a key factor contributing to NIHL is oxidative stress damage to body's sensory hair cells (Yuan et al. 2015). Oxidative stress is a state of imbalance between oxidation and antioxidant defenses, thus resulting in oxidative damage (Sies 1997). Available studies showed that reactive oxygen species (ROS) may play an essential role in regulating cellular stress and defense pathways. Excessive production of ROS was thought to be a

key pathological mechanism involving in the process of inner ear injury, such as exposure to noise and ototoxic drug therapy (Ohlemiller et al. 1999, Yamashita et al. 2004). In addition, related studies found that ROS has the capacity to induce cell defense pathways like autophagy. Autophagy is an important defense process that could pass impaired cell components to lysosomes degradation (Wang &Klionsky 2003, Yang &Klionsky 2009). In our previous studies, we found that inflammation-related genes polymorphisms associated with the susceptibility to NIHL, revealing inflammation is an essential stress to the pathogenesis of noise-induced cochlear impairment (Miao et al. 2021a, Miao et al. 2021b). Nevertheless, the related metabolic profiles in occupational noise-exposed workers are still not clear and whether autophagy is involved in the development of NIHL has yet to be established and need to be further explored.

In this study, metabolomics of plasma samples from occupational noise-exposed workers with hearing loss and normal hearing controls was performed to identify potential metabolic biomarkers and pathways participating in the process of NIHL development. We further conducted quantitative PCR reaction to determine the mRNA expression levels of a few crucial genes in autophagy pathway.

Materials And Methods

Subjects

The study subjects were the workers who exposed to occupational noise in the factories. According to requests, all workers were requested to receive annual occupational health examination, such as general physical examination, peripheral blood collection and pure-tone audiometry (PTA) test. The questionnaire survey was performed to collect important information of subjects, including general family history of disease, personal disease history, smoking and alcohol consumption, and drug use history. In this study, the criteria for inclusion of study subjects were: (1) workers who exposed to occupational noise for more than one year; (2) workers only exposed to occupational noise; (3) Chinese Han workers. Nevertheless, the exclusion criteria were as follows: (1) workers who had family history of deafness; (2) workers who carried diseases that could affect normal hearing (e.g., otitis media, tinnitus, and skull trauma); (3) workers had recently treatment with ototoxic drugs (e.g., aminoglycosides, quinolones, aspirin) that damages the normal function of the inner ear; (4) workers had metabolic diseases (e.g., diabetes, hypertension, hyperlipidemia, etc.).

This study was approved by the Ethics Committee of Zhongda Hospital Affiliated to Southeast University and written informed consent was also acquired from all study subjects.

Noise exposure evaluation

Exposure level of noise was estimated according to equivalent continuous dB(A)-weighted sound pressure levels (L_{Aeq}, 8 h) with the TES 1350A sound level meter (TES, Taiwan). Cumulative noise exposure (CNE) was used to reflect the true individual noise exposure level based on L_{Aeq}.

Pure-tone audiometric examination

After stopping noise exposure for over 12 h, each study subject has to accept the PTA examination conducted by professional doctor using Voyager 522 audiometer (Madsen, Taastrup, Denmark) at frequencies of 0.5, 1, 2, 3, 4, and 6 kHz in a noise-proof room. Referring to GB/T7582-2004, the obtained raw data were adjusted by sex and age.

Selection of NIHL cases and normal hearing controls

Referring to the Chinese National Occupational Health Standard (GBZ49-2007), NIHL cases of this current study were define as occupational noise-exposed workers with binaural high frequencies (3, 4 and 6 kHz) hearing threshold level more than 25dB (A). Nevertheless, those with binaural high frequencies hearing threshold level less than 25dB (A) were included as the normal hearing controls. Totally, 124 subjects including 62 NIHL cases and 62 normal hearing controls were recruited in this study.

Chemicals and Reagents

Liquid chromatography grade methanol and acetonitrile were purchased from Merck (Darmstadt, Germany). Both formic acid and ammonium formate were purchased from Thermo Fisher Scientific (Waltham, USA). Ultrapure water was prepared by Milli-Q water purify system (Millipore, USA).

