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Antioxidant enzymes, presbycusis, and ethnic variability

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Abstract

INTRODUCTION—A proposed mechanism for presbycusis is a significant increase in oxidative stress in the cochlea. The enzymes glutathione s-transferase (*GST*) and N-acetyltransferase (*NAT*) are two classes of antioxidant enzymes active in the cochlea.

OBJECTIVE—Investigate the association of different polymorphisms of *GSTM1*, *GSTT1*, and *NAT2* and presbycusis, and analyze if ethnicity has an effect in the genotype-phenotype associations.

STUDY DESIGN—Case-control study of 134 DNA samples.

SETTING—University-based tertiary care center.

SUBJECTS AND METHODS—Clinical, audiometric, and DNA testing of 55 adults with presbycusis and comparison with 79 normal-hearing controls.

RESULTS—The *GSTM1* null genotype was present in 77% of White Hispanics and 51% of White non-Hispanics (Fisher's exact test, 2-tail, $p = 0.0262$). The *GSTT1* null genotype was present in 34% of controls and in 60% of White presbycusis subjects ($p = 0.0067$, OR=2.843, CI=1.379, 5.860). The *GSTM1* null genotype was more frequent in presbycusis subjects, 48% of controls and 69% of White subjects carrying this deletion ($p = 0.0198$, OR=2.43, CI=1.163, 5.067). The *NAT2*6A* mutant genotype was more frequent among subjects with presbycusis (60%) than in controls (34%) ($p = 0.0086$, OR=2.88, CI=1.355, 6.141).

DISCUSSION—We showed an increased risk of presbycusis among Caucasians carrying the *GSTM1* and the *GSTT1* null genotype and the *NAT*6A* mutant allele. Individuals with the *GSTT1* null genotypes are almost three times more likely to develop presbycusis than those with the wild type. The *GSTM1* null genotype was more prevalent in White Hispanics than in White non-Hispanics, but the *GSTT1* and *NAT2* polymorphisms were equally represented in the two groups.

Introduction

Presbycusis, or age-related hearing loss, is a progressive, bilateral, primarily sensorineural hearing loss that occurs with aging. Presbycusis is the most common of human auditory

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disorders.¹ Of the 28 million Americans with hearing loss, nearly 10 million are over the age of 65, and the prevalence of hearing loss increases more dramatically with further advancing age such that while 31% of people over age 65 are affected, that percentage increases to 70% for those over 85 years of age.² Previous literature has proposed that presbycusis is due to a series of cumulative effects and insults such as loud noise exposure, systemic illnesses, medications, and genetic susceptibility.³⁻⁷ Ultimately, the exact pathophysiological mechanism of presbycusis is not known.

A potential pathophysiological mechanism of presbycusis could be oxidative stress. Oxidative stress plays a fundamental role in the overall aging process and the diseases associated with aging.⁸ Oxidative stress is the result of the accumulation of damage from reactive oxygen species (ROS) and free radicals which are a natural byproduct of aerobic metabolism.⁹ The cochlea itself is not exempt from oxidative stress and its role in presbycusis has been shown in the mouse model.^{10,11} ROS are thought to upregulate apoptotic genes in response to hypoxic injury in the inner ear as was demonstrated by Riva et al in the mouse model.¹¹ We hypothesized therefore that oxidative stress in the inner ear secondary to the decreased elimination of ROS by certain polymorphisms of antioxidant enzymes in humans would then make these individuals more susceptible to presbycusis.

Several antioxidant enzymes have been demonstrated to be active in the adult cochlea. For instance, enzymes involved in glutathione metabolism (glutathione S-transferase, glutathione peroxidase, glutathione reductase) and enzymes involved in the breakdown of superoxide anions (catalase).⁹ Specifically, glutathione S-transferase (*GST*) enzymes catalyze the conjugation of glutathione with xenobiotics, compounds foreign to human metabolism, and are considered to play an important role in the antioxidant protection of the cochlea. *GST* comprises several gene classes including *GSTM* and *GSTT*. *GSTM1* and *GSTT1* genes show genetic variability in humans. Individuals who are null genotypes for *GSTM1* and *GSTT1* cannot conjugate metabolites specific for these enzymes. These individuals are thus thought to be more prone to damage caused by oxidative stress and possibly more susceptible to presbycusis. Previous studies, however, have not demonstrated an association between individuals who are null genotypes for *GSTM1* and *GSTT1* and presbycusis.^{12, 13}

