Analysis of polymorphisms associated with base excision repair in patients susceptible and resistant to noise-induced hearing loss

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Research article

Keywords: base excision repair; noise-induced hearing loss; polymorphism

Posted Date: August 21st, 2019

DOI: https://doi.org/10.21203/rs.2.13320/v1

Version of Record: A version of this preprint was published at Disease Markers on November 14th, 2019.
See the published version at https://doi.org/10.1155/2019/9327106.

Abstract

Objective We aim to investigate whether genetic mutations in three important base excision repair genes (OGG1, APEX1, and XRCC1) may influence susceptibility to noise-induced hearing loss. Methods Three SNPs in OGG1, APEX1, and XRCC1 were genotyped from noise exposed workers who were classified into susceptible and resistant individuals. Results: Results showed that the rs1799782 TT genotype located in the XRCC1 coding region and rs1130409 GG/GT in the APEX1 coding region were associated with increased risk for noise-induced hearing loss. Compared to the rs1799782 C allele frequency, the T allele frequency increased in the sensitive group (OR = 1.51). Rs1130409 G allele frequency also increased in the sensitive group compared to the resistant group (OR = 1.59). Conclusions XRCC1 rs1799782 and APEX1 rs1130409 may have potential as biomarkers for screening susceptibility to NIHL in workers exposed severe noise.

Introduction

Noise-induced hearing loss (NIHL) has been the second most common form of severe sensorineural hearing impairment, besides age-related hearing loss (ARHL). It is one of the leading occupational diseases both in developed and industrialized countries [1].

NIHL is a complex occupational disease, caused by an interaction between genetic and environmental factors, and there exists a large difference in occurrences of hearing loss after similar noise exposure [2, 3]. This inter-individual variability has been considered as an interaction between genetic, environmental factors and living habits. It is believed that besides noise, ototoxic substances, heat, vibrations, and individual factors such as age, smoking and blood pressure have their effect on the development of NIHL [4]. Numerous variations in susceptibility to noise loss have been reported. Single nucleotide polymorphisms (SNPs) are known as the most common form of genetic variation in the mammalian genome, with about 15 million SNPs found among all humans. So far, SNPs in genes such as *FOXO3*, *DNMT*, *HSP70*, *CAT, Notch* and *KCNQ4* have been identified in many association studies involved with human subjects [5-10].

DNA repair is the most important defense mechanism against DNA lesions which are caused by environmental factors and normal metabolic activities in humans [11]. DNA damage is identified and processed by a variety of distinct pathways called the “DNA damage response (DDR)” pathways [12]. DDR includes direct repair (DR), mismatch repair (MMR), double-strand break repair (DSBR), nucleotide and base excision repair (NER and BER), and DNA interstrand crosslink repair mechanisms [13, 14]. Base-excision repair (BER), a key mechanism of the DNA repair pathway, mainly plays a role by repairing single bases in damaged DNA molecules. BER is the main guard against DNA damage as a result of both normal and abnormal cellular metabolism, including methylation, deamination, hydroxylation, reactive oxygen radicals, and physical and chemical factors (such as X-rays, alkylating agents, and so on) [15]. The BER pathway is the primary mechanism that defends against oxidative induced DNA damage in cells. BER is known to act on small DNA lesions or modified bases to repair damage by removing and

replacing damaged base-pairs. Enzymes involved in BER include human 8-oxoG DNA glycosylase1 (*hOGG1*), apurinic/apyrimidinic endonuclease 1 (*APE1* or *APEX1*), and X-ray repair cross-complementing group 1 (*XRCC1*). Variations that occur in BER-related gene regions can lead to abnormality of repair functions, increasing the probability of developing diseases [16].