Plasma sample collection and preparation

Considering food intake and drinking may result in the alteration of human metabolome, morning peripheral blood (collected after 12 h-fasting) is collected for plasma collection. Two milliliters of peripheral venous blood were collected from each study subject and transferred to tubes containing ethylenediaminetetraacetic acid. Plasma samples were isolated and centrifuged at 3500 rpm at room temperature for 15 min. Subsequently, all plasma samples were stored at -80 °C until metabolomics analysis.

All collected plasma samples were thawed at 4 °C and then vortexed for 20 s. A 100 μL aliquot of plasma sample was mixed using three times volume of methanol for protein precipitation. Then, the mixture was further vortexed for 30 s and was centrifuged at 13000 rpm at 4 °C for 20 min. The liquid supernatant was obtained for centrifugation at 13000 rpm at 4 °C for 20 min. Finally, a total of 20 μL aliquot of the supernatant was transferred into a sample vial for metabolomics analysis.

Chromatographic and mass spectrometric analysis

Metabonomic profiles of plasma samples from occupational noise-exposed workers was performed by ACQUITY Ultra Performance Liquid Chromatography (UPLC) (Waters, Milford, USA) system equipped with AB SCIEX Triple TOF 5600 System (AB SCIEX, Framingham, USA).

Chromatographic separation was implemented on an ACQUITY UPLC BEH C8 column (2.1 mm × 100 mm × 1.7 μm, Waters, Milford, USA). For positive ion mode, the mobile phase was composed of water with 0.1% formic acid (A) and acetonitrile (B). For negative ion mode, the mobile phase was 5 mM ammonium formate aqueous solution (C) and acetonitrile (D). The procedures of gradient elution were as follows: 5% solution B for 0-0.5 min, 5-20% solution B for 0.5-2 min, 20-25% solution B for 2-4 min, 25-60% solution B for 4-10 min, and 60-100% solution B for 10-15 min, 100% solution B for 15-16 min, and 5% solution B for 16-19 min. The delivery flow rate was set at 0.3 mL/min. Besides, the injection volume was 5 μL. All analyzed samples were kept at 4 °C, and the temperature of column was set at 40 °C.

Mass spectrometry (MS) was conducted with AB SCIEX Triple TOF 5600 System (AB SCIEX, Framingham, USA) coupled with electrospray ionization source. The parameters were as follows: collision energy, 35 V/-35 V; declustering potential, 80 V/-80 V; ion spray voltage, 5000 V/-4500 V; pressure of nebulizer gas, 50 psi; and curtain gas, 35 psi. Besides, the interface heater temperature was 500 °C and 550 °C, respectively. The scanning range of data acquisition was 50-1200 m/z.

Data processing and analysis

Firstly, the original data were processed using Progenesis QI software for baseline removing, peak identification, peak alignment and integration, and retention time adjustment. Then, a data matrix composed of retention time, mass-to-charge ratio, intensity of peak and information of sample was performed for further statistical analyses. For each plasma sample, the peak intensity corresponding to metabolite was further adjusted with the total intensity of peak of sample.

SIMCA-P 14.1 (Umetrics, Umea, Sweden) was applied for multivariate statistical analyses. Both principal component analysis (PCA) and orthogonal partial least square-discriminant analysis (OPLS-DA) were applied for distinguishing normal hearing controls and NIHL patients after unit variance (UV) scaling. The metabolites with variable importance of projection (VIP) values > 1 calculated by SIMCA-P were considered to be the potential biomarkers. Furthermore, a heatmap of metabolites was generated with Multi Experiment Viewer (MEV) software.

Some further analyses were also conducted in this study. MetaboAnalyst was applied for related metabolic pathway analysis (http://www.metaboanalyst.ca). Pathway plots were based on the Kyoto Encyclopedia of Genes and Genomes (KEGG) database. Gene Set Enrichment Analysis Software (GSEA, Broad Institute, Cambridge, MA, USA) was used to gene enrichment analysis. Furthermore, to characterize the gene functions, Human metabolome database (HMDB) was performed.