N-acetyltransferases (NAT) are also known to be involved in the detoxification of harmful xenobiotics. The frequency of *NAT2* alleles and phenotypes varies amongst different ethnicities. Genotypes can be classified as null genotypes, heterozygotes, or wild type, based on whether they are homozygotes for the deletion, heterozygotes, or if they do not possess the deletion. Half of the Caucasian and African-American population are homozygotes for the deletion (null genotype).¹⁴ In fact the percentage of the population carrying the null genotype varies amongst different geographic regions and ethnicities.¹⁵ Unal et al demonstrated that polymorphisms of the gene encoding for *NAT2* enzyme are associated with presbycusis in the Turkish Caucasian population.¹⁶ In the United States, individuals of Hispanic ethnic origin make up the largest minority. Hispanics constituted 15.4% of the US population according to the 2008 census. It is projected that by 2050 there will be approximately 132 million Hispanics in the US, making Hispanics the fastest growing minority group. In South Florida, Hispanics account for 57% of the population.¹⁷

Given the proposed associations between antioxidant enzymes' polymorphisms and presbycusis, our study sought to further investigate these associations, across subjects of different ethnicities, particularly the Hispanic population. No previous study in the literature has shown the proportion of Hispanic individuals with the *NAT2* null genotype. Our study provides a novel analysis of antioxidant enzymes and their possible genetic and phenotypic distinctions and how these relate to presbycusis in different ethnicities.

Methods

The study was approved by the University of Miami institutional review board. This was a hospital based, case-control study. We recruited subjects from adults attending the outpatient clinic of the University of Miami Ear Institute. All subjects underwent a medical history and physical examination including otoscopy. Subjects completed a questionnaire on demographic information including ethnicity and on medical history focused on the identification of factors with known influence on hearing such as age, gender, excess noise, solvents, ototoxic drugs, ear trauma, radiation exposure or surgery, ear diseases, hearing nerve neoplasm, chronic illness (i.e., cardiovascular disease, diabetes), manifestations of syndromic deafness (i.e., blindness from retinitis pigmentosa, tegumentary and craniofacial anomalies), and family history of hearing loss. At least a three-generation family history was obtained for each subject and the case classified based on the inheritance pattern in autosomal dominant, autosomal recessive, X-linked, mitochondrial DNA, or sporadic case. Recruited subjects with presbycusis underwent audiological examination according to current clinical standards (ISO 8253-1, 1989). The minimal audiological examination consisted of measurement of air and bone conduction pure-tone thresholds at 500, 1000, 2000, and 4000 Hz.

The inclusion criteria for subjects with presbycusis included 1) age greater than 40 years; 2) greater than 30 dB HL hearing loss (bone conduction pure-tone average of frequencies 500, 1000, 2000, and 4000 Hz). Exclusion criteria included 1) previous history of exposure to occupational or excess environmental noise; 2) exposure to toxins or drugs with known ototoxic effect; 3) history of temporal bone trauma; 4) history of otologic disorders such as Meniere's disease, autoimmune hearing loss, etc.; 5) average conductive hearing loss greater than 15 dB hearing loss (HL) in one or two ears, measured at 500, 1000 and 2000 Hz; 6) unilateral or significantly asymmetric (greater than 25 dB difference in interaural pure-tone average, PTA) hearing loss; 7) syndromic hearing loss. Control DNA samples were comprised of a Caucasian diversity panel of unrelated individuals with normal hearing aged 40-81 years (Coriell cell repositories, Camden, NJ).

DNA isolation

Blood samples were collected, and genomic DNA was extracted using a standard extraction method.

Genotyping

GSTM1 and GSTT1—The genetic polymorphism analyses for the *GSTM1* and *GSTT1* genes was determined by the multiplex polymerase chain reaction (PCR) procedure using previously described primers of Kyung A. Lee et al.¹⁸ The PCR products were analyzed electrophoretically on an ethidium bromide-stained 1.5% agarose gel (Fig 1).

NAT2—Genotyping of the *NAT2* polymorphisms (*NAT2*5A*, *NAT2*6A*, *NAT2*7A*, *NAT2*7B*, *NAT2*14A* and *NAT2*14B*) was performed using Restriction Fragment-Length Polymorphism (PCR-RFLP), Allele-Specific Amplification and Direct sequencing.