Numerous studies have reported that the associations of genetic factors, including DNA synthesis-related genes, DNA repair pathways, cell cycle control, and apoptosis, with NIHL individual susceptibility of workers exposed to industrial noise. *APEX1* rs1130409 polymorphism and *hOGG1* rs1052133 polymorphism were reported to contribute to the susceptibility of NIHL in Chinese populations by Shen et al. [17, 18]. However, the conclusions remain unclear due to an insufficient number of samples and polymorphic sites. Also, polymorphisms of the *XRCC1* gene and NIHL susceptibility were not reported before.

In this study, we aim to investigate whether BER genes are associated with susceptibility to NIHL in 117 sensitive and 117 resistant individuals within 1170 noise-exposed workers. By using the Single Nucleotide Polymorphism Database (dbSNP data), three putative SNPs in *hOGG1* (rs2072668), *APEX1* (rs1130409), and *XRCC1* (rs1799782) were selected and the genetic interactions of these three polymorphisms and their relation to NIHL risk among the Northern Han Chinese population were evaluated.

Patients And Methods

2.1 Patients

A total of 1170 noise-exposed workers from a single factory located in northern China were enrolled in the current study in December 2017. Informed consent was obtained from all individual participants, and research was approved by the ethical committee of the XXX. Data concerning the general information, lifestyle, past medical history, and exposure to chemical/physical factors was gathered. To exclude confounding factors other than genetic susceptibility as much as possible, we selected the 10% most susceptible and 10% most resistant subjects of 1170 workers (based on a high-frequency hearing threshold) for genetic analysis.

2.2 Pure tone audiometry and environmental noise measurement

As described in a previous study [17], 500, 1,000, 2,000, 3,000, 4,000, and 6,000 Hz pure tone air hearing threshold tests were conducted in a sound-attenuating chamber by an otolaryngologist. The subjects were required to avoid loud noise exposure (> 85d) for at least 12 hours before pure tone audiometry. An ascending method in 5 dB(A) steps was adopted to ascertain the hearing threshold levels of both ears according to the Diagnostic Criteria of Occupational Noise-Induced Hearing Loss of China [10].

Individual sound pressure noise meters (Noise-Pro, Quest, Oconomowoc, WI USA) were used to measure noise exposure levels for each individual in the workplace at 10am, 3pm, and 5pm for three consecutive

days.

2.3 SNP Selection

For the aim of the current study, the analysis of the genotyping data was focused on candidate SNPs located in genes involved in the base-excision repair pathway. First, SNPs were selected based on the data of 1000 Genomes Project and dbSNP (http://www.ncbi.nlm.nih.gov/) and primary literature review. The criteria for identifying SNPs included a minor allele frequency (MAF) in the Han Chinese population (CHB) > 0.10 and the linkage disequilibrium (LD) r^2 > 0.8. Then, we screened out the SNPs which were in functional regions (missense, 3'UTR and 5'UTR) or reported in human diseases previously. Finally, rs2072668, rs1130409 and rs1799782 met our requirements and were applied to the following research.

2.4 SNP Genotyping

Genomic DNA was extracted from 200μl of peripheral blood samples using QIAcube HT and QIAamp 96 DNA QIAcube HT Kits (Qiagen, Dusseldorf, Germany). The three SNPs, rs2072668, rs1130409, and rs1799782, were genotyped using ABI TaqMan SNP genotyping assays on the ABI 7900HT system (Applied Biosystems, Foster City, CA, USA). The genotyping results were analyzed using ABI SDS 2.4 Software (Applied Biosystems).

2.5 Statistical Analysis

The chi-square goodness-of-fit test was used to evaluate the deviation of the genotype frequencies of the three SNPs from Hardy-Weinberg equilibrium (HWE) in the control subjects. Comparison of the distribution of *hOGG1*, *APEX1*, and *XRCC1* genotypes between sensitive and resistant individuals were conducted using Pearson's chi-square test. Multivariate unconditional logistic regression adjusting for age, sex, tobacco use, and alcohol consumption was performed to estimate the odds ratio (OR) and 95% confidence interval (95% CI) for the associations of the selected SNPs with NIHL risk. Generalized multifactor dimensionality reduction (GMDR), a generalized combinatorial approach for detecting gene-by-gene and gene-by-environment interactions, adopts dimension reduction strategy to discover the interactions [19]. GMDR v0.9 software was used to explore the interactions of the three selected SNPs with environment. SHEsis platform was applied to haplotype analysis. (http://www.nhgg.org/analysis/) [20]. All statistical analyses were performed using SPSS 24.0 software (IBM, NYC, USA), and values of $P < 0.05$ were considered significant.