RNA extraction and RT-qPCR analysis

TRIZOL reagent (Invitrogen, Carlsbad, CA) was employed for RNA extraction. Double-stranded cDNA was synthesized with Takara Prime Script RT Reagent Kit (Takara Bio Inc., Clontech, Japan) referring to the instructions. Subsequently, RT-qPCR was conducted with SYBR Green real-time PCR kits (Toyobo, Osaka, Japan). The following primers were used for mRNA expression levels detection: PI3K, forward: 5′-CCACGACCATCATCAGGTGAA-3′, reverse: 5′-CCTCACGGAGGCATTCTAAAGT-3′; AKT, forward: 5′-GCAAGGTGATCCTGGTGAA-3′, reverse: 5′-TCGTGGGTCTGGAAAGAGTA-3′; ATG5, forward: 5′-AAAGATGTGCTTCGAGATGTGT-3′, reverse: 5′-CACTTTGTCAGTTACCAACGTCA-3′; and *β-actin*, forward: 5′-CTACCTCATGAAGATCCTCACCGA-3′, reverse: 5′-TTCTCCTTAATGTCACGCACGATT-3′.

Statistical analyses

Statistical analysis was carried out to ensure the potential metabolites significantly changed between NIHL cases and controls by using paired non-parametric test on MATLAB software (MathWorks). Otherwise, statistical analysis was conducted with the SPSS 23.0 software (SPSS, Chicago, Illinois, USA). The statistical significance criterion was set with a two-sided P value < 0.05.

Results

Characteristics of subjects

A total of 62 NIHL cases and 62 healthy controls were recruited in this study. In terms of age, sex, time with noise exposure, habit of smoking and drinking, systolic blood pressure (SBP) and diastolic blood pressure (DBP) levels, and exposure level with noise, no significant differences were found between the case group and control group (P > 0.05). Nevertheless, a significant difference of the high frequency hearing threshold were observed between two groups. The results showed that NIHL patients had a significantly higher hearing threshold of high frequency in both ears (53.24 ± 13.01) compared with the controls (18.73 ± 3.96; P < 0.001). The information of all study subjects are summarized in Table 1.

Plasma metabonomic profiling of NIHL

The non-targeted metabolomic profile was explored in 124 plasma samples obtained from 62 NIHL patients and 62 controls using UPLC-Q-TOF/MS with ESI positive ion mode and negative ion mode. Due to the metabolites detected by positive ion mode and negative ion mode were complementary, we combined the data obtained from two modes into a matrix for analysis. After removing missing values > 50% ion peaks, 6777 ion peaks in positive ion mode and 5404 ion peaks in negative ion mode were obtained. A total of 4009 metabolites (2277 in positive ion mode and 1732 in negative ion mode) were detected by accurate mass, fragmentation patterns and retention time. Univariate statistical analyses were further performed for all metabolite to determine the changes of plasma metabolome between two groups. Based on OPLS-DA model, an obviously separation was shown in NIHL patients and normal hearing (Figure 1).

Identification of changed endogenous metabolites

Paired non-parametric test was conducted to identify significant differential metabolites for plasma between two groups. Totally, 207 differential metabolites ($P < 0.05$) were identified, including 136 up-regulated and 71 down-regulated metabolites. We further explored which significant differential metabolites contributing to the differences described above by setting comparatively low stringency ($P < 0.05$ and VIP > 1). In this way, it showed that 59 metabolites were significantly alterations in NIHL patients compared with the controls. Through further examining the original data and feature of ion peaks, 20 metabolites were identified and considered potential biomarkers, including 12 up-regulated and 8 down-regulated metabolites (Table 2). Besides, a visual heat map was generated based on the 20 plasma differential metabolites, showing a considerable difference between NIHL and controls (Fig. 2). The up-regulated metabolites were organic acids, including homodeoxycholic acid, quinolacetic acid, etc. Nevertheless, most of down-regulated metabolites were lipids, such as glycerophospholipids and saccharolipids, etc. KEGG pathway analysis showed that seven metabolic pathways were enriched, including glycerophospholipid metabolism, glycosylphosphatidylinositol (GPI)-anchor biosynthesis, autophagy, choline metabolism, alpha-linolenic acid metabolism and linoleic acid metabolism, and retrograde endocannabinoid signaling pathway, suggesting these pathways may be involved in the development of NIHL (Table 3).