DNA amplification for RFLP analysis

Polymerase chain reaction (PCR) was performed in a 25ul reaction with 80ng of genomic DNA, 10 pmol of each primer, 200uM dNTPs, 1.5mM MgCl₂ and 1U of TaqDNA polymerase. Two sets (A and B) of primers were used. The 369 bp (primers Set A) and 547 bp (Primers Set B) PCR products were subjected to RFLP analysis. Primer sequences and PCR conditions are available upon request.

Allele-specific amplification

The presence or absence of the T341C nucleotide substitution was detected using the allele-specific PCR method described by Vatsis et al.¹⁴ G191A, C282T, C341T, C480T, G590A and G857 substitutions were confirmed by direct sequencing on an automated sequencer (ABI 3100).

Statistical Analysis

Student's t test was used to compare differences in terms of age between sample groups. Non-parametric statistical tests (Chi-square or Fisher's exact test) were used to determine differences between the frequency of *GSTM1*, *GSTT1*, and *NAT2* mutant alleles in the controls versus subjects. Odds ratio (OR) and 95% confidence intervals (CI) were used to analyze the occurrence of the high-risk genotypes (all except the "low-risk" or wild type genotype = +/+) in the population samples. The hearing level measured as the PTA for 500, 1000, 2000 and 4000 Hz was compared between subjects with low- and high-risk genotypes by Student's t test. The level of significance α was 0.05. All calculations were performed with the JMP IN statistical software package 8.0 (SAS Institute, Inc., Belmont, CA).

Results

The study consisted of 55 subjects and 79 controls who met the inclusion criteria. The average age of the subjects was 59 ± 10 years and that of the controls was 56 ± 11 years with a male to female ratio of 1:1 for the subjects and 1.3:1 for the controls. There were no statistically significant differences in terms of age and gender ratio between subjects and controls. Among subjects, 26 subjects were White non-Hispanics (Northern European), 26 were White Hispanics, 2 were Asians, and 1 was Black. All of the control samples were of White non-Hispanic descent. For comparisons of allele distribution between subjects and control we only used data from Caucasians (White non-Hispanics and White Hispanics). Table 1 summarizes the demographic data.

Analysis of frequency of allele distribution by ethnicity (White non-Hispanics versus White Hispanics)

We encountered that the *GSTM1* null genotype was present in 77% of White Hispanics and in 51% of White non-Hispanics, and this difference was statistically significant (Fisher's exact test, 2-tail, $X=5.826$, $p=0.0262$. OR = 3.148, 95% CI = 1.170, 8.467). In contrast, there were no significant differences in the frequency of the *GSTT1* null genotype and the studied *NAT* polymorphisms between White Hispanics and White non-Hispanics.

Analysis of the frequency of allele distribution between White subjects and controls

Table 2 shows the *GSTT1* null genotype was present in 34% of controls and in 60% of White presbycusis subjects, and this difference was statistically significant ($X=8.263$, $p=0.0067$, OR=2.843, CI=1.379, 5.860). Similarly, the *GSTM1* null genotype was found more frequently in presbycusis subjects, with 48% of controls and 69% of White subjects carrying this deletion ($X=5.798$, $p=0.0198$, OR=2.43, CI=1.163, 5.067).

Results of the distribution of the *NAT2* gene polymorphisms between subjects and controls are shown in table 3. The frequency of gene polymorphisms of *NAT2*5A*, *NAT2*7A*, and *NAT2*14A* in subjects with presbycusis were not statistically significantly different from the controls ($p > 0.05$). However, the frequency of the *NAT2*6A* mutant genotype (heterozygous and null genotype combined) was more frequent among subjects with presbycusis (60%) than in controls (34%) ($X=7.807$, $p=0.0086$. OR=2.88, CI=1.355, 6.141).

Comparison of audiometric data

The PTA for air conduction thresholds at 500, 1000, 2000 and 4000 Hz was calculated for groups of individuals with putative low-risk (wild type) and high-risk (heterozygous or null) genotypes. Table 4 depicts audiometric data for these individuals categorized by genotype. The only two comparisons with differences of statistical significance were in the *GSTT1* and *NAT2*6A* groups. The mean PTA and standard deviation of the group with the *GSTT1* null genotype were 38.9 ± 22 dB HL and the corresponding values for the *GSTT1* wild-type genotype were 29.9 ± 19 dB HL (One-way ANOVA, degrees of freedom DF=1, F ratio=6.1697, n=131, $p=0.0143$). The wild-type *NAT2*6A* polymorphism had a mean PTA and standard deviation value of 31.3 ± 19 dB HL, and the “high-risk” *NAT2*6A* genotype (i.e., heterozygous or null genotype) had a mean PTA and standard deviation of 41 ± 22 dB HL (One-way ANOVA, DF=1, F ratio=5.7660, n= 117, $p=0.018$). The hearing level of individuals with wild type *GSTM1* genotype was 29.8 ± 18 dB HL and the hearing level of individuals with the *GSTM1* null genotype was 37 ± 22 dB HL, but this difference only approached statistical significance (DF=1, F ratio=3.7780, n= 131, $p=0.054$).