Results

3.1 Demographic characteristics of the study subjects and Hardy-Weinberg test

General demographic and lifestyle features (age, sex, tobacco, and alcohol consumption habits), duration of noise-exposed work time, noise intensity, and high-frequency hearing threshold of the sensitive and resistant samples are shown in **Table 1**. There is no significant difference between sensitive and resistant

samples regarding the general characteristics and lifestyle features, duration of noise-exposed work time, and noise intensity ($P > 0.05$). The average high-frequency hearing threshold was significantly higher in sensitive samples (52.35 ± 6.63 dB) than for resistant samples (8.98 ± 2.27 dB) ($P < 0.001$). General data of the selected SNPs and Hardy-Weinberg test results are shown in **Table 2**. Rs2072668 of *hOGG1*, rs1799782 of *XRCC1*, and rs1130409 of *APEX1* are intron, missense, and missense variants, respectively. All selected SNPs have minor allele frequencies ≥ 5% and are within Hardy-Weinberg equilibrium (HWE) ($P > 0.05$).

3.2 Single SNP analysis

Table 3 shows genotype frequencies of the sensitive and resistant groups. The *P*-values resulting after statistical analysis of the single SNPs were also presented. In the codominant model, rs1799782 TT, rs1130409 GG and rs1130409 GT were more frequent in the sensitive group (P= 0.005, OR = 8.92, 95% CI = 1.91 to 41.63; P= 0.039, OR= 2.21, 95% CI = 1.04 to 4.70; P= 0.004, OR = 2.48, 95% CI = 1.34 to 4.61, respectively). For the rs1130409 dominant model, genotype GG and GT was more frequent in the sensitive group (76.1%) compared to the resistant group (58.1%) (P= 0.003, OR = 2.39, 95% CI = 1.34 to 4.27). Genotype TT was more frequent in the sensitive group (12.0%) compared to the resistant group (1.7%) for the rs1799782 recessive model with odds ratio (OR) = 8.83 (P= 0.005, 95% CI= 1.93 to 40.36). Furthermore, compared to the rs1799782 C allele frequency, the T allele frequency increased in the sensitive group (P= 0.046, OR = 1.51, 95% CI = 1.01 to 2.26). Rs1130409 G allele frequency also increased in the sensitive group compared to the resistant group (P= 0.015, OR = 1.59, 95% CI = 1.10 to 2.31).

3.3 Stratification analysis

Stratified analyses of SNPs were conducted under allelic model and the results were presented in **Table 4**. An increased risk was evident in > 95 dB(A) cumulative noise exposure individuals who carried the *XRCC1* rs1799782 T allele (adjusted OR = 1.76, 95% CI = 1.05 to 2.98).

3.4 Haplotypes analysis

Table 5 presented eight haplotypes (frequency > 2%) originated from the target SNPs. The results showed that the haplotype CTG (rs2072668-rs1799782-rs1130409) increase the susceptibility to NIHL (OR= 2.71, 95%CI= 1.23 to 6.05) while the haplotype GCT (rs2072668-rs1799782-rs1130409) was found to be a protective factor for NIHL (OR= 0.51, 95% CI= 0.32 to 0.80).