Autophagy related-gene expression in NIHL patients and controls

Among the identified differentially regulated metabolites, PE(15:0/20:2(11Z,14Z)), also known as Phosphatidylethanolamine in HMDB, was found to be reduced in plasma samples in NIHL patients, which was involved in autophagy metabolism pathway. In addition, the another metabolite, namely, PC(15:0/18:1(11Z)) (called Phosphatidylcholine in HMDB) was also decreased in plasma samples of NIHL patients and was involved in autophagy metabolism pathway. To explore the biological effects of noise exposure on autophagy pathway in NIHL patients, three autophagy-related genes (PI3K, AKT, and ATG5) were included and the expression levels were determined in peripheral white blood cells (WBCs) of NIHL patients and controls. Results indicated that individuals with NIHL had significantly lower levels of PI3K, AKT, and ATG5 than those with normal hearing (Fig. 3).

Discussion

NIHL is one of the serious harmful health effects induced by high-intensity noise exposure and has become a leading occupation-related disease in the world (Miao et al. 2019). In the present study, we compared the plasma metabolomic characteristics of NIHL patients with normal hearing controls by using non-targeted metabolomics, which provides a theoretical basis for further exploring the pathogenesis of harmful effects induced by noise. Totally,

twenty significantly changed metabolites were identified, revealing disturbances of a variety of biological pathways in the development of NIHL. We performed three autophagy related-gene expression levels by conducting RT-qPCR and found that PI3K, AKT, and ATG5 were significantly downregulated in NIHL patients compared with controls, suggesting that autophagy pathway plays an essential role in development of NIHL. Therefore, our study firstly provides a new perspective to understand the mechanism and identifies potential biomarkers closely correlated with NIHL and verified the critical role of autophagy pathway in NIHL.

Metabolomics is a powerful platform for exploring disease phenotype, which provides a wealth of information for the discovery of biomarker, pathogenesis and personalized treatment (Ye et al. 2014). So far, some metabolomics studies have been performed in noise exposure field (Floegel et al. 2013, Huang et al. 2018, Wang et al. 2017, Zhang et al. 2016). Pudrith *et al*. (Pudrith &Dudley 2019) found five metabolites related to glutathione-dependent mercapturic acids in urine, while significant associations were just only found in non-noise exposure subjects. Fujita *et al*. (Fujita et al. 2015) revealed that ten metabolites exhibited statistically significant changes in inner ear fluid of guinea pig that exposed to loud noise, including amino acid catabolites and lipid compounds. Also, a study by He *et al*. (He et al. 2017)demonstrated that multiple metabolic pathways are involved in acoustic trauma, such as arginine, proline, and purine metabolic pathways. However, little studies were conducted to investigate the metabolic signatures induced by occupational noise in humans. Thus, we analyzed the plasma samples of 62 NIHL patients and 62 normal hearing controls to explore the metabolomic profiles on NIHL. Eventually, 20 differential metabolites previously unknown were identified to be correlated with NIHL.

Bilirubin is one of important products of heme catabolism and is an effective antioxidant that removes hydrogen peroxide (Minetti et al. 1998). Bilirubin and glutathione were reported to have complementary cell-protective and antioxidant effects (Sedlak et al. 2009). Findings from previous studies indicated that serum bilirubin was closely related to cardiovascular disease-related factors, including body mass index, metabolic syndrome, and diabetes (Cheriyath et al. 2010, Horsfall et al. 2012). In addition, studies found that the plasma and serum bilirubin level significantly increased in benzene-exposed workers compared with the controls (Neghab et al. 2015). In our current study, low plasma bilirubin in NIHL patients may act in conjunction with glutathione to protect cells from oxidation. However, the concrete role of bilirubin in the development of NIHL needs to be further explored.