Discussion

Recently, the effects of free radicals, also known as reactive oxygen species (ROS) and their damaging cellular effects have received much attention. Among the anti-oxidant enzymes thought to be active in the cochlea are enzymes involved in the metabolism of glutathione such as glutathione S-transferase, *GST*. Also, the enzymes N-acetyltransferases (*NAT*) are known to be active in detoxifying xenobiotic toxins.¹⁶ Our study set out to evaluate the potential association between different *GST* and *NAT* genotypes and presbycusis. Hispanic Americans comprise the fastest growing minority group in the US. Among Hispanics, 80% claimed at least two races.¹⁹ Our study is the first in the literature to study a majority of Caucasians of Hispanic descent and analyze how ethnic variability may play a role in genotype-phenotype associations, antioxidant enzymes, and presbycusis.

Our study showed an increased risk of presbycusis among Whites carrying a *NAT*6A* mutant allele. This agrees with Unal et al who most recently analyzed the association of N-acetyltransferase 2 gene polymorphisms and presbycusis. They studied a population of 68 Turkish Caucasian adults with presbycusis and found a 15.2-fold increased risk for the development of presbycusis in individuals with a *NAT2*6A* mutant alleles.¹⁶ Our data showed that individuals with the high-risk genotype (i.e., heterozygous and null genotypes) had worse hearing than those individuals with the low-risk (wild type) genotype. In this study's population of White subjects with presbycusis, Hispanics and non-Hispanics were equally represented, and there were no difference in the frequency of *NAT* polymorphisms among these two racial variants. The findings that the *NAT* slow-acetylator status appears to be a risk of presbycusis in this and other studies suggests a significant association. Ultimately, epidemiological studies with much greater number of subjects and with improved methodological designs are needed to establish a definite association.

Glutathione-S-transferase consists of several gene classes including *GSTM* and *GSTT* coding for cytosolic enzymes.²⁰ *GSTM1* and *GSTT1* genes show genetic variability in humans. Up to 50% of the Caucasian population is null genotypes for *GSTM1*.²¹ Individuals who are null genotypes for *GSTM1* cannot conjugate metabolites specific for these enzymes. Individuals with null *GSTM1* genotypes had lower amplitudes of high frequency distortion product otoacoustic emissions (DPOAEs) compared to individuals possessing the gene, and this fact supports that *GSTM1* null individuals might be more susceptible to presbycusis.²² Data presented in this study revealed a modest association between the presence of either the *GSTT1* or *GSTM1* null genotypes and presbycusis. Of clinical importance, individuals with the *GSTT1* null genotypes are almost three times more likely to develop presbycusis.

Furthermore, individuals with the putative “high-risk” genotype in our study population had statistically significant worse hearing level (mean PTA) than those with the *GSTT1* wild type genotype. These results are in contrast with previously published data. In a study of 68 Caucasian subjects of Turkish descent, Ates et al did not find a statistically significant correlation between individuals with a *GSTM1* or *GSTT1* null genotypes and 69 control subjects.¹³ Similarly, Unal et al did not find an association between the *GSTT1* and the *GSTM1* null genotypes and presbycusis in Turkish Caucasians. The reported frequency of the *GSTM1* mutant genotypes ranges from 23% to 62% in different populations and is common in Caucasians with a frequency in this group of approximately 50%, while the *GSTT1* mutant genotype is less common and ranges from 15% to 20% in Caucasians.²³ In our cohort, the *GSTT1* null genotypes was present in 34% of Caucasians with normal hearing, and in 60% of Caucasians with presbycusis; the *GSTM1* null genotype was present in 48% of normal-hearing and in 69% of hearing-impaired Caucasians. Our cohort of hearing-impaired subjects is comprised of Caucasians from different European origins and reflects the demographic composition of South Florida. When comparing our study to those from Mersin, Turkey, phenotypical heterogeneity is expected to exist between anti-oxidant enzyme gene mutations and the subsequent development of presbycusis. In contrast to the Turkish studies, our study showed a clinically significant correlation for the development of presbycusis among individuals with *GSTT1* mutant allele and the *GSTM1* mutant allele. Our study has some important limitations. First of all, although we used age- and gender-matched controls, these samples were not derived from the same geographical area as that of the subjects. Secondly, the control:subject ratio was almost 1.5 (79 controls and 52 subjects) instead of 2:1 ratio which provides for a more efficient design. Despite these limitations, our results are similar albeit more modest than those reported by Unal et al in Turkish Caucasian.