3.5 Gene and environment interaction analysis

We applied the GMDR v0.9 software to detect the interaction of the three selected SNPs with environment. **Table 6** shows the best fit model, testing balanced accuracy, cross-validation (CV) consistency, and *P*-values. In all of the models, rs1130409, rs1130409-drinking, and rs1799782-rs1130409-smoking were the best fit models. The analysis showed that rs1130409 and drinking had a statistically significant interaction (P = 0.0002, OR = 2.77, 95% CI = 1.61 to 4.77). Rs1799782, rs1130409,

and smoking also had a statistically significant interaction with $P < 0.0001$ (OR = 3.71, 95% CI = 2.16 to 6.38). Diagrams of the best fit model are shown in **Figure 1**.

Discussion

In the current study, our results showed significant statistical association of the rs1799782 TT genotype located in the *XRCC1* coding region and the rs1130409 GG/GT in the *APEX1* encoding region with increased risk of noise-induced hearing loss in a Chinese population. One interesting finding is that the *APEX1* rs1130409 polymorphism has been previously reported to contribute to the susceptibility of NIHL in an eastern Chinese population by Shen et al., hence, providing additional support that *APEX1* rs1130409 is a potential NIHL susceptibility gene. Rs1799782 *XRCC1* was found to be associated with NIHL susceptibility in a Chinese population for the first time.

The *XRCC1* is a 33kb long gene located in the chromosome 19q13.3 region. It consists of 17 exons and encodes a 2.2kb transcript, producing X-ray cross-complementing group 1. It has potential interaction with DNA polymerase Beta (POLB), Poly ADP ribose Polymerase (PARP), and DNA ligase III in the BER pathway. Rs1799782 (Arg194Trp, 580C>T) mutation within the *XRCC1* gene leads to changes of amino acids. These changes may alter the efficiency of XRCC1 in DNA repair and may have vital functional significance. Previously published research showed that the *XRCC1* gene codon 194 (rs1799782) is located at a conserved residue in the human genome, indicating that this polymorphism may have functional significance [21]. There are studies that suggest that protein ability can be affected by amino acid substitutions in evolutionary conserved regions [22]. The functional effect of *XRCC1* rs1799782 has not been well illuminated yet.

Another enzyme that plays a primary role in base excision repair is *APEX1*. *APEX1* completes the restoration through excising abasic residues and poly polymerase-1 binding in DNA containing strand breaks, DNA polymerase-β, polynucleotide kinase, and DNA ligase III. For the rs1130409 (Asp148Glu, -656T>G) polymorphism of *APEX1*, functional studies suggest that mutation to a G allele may alter endonuclease DNA-binding activity, reduced ability to communicate with other base excision repair proteins, and decreased capacity to repair DNA oxidative damage [23, 24].

Some studies have also reported adverse effects of smoking on hearing ability [25, 26]. Our results showed an interaction between cigarette use and SNPs (rs1799782 and rs1130409) with a NIHL risk of OR= 3.71. Adverse effects were also observed between alcohol consumption and NIHL in this study. However, there is controversy about the effects of smoking and drinking on hearing loss; more study is required to confirm these possibilities [27, 28].

Our study was the first to investigate the association between *XRCC1* rs1799782 and *APEX1* rs1130409 polymorphisms and NIHL risk. One limitation of our study was that workers who were exposed to steady noise for more than 20 years, but less to other occupational hazards, were enrolled in our study. Moreover, the NIHL workers with both low and high frequency hearing ranges worse than 25 dB were all transferred from noisy environments; therefore, selection bias may exist in our study.

Conclusion

Our findings support potential association for variants in *XRCC1* rs1799782 and *APEX1* rs1130409 with inherited susceptibility of noise-induced hearing loss. However, the concrete mechanism underlying NIHL of *XRCC1* rs1799782 and *APEX1* rs1130409 will need to be investigated in future studies.

Abbreviations

NIHL: Noise induced hearing loss; PTA: Pure-tone audiometry; SNP: Single nucleotide polymorphism; HC: Hair cells; MAF: Minor allele frequency; OR: Odds ratios.

Declarations

Acknowledgements

Not applicable

Funding

This research was funded by Jiangsu Province's Outstanding Medical Academic Leader program (CXTDA2017029).