Among the identified differential metabolites, some were phospholipids (PLs), which are major components of cellular membranes and vital bioactive molecules (Cvetkovic et al. 2017). In this study, plasma levels of a few of crucial PLs (PE(15:0/20:2(11Z,14Z)), PC(15:0/18:1(11Z)), and PI(O-20:0/18:0)) were significantly decreased in NIHL patients compared with controls. It is well known that lipid metabolism is frequently occurred in a variety of diseases, but the most recent evidence found that lipid-related genes may be involved in inflammatory and metabolic diseases (Hirsch et al. 2010). Previous studies have found that the alterations of PLs levels in tissue or plasma may be associated with the risk and progression of all kinds of diseases (Sun et al. 2018). Cvetkovic et al. (Cvetkovic et al. 2017) had reported that the abnormal alterations of PLs profile were associated with non-Hodgkin's lymphoma. In addition, lipidomic studies suggested that the altered levels of PLs composition could results in changes in membrane integrity, permeability, cell damage, as well as cell intimal transport (Leamy et al. 2014). Metabolites PE, PC and PI, also known as phosphatidylethanolamine, phosphatidylcholine and phosphatidylinositol, a group of antioxidants, were involved in cell morphology, metabolism regulation, signal transduction, and a variety of physiological functions of cells (Hidalgo et al. 2005). In this study, the decreased levels of three metabolites in plasma of NIHL patients may be due to the overproduction of ROS during noise exposure, thereby being consumed to maintain the balance between ROS and antioxidant defenses system. Meanwhile, this finding may indicate that oxidative stress is a key factor and important mechanism contributing to NIHL.

In addition, the results obtained from our study showed that the abnormal metabolites, both PE and PC were involved in autophagy pathway, indicating autophagy may be closely associated with the development of NIHL. Recent findings demonstrated that PE is an important substrate for the GPI-anchor biosynthesis that is essential for immune response and plays an essential role in the initiation of autophagy by attaching to the autophagy protein to initiate autophagosome formation (Fracchiolla et al. 2017). The findings from previous studies indicated that ROS have the capacity to induce cell defense pathways like autophagy, which is a protective process that delivers negative cellular components to lysosomes for degradation (Wang &Klionsky 2003, Yang &Klionsky 2009). Abnormal level of metabolites in autophagy pathway was observed in NIHL patients, indicating that autophagy may play a potential biological role in NIHL. Therefore, we conducted RT-qPCR to explore the mRNA expression of important genes in autophagy metabolism pathway. PI3K/AKT pathway is an upstream major modulator of autophagy and they participate in extensive cellular process, including cell growth, proliferation, survival and metabolism (Heras-Sandoval et al. 2014). ATG5, as an essential autophagy related protein, is involved in autophagy at the molecular level (Zheng et al. 2019). Taken together, these three genes were used for further validation. Our results displayed that the mRNA levels of PI3K, AKT and ATG5 were decreased in the WBC of NIHL patients. Substantial evidence has suggested that the reduction of PI3K/AKT signaling pathways is correlated with hair cell death and hearing loss after various of injuries and stimuli (Chen et al. 2015). Data from animal studies showed that deletion of ATG5 could cause hair cells degeneration and profound congenital hearing loss in mice (Fujimoto et al. 2017). In addition, the most data from a review showed that autophagy is a key factor for the auditory cell fate, but autophagy deficiency may be one of leading causes of hearing impairment (Hayashi et al. 2020). Our results are in accord with the previously reported findings, indicating that autophagy could be considered an essential signaling pathway involved in NIHL development.

Conclusions

In summary, our study firstly provides evidence that metabolomics can characterize the metabolites and provide novel insights into the molecular events triggered by NIHL. We found that the plasma metabonomic profiles significantly altered in NIHL patients compared with normal hearing controls. Twenty identified metabolites were significantly altered in NIHL patients. The metabolic signature alterations of metabolites indicated that some specific pathways were involved in NIHL, including

glycosylphosphatidylinositol (GPI)-anchor biosynthesis, autophagy, choline metabolism, alpha-linolenic acid metabolism and linoleic acid metabolism, retrograde endocannabinoid signaling, and glycerophospholipid metabolism. These altered metabolites might act as potential biomarkers of NIHL diagnosis for Chinese noise-exposed workers. In addition, autophagy pathway might play an essential role in the occurrence and development of NIHL. This study provides a new perspective for the progress of NIHL and offers novel clues on the mechanisms underlying NIHL.

Declarations

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Authors' contributions

YP designed the research study. LM and BW performed the research and analyzed the data. LM drafted the manuscript. JZ, LY critically reviewed and revised the manuscript. All authors read and approved the final manuscript.

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Data availability

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

Compliance with ethical standards

Conflict of interest

The authors declare that they have no conflicts of interest.