In summary, the present study highlights the genotypic variabilities of anti-oxidant enzymes that exist amongst different ethnic populations. We are the first study to emphasize these ethnic discrepancies and how they relate to presbycusis. We demonstrated an increased risk of presbycusis among Caucasians carrying the *GSTM1* and the *GSTT1* null genotype and the *NAT*6A* mutant allele. Furthermore, we showed that individuals with the *GSTT1* null genotypes are three times more likely to develop presbycusis than those with the wild type. Of clinical importance, recent animal studies have shown the potential importance of anti-oxidant enzyme supplementation in the prevention of presbycusis as illustrated most recently by Bielefeld et al.²⁴ Future studies will need to analyze the apparent phenotypic difference among subpopulation of Caucasians and the development of age-related hearing loss.

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References

1. US Department of Health and Human Services, National Institute of Health, National Institute on Deafness and Other Communication Disorders. National Strategic Research Plan. Bethesda, MD: National Institute of Health; 1993.
2. Agrawal Y, Platz EA, Niparko JK. Prevalence of Hearing Loss and Differences by Demographic Characteristics Among US Adults. Arch Intern Med 2008;168:1522–30. [PubMed: 18663164]
3. Ding DL, McFadden S, Wang J, et al. Age and strain-related differences in dehydrogenase activity and glycogen levels in CBA and C57 mouse cochleas. Audiol Neurotol 1999;4:55–63.

4. Erway LC, Shiau YW, Davis RR, et al. Genetics of age-related hearing loss in mice. III. Susceptibility of inbred and F1 hybrid strains to noise induced hearing loss. *Hear Res* 1996;93:181–7. [PubMed: 8735078]
5. Gabaizadeh R, Staecker H, Liu W, et al. Protection of both auditory hair cells and neurons from cisplatin induced damage. *Acta Otolaryngol* 1997;117:232–8. [PubMed: 9105457]
6. Gates GA, Couropmitree NN, Myers RH. Genetic association in age-related hearing thresholds. *Arch Otolaryngol Head Neck Surg* 1999;125:654–9. [PubMed: 10367922]
7. Guegan C, Ceballos-Picot I, Chevalier E, et al. Reduction of ischemic damage in NGF-transgenic mice: correlation with enhancement of antioxidant enzyme activities. *Neurobiol Dis* 1999;6:180–9. [PubMed: 10408807]
8. Sohal RS, Weindruch R. Oxidative stress, caloric restriction, and aging. *Science* 1996;273:59–63. [PubMed: 8658196]
9. Liu XZ, Yan D. Ageing and hearing loss. *J Pathol* 2007;211:188–97. [PubMed: 17200945]
10. Staecker H, Zheng QY, Van de Water TR. Oxidative stress and in aging in the C57B16/J mouse cochlea. *Acta Otolaryngol* 2001;121:666–672. [PubMed: 11678164]
11. Riva C, Donadieu E, Lavieille JP. Age-related hearing loss in CD/1 mice is associated to ROS formation and HIF target proteins up-regulation in the cochlea. *Exp Gerontol* Apr;2007 42(4):327–36. [PubMed: 17141999]
12. Pemble S, Schroeder ER, Spencer SR, et al. Human glutathione S-transferase theta (GSTT1): cDNA cloning and the characterization of a genetic polymorphism. *Biochem J* 1994;300:271–76. [PubMed: 8198545]
13. Ates NA, Unal M, Tamer L, et al. Glutathione S-transferase gene polymorphisms in presbycusis. *Otol Neurotol* 2005;26:392–397. [PubMed: 15891640]
14. Vatsis KP, Weber WW. Structural heterogeneity of Caucasian N-acetyltransferase at the NAT1 gene locus. *Arch Biochem Biophys* 1993;301:71–76.
15. Blum M, Demierre A, Grant DM, et al. Molecular mechanism of slow acetylation of drugs and carcinogens in humans. *Proc Natl Acad Sci USA* Jun 15;1991 88(12):5237–41. [PubMed: 1675794]
16. Unal M, Tamer L, Dogruer ZN, et al. N-acetyltransferase 2 gene polymorphism and presbycusis. *Laryngoscope* 2005;115:2238–41. [PubMed: 16369173]
17. U.S. Census Bureau: State and County QuickFacts. 2008. <http://quickfacts.census.gov/qfd/states/12/12086.html>
18. Lee KA, Kim SH, Woo HW, et al. Increased frequencies of glutathione S-transferase (GSTM1 and GSTT1) gene deletions in Korean patients with acquired aplastic anemia. *Blood* 2001;98:3483–85. [PubMed: 11719393]
19. U.S. Bureau of Census. Profile of General Demographic Characteristics. [Accessed December 2009]. 2000 Available at <http://www.labormarketinfo.com/index.htm>
20. Mannervik B, Awasthi YC, Board PG, et al. Nomenclature for human glutathione transferases. *Biochem J* 1992;282:305–306. [PubMed: 1540145]
21. Board PG. Biochemical genetics of glutathione-S-transferase in man. *Am J Hum Genet* 1981;33:36–43. [PubMed: 7468592]
22. Rabinowitz PM, Wise J Pierce Sr, Mobo B Hur, et al. Antioxidant status and hearing function in noise-exposed workers. *Hearing Res* 2002;173:164–171.
23. Cotton SC, Sharp L, Little J, et al. Glutathione S-transferase polymorphisms and colorectal cancer: a HuGE review. *Am J Epidemiol* 2000;10:781–8.
24. Bielefeld EC, Tanaka C, Guang-di, et al. Age-related hearing loss: Is it a preventable condition? *Hear Res*. Sep. 6;2009 (Epub ahead of print).