Availability of data and materials

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

Author Contributions

ED performed the experiments and wrote the paper. JL and HS collected the specimens. ED, HZ, WG and HS statistically analyzed the data. B.Z. designed the research and wrote the paper. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Institutional Review Board of the Jiangsu Provincial Center for Disease Prevention and Control granted ethical clearance for the study. Written informed consent was sought from all participants.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests regarding the publication of this paper.

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Tables

Table 1. Demographic characteristics of study subjects

Variables	Sensitive group (n = 117)		Resistant group (n = 117)		*P*
	n	%	n	%	
Age (years)					
Mean± SD	40.72± 6.60		41.87± 4.56		0.121[a]
Sex					
Male	112	95.7	109	93.2	0.392[b]
Female	5	4.3	8	6.8	
Tobacco use					
Now	59	50.4	61	52.1	0.249[b]
Ever	3	2.6	8	6.8	
Never	55	47.0	48	41.0	
Alcohol consumption					
Now	40	34.2	51	43.6	0.374[c]
Ever	3	2.6	3	2.6	
Never	74	63.2	2.6	53.8	
Work time with noise (years)					
Mean± SD	191.81± 7.67		18.79± 6.94		0.288[a]
Expose level with noise (dB)					
Mean± SD	87.01± 8.11		87.01± 6.37		1.000[a]
High frequency hearing threshold (dB)					
Mean± SD	52.35± 6.63		8.98± 2.27		**<0.001**[a]
< 26	0	0	117	100.0	
≥ 26	117	100	0	0.0	

[a] *Students'* t-test

[b] Two-sided χ^2 test

[c] Fisher's Exact Test.

Table 2. General information of selected SNPs and Hardy-Weinberg test

Gene	SNP	Alleles	Chromosome	Functional Consequence	MAF		*P* for HWE[b]
					Control[a]	Database	
hOGG1	rs2072668	C/G	3:9756456	intron variant	0.376	0.378	0.926
XRCC1	rs1799782	C/T	19:43553422	missense	0.296	0.267	0.149
APEX1	rs1130409	G/T	14:20456995	missense	0.438	0.452	0.529

[a] Data from NCBI dbSNP

[b] *P* value of Hardy-Weinberg test.

Table 3. Distribution of three polymorphisms and the association with NIHL

Genetic models	Genotypes	Sensitive group		Resistant group		Adjusted P^a	Adjusted OR (95% CI)[a]
		n = 117	%	n = 117	%		
rs2072668							
Codominant	GG	34	29.1	39	33.3		1.00 (Ref.)
	CC	15	14.5	17	14.5	0.874	1.07 (0.45-2.55)
	CG	68	52.1	61	52.1	0.359	1.32 (0.73-2.38)
Dominant	GG	34	29.1	39	33.3		1.00 (Ref.)
	CC+CG	83	70.9	78	66.7	0.414	1.27 (0.72-2.25)
Recessive	CG+GG	102	87.2	100	85.5		1.00 (Ref.)
	CC	15	12.8	17	14.5	0.766	0.89 (0.41-1.92)
Alleles	G	136	58.1	139	59.4		1.00 (Ref.)
	C	98	41.9	95	40.6	0.695	1.08 (0.74-1.57)
rs1799782							
Codominant	CC	51	43.6	59	50.4		1.00 (Ref.)
	CT	52	44.4	56	47.9	0.940	1.02 (0.59-1.76)
	TT	14	12.0	2	1.7	**0.005**	8.92 (1.91-41.63)
Dominant	CC	51	43.6	59	50.4		1.00 (Ref.)
	CT+TT	66	56.4	58	49.6	0.344	1.29 (0.76-2.17)
Recessive	CC+CT	103	88.0	115	98.3		1.00 (Ref.)
	TT	14	12.0	2	1.7	**0.005**	8.83 (1.93-40.36)
Alleles	C	154	65.8	174	74.4		1.00 (Ref.)
	T	80	34.2	60	25.6	**0.046**	1.51 (1.01-2.26)
rs1130409							
Codominant	TT	28	23.9	49	41.9		1.00 (Ref.)
	GG	26	22.2	21	17.9	**0.039**	2.21 (1.04-4.70)
	GT	63	53.8	47	40.2	**0.004**	2.48 (1.34-4.61)
Dominant	TT	28	23.9	49	41.9		1.00 (Ref.)
	GG+GT	89	76.1	68	58.1	**0.003**	2.39 (1.34-4.27)
Recessive	GG	26	22.2	21	17.9		1.00 (Ref.)
	GT+TT	91	77.8	96	82.1	0.428	1.30 (0.68-2.51)
Alleles	T	119	50.9	145	62.0		1.00 (Ref.)
	G	115	49.1	89	38.0	**0.015**	1.59 (1.10-2.31)