Ethical approval

The present study was approved by the Ethics Committee of Zhongda Hospital, Affiliated Hospital of Southeast University.

Consent to participate

Informed consent was obtained from all individual participants included in the study.

Consent to publish

The participant has consented to the submission of the case report to the journal.

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Tables

Table 1 Demographic characteristics of NIHL patients and control subjects

Characteristic	Cases (n = 62)		Controls (n = 62)		*P*
	n	%	n	%	
Age (years)					0.832[b]
Mean ± SD	42.11 ± 10.15		41.73 ± 10.07		
Sex					-
Male	62	100.0	62	100.0	
Female	0	0.0	0	0.0	
Work time with noise (years)					0.951[b]
Mean ± SD	18.19 ± 11.94		18.32 ± 11.71		
Smoking status					0.844[a]
No	18	29.0	19	30.6	
Yes	44	71.0	43	69.4	
Drinking status					0.469[a]
No	29	46.8	25	40.3	
Yes	33	53.2	37	59.7	
SBP					0.555[b]
Mean ± SD	124.42 ± 17.03		126.15 ± 15.38		
DBP					0.681[b]
Mean ± SD	80.27 ± 11.35		81.18 ± 13.00		
High frequency hearing threshold (dB)					< 0.001[b]
Mean ± SD	53.24 ± 13.01		18.73 ± 3.96		
Expose level with noise (dB)					0.971[b]
Mean ± SD	89.11 ± 3.84		89.08 ± 5.86		

[a] Two-sided χ^2 test.

[b] Students' *t*-test.

SD, standard deviation; dB, decibel; SBP, systolic blood pressure; DBP, diastolic blood pressure

Table 2 Identified plasma differential metabolites associated with NIHL

Accuracy mass (m/z)	Retention time (min)	Metabolites	VIP-value	*P*-value	FC
Positive ion mode					
311.1852569	10.4648	Botrydial	1.27286759	0.03894814	1.28783125
185.0443214	6.67985	3-O-Methylgallate	2.31339463	0.02983198	1.70865564
459.2490377	8.34475	Homodeoxycholic acid	1.18948582	0.01139502	1.53923459
169.049315	8.169933333	Quinolacetic acid	3.29015824	0.03374699	1.70844877
239.1643354	12.32853333	7-oxo-11E,13-Tetradecadienoic acid	1.57982426	0.03663327	1.33969105
237.1482017	10.25615	12,13-Dimethyl-5,14-dioxabicyclo[9.2.1]-tetradeca-1(13),11-dien-4-one	1.04849816	0.00652026	1.45013866
540.3658642	12.40703333	PS(O-20:0/0:0)	1.06692078	0.03151553	0.84758463
167.033526	5.928883333	3,4-Dihydroxymandelic acid	1.7126814	0.03719973	1.51393039
708.4889779	12.71418333	Butyl 4'-O-butanoyl-6-O-hexadecanoyl-neohesperidoside	2.0716998	0.03969699	0.56778489
752.5186207	12.68268333	PE(15:0/20:2(11Z,14Z))	2.14374406	0.0216304	0.60668356
313.0358286	0.7735	4-Hydroxy-5-(3'-hydroxyphenyl)-valeric acid-3'-O-sulphate	1.49168798	0.0243405	1.14578276
838.2387531	7.86895	({2-[2-(2-hydroxypropan-2-yl)-7-oxo-2H,3H,7H-furo[3,2-g]chromen-6-yl]-2-methylbut-3-en-1-yl}oxy)sulfonic acid	3.61397217	0.02653202	2.32513639
837.4362666	7.86895	Mabioside D	3.93298292	0.02712122	2.37605848
763.5942952	11.72606667	PC(15:0/18:1(11Z))	1.41579684	0.01678653	0.40772182
616.2519865	9.662016667	12S-acetoxy-punaglandin 1	1.02041589	0.04217161	0.70668393
881.6498	8.8424	PI(O-20:0/18:0)	1.60990954	0.03281649	0.74938231
Negative ion mode					
583.2554212	7.870583333	Bilirubin	5.94802089	0.01894616	0.75876997
245.0484576	7.240283333	[3-(4-methoxyphenyl)propoxy]sulfonic acid	1.77471751	0.02071462	2.1502221
615.244282	7.947083333	Myricanene B 5-[arabinosyl-(1->6)-glucoside]	1.74800988	0.02618671	0.74123558
311.29438	16.92686667	Stearic Acid ethyl ester	1.16524736	0.00478247	1.62621185