Figure 1.
The presence or absence of GSTM1 and GSTT1 genes were analyzed on 1.5% agarose gel.
Details described by Kyung A. Lee et al.¹⁸

Table 1

Demographic data.

	Presbycusis	Controls
N	55	79
Age (mean, SD)	59, 10	56, 11
Gender		
Female	27	34
Male	28	45
Race		
White, non Hispanic	26	79
White, Hispanics	26	0
Black	1	0
Asian	2	2

Table 2

Frequency of the *GSTT1* and *GSTM1* null genotypes in Caucasians: 52 presbycusis subjects and 79 control individuals. Results of the 2-tail Fisher's exact test (CI = Confidence Intervals).

—/— genotype	Presbycusis	Control	P value	Odds ratio (95% CI)
<i>GSTT1</i>	60%	34%	0.0067	2.84 (1.379, 5.860)
<i>GSTM1</i>	69%	48%	0.0198	2.43 (1.163, 5.067)

Table 3

Distribution of the N-acetyltransferase genotype polymorphisms in controls and presbycusis subjects.

NAT2	Presbycusis (%) N=51	Control (%) N=79
<i>NAT2*5A</i>		
+/+	25	26
+/-	60	58.5
-/-	15	15.5
<i>NAT2*6A</i>		
+/+	40	66
+/-	48	34
-/-	12	0
<i>NAT2*6B</i>		
+/+	42	50
+/-	48	42
-/-	10	8
<i>NAT2*7A</i>		
+/+	94	89
+/-	6	11
-/-	0	0
<i>NAT2*7B</i>		
+/+	94	92
+/-	6	8
-/-	0	0
<i>NAT2*14A</i>		
+/+	96	98
+/-	4	2
-/-	0	0
<i>NAT2*14B</i>		
+/+	94	100
+/-	6	0
-/-	0	0

Table 4

Hearing level measured as the average of the 4-frequency (500, 1000, 2000, 4000 Hz) air conduction pure-tone thresholds by high “putative-risk” and “low-risk” genotypes. The “low-risk” genotype is the wild type genotype and the “high-risk” genotypes are the heterozygous or null genotypes. Statistical comparisons by ANOVA with significance level of 0.05.

Gene	“Low-Risk” genotype	“High-Risk” genotype	p value	n
<i>GSTT1</i>	29.9 ± 19 dB	38.9 ± 22 dB	0.0143	131
<i>GSTM1</i>	29.8 ± 18 dB	37 ± 22 dB	0.054	131
<i>NAT2*6A</i>	31.3 ± 19 dB	41 ± 22 dB	0.018	117