[a] Adjusted for age, sex, tobacco use and alcohol consumption in logistic regression model.

Table 4. Stratified analysis of SNPs in allelic model

SNPs	Group	Alleles	Cumulative noise exposure (dB) ≤ 95	> 95
rs2072668	Sensitive group	C	17	81
		G	29	107
	Resistant group	C	46	49
		G	64	75
	Adjusted P[a]		0.613	0.390
	Adjusted OR (95% CI)[a]		0.83 (0.39-1.73)	1.23 (0.76-1.99)
rs1799782	Sensitive group	C	31	123
		T	15	65
	Resistant group	C	79	95
		T	31	29
	P[a]		0.611	**0.034**
	Adjusted OR (95% CI)[a]		1.22 (0.57-2.63)	1.76 (1.05-2.98)
rs1130409	Sensitive group	G	20	95
		T	26	93
	Resistant group	G	38	51
		T	72	73
	P[a]		0.309	0.126
	Adjusted OR (95% CI)[a]		1.46 (0.71-3.03)	1.44 (0.90-2.30)

dB, decibel.

[a] Adjusted for age, sex, tobacco use and alcohol consumption in logistic regression model.

Table 5. Major haplotype frequencies of genes in the sensitive and resistant groups

Haplotypes[a]	Senstive group (n=117*2)		Resistant group (n=117*2)		P[b]	OR (95% CI)[c]	Global P
	n	%	n	%			
CCG	25.91	11.1	28.70	12.3	0.689	0.89 (0.51-1.57)	**0.010**
CCT	37.24	15.9	37.33	16.0	0.991	1.00 (0.61-1.64)	
CTG	22.41	9.6	8.79	3.8	**0.012**	**2.71 (1.23-6.05)**	
CTT	12.44	5.3	20.18	8.6	0.160	0.60 (0.29-1.24)	
GCG	53.77	23.0	44.85	19.2	0.312	1.26 (0.81-1.97)	
GCT	37.07	15.8	63.13	27.0	**0.003**	**0.51 (0.32-0.80)**	
GTG	12.90	5.5	6.66	2.8	0.150	1.99 (0.77-5.17)	
GTT	32.25	13.8	24.36	10.4	0.264	1.38 (0.79-2.41)	

[a] The alleles of haplotypes were arrayed as rs2072668-rs1799782-rs1130409.

[b] Two-sided c^2 test.

[c] Generated by permutation test with 1000 times of simulation.

Table 6. Analysis of the interaction by GMDR

Best model	Training balanced accuracy	Testing balanced accuracy	Cross-validation consistency	*P*	OR（95%CI）
rs1130409	0.5897	0.5897	10/10	0.0037	2.29 (1.31-4.02)
rs1130409*Drink	0.6211	0.5641	7/10	0.0002	2.77 (1.61-4.77)
rs1799782*rs1130409*Smoke	0.6629	0.5513	5/10	<0.0001	3.71 (2.16-6.38)