VIP, variable importance of projection; FC, fold change

Table 3 Identified differential metabolic pathways involved in NIHL		
Pathway name	*P*-value	FDR correction
Retrograde endocannabinoid signaling	1.51E-04	2.27E-03
Glycerophospholipid metabolism	1.16E-03	5.79E-03
Glycosylphosphatidylinositol (GPI)-anchor biosynthesis	3.26E-03	9.79E-03
Autophagy	4.35E-03	1.09E-02
Choline metabolism	1.19E-02	2.24E-02
Linoleic acid metabolism	3.01E-02	5.02E-02
Alpha-Linolenic acid metabolism	4.71E-02	6.42E-02
FDR, false discovery rate		

Figures

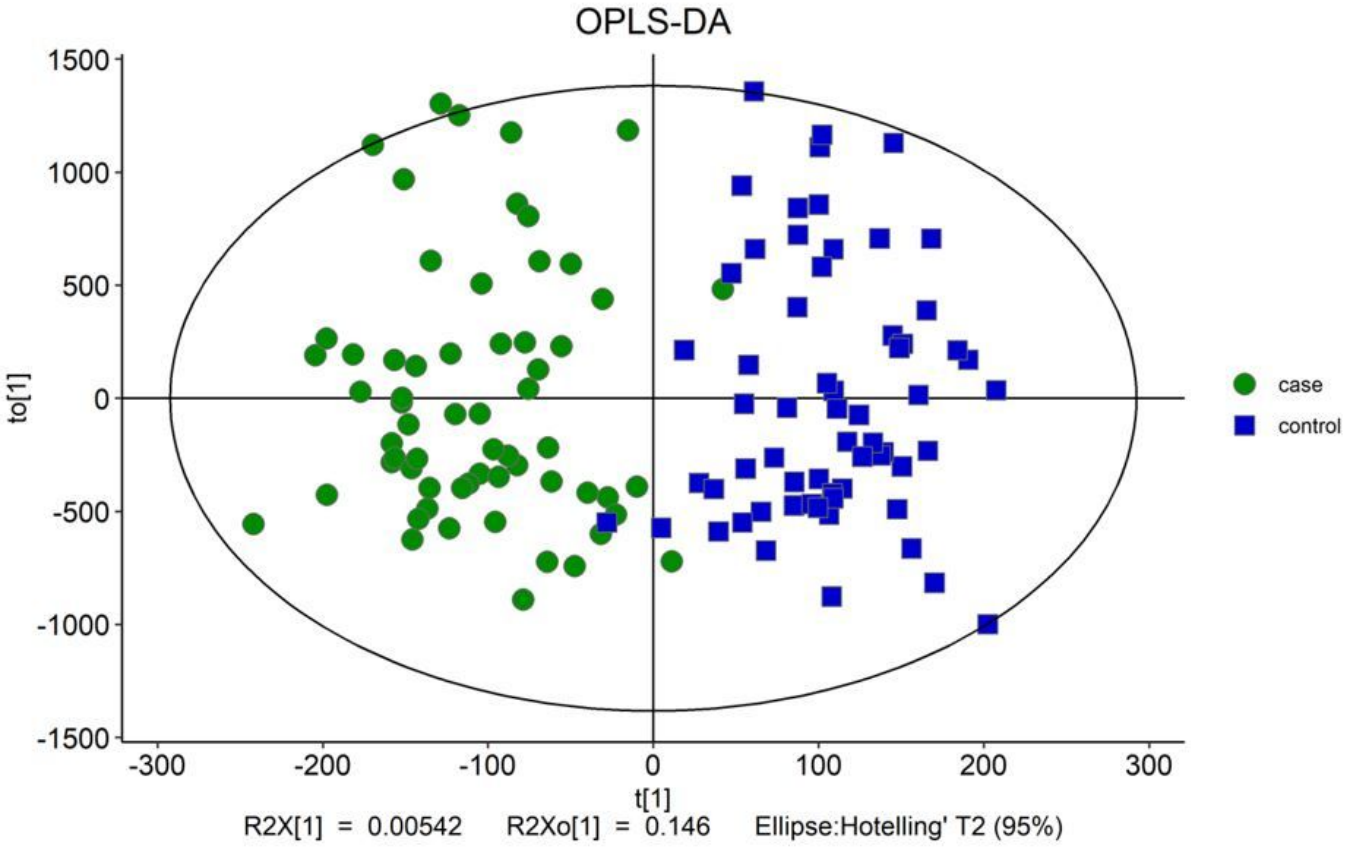

Figure 1

Scoring plots for OPLS-DA model. OPLS-DA score plot of healthy control group (blue) and NIHL group (green). Data from positive ion mode and negative ion mode were combined.

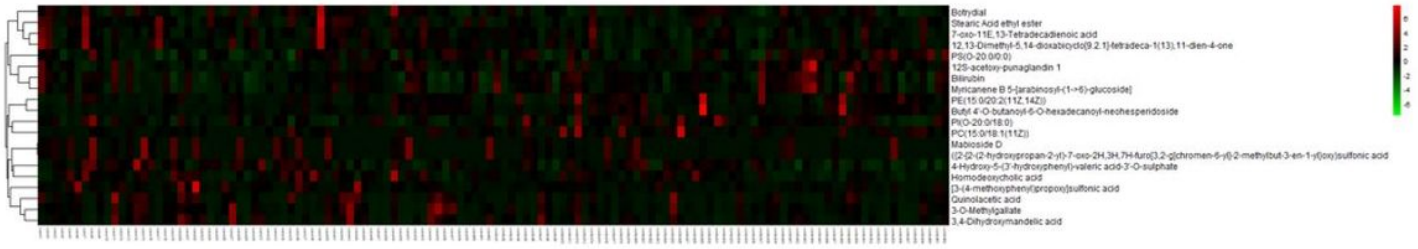

Figure 2

Heat map of twenty significantly changed metabolites. Red color indicates a high level of metabolites and green color indicates a low level of metabolites, while black color means an equal level in both groups.

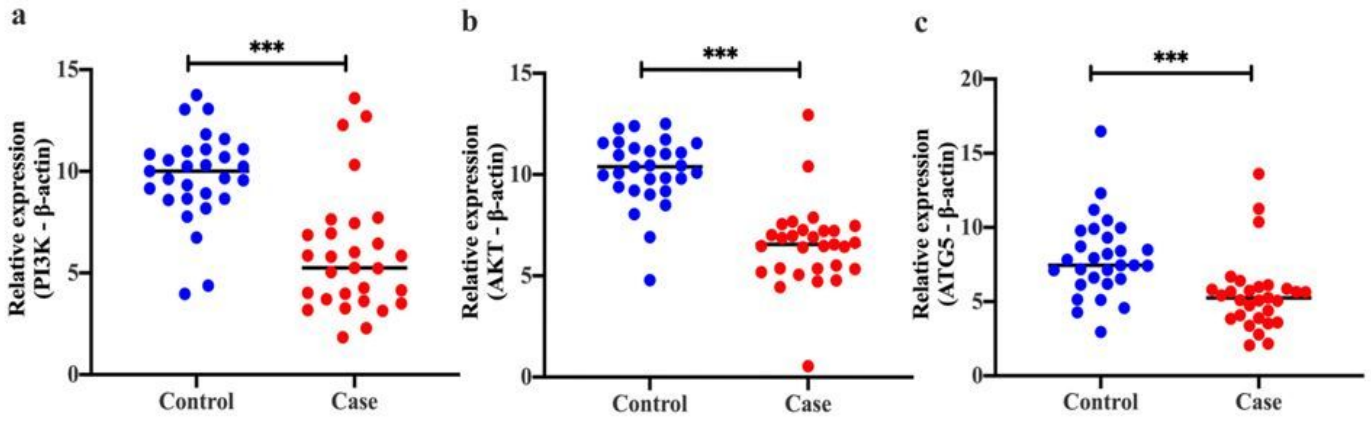

Figure 3

mRNA levels of PI3K, AKT and ATG5 in NIHL patients and controls were detected by RT-qPCR analysis. ***P < 0.001.