Figures

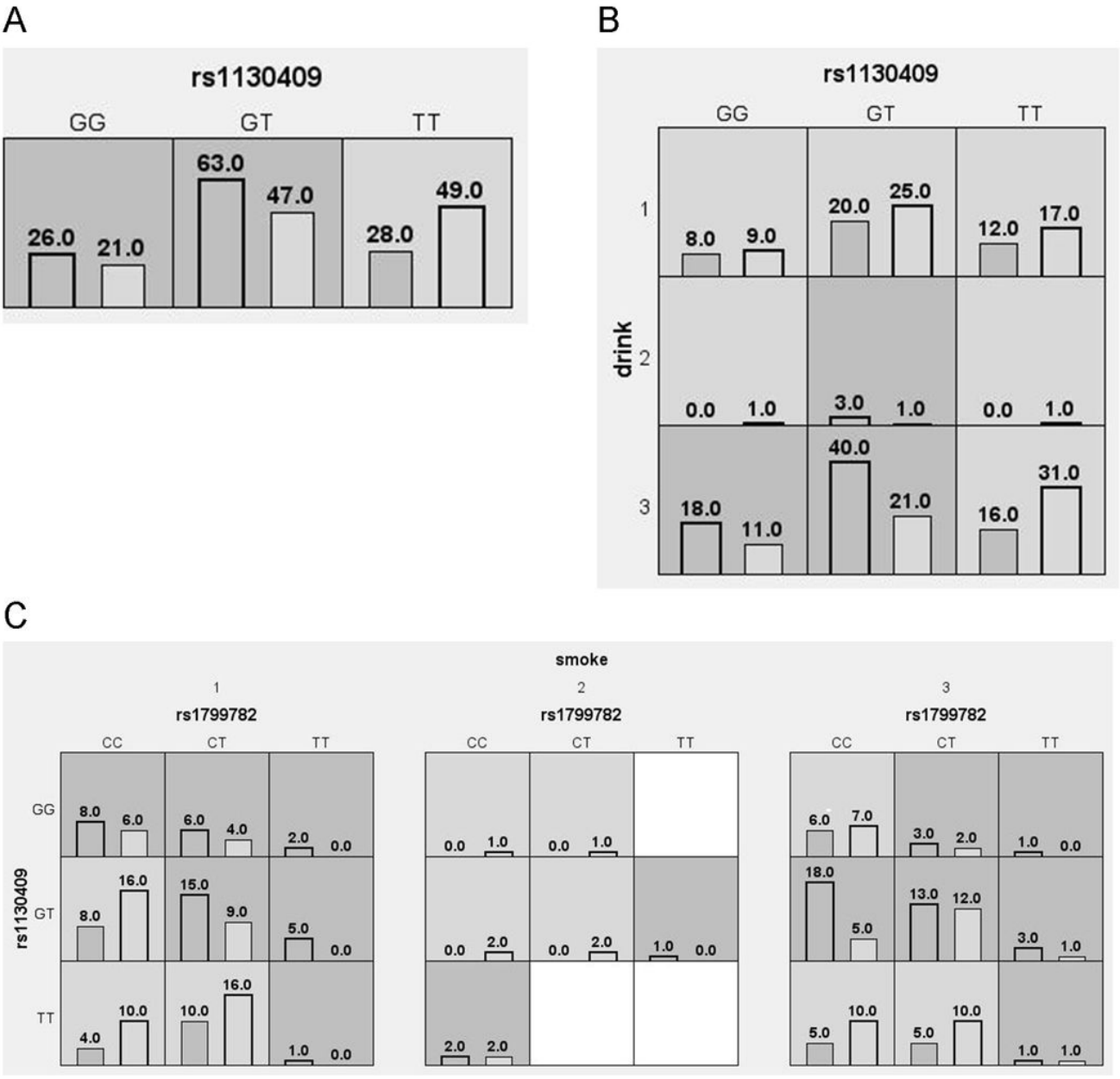

Figure 1

Best fit model gained by the analysis of GMDR. The implications of bars and background color in each multifactor cell are as follows. The left bars represent the sum of scores in case and the right represent the control. High risk cells are expressed by black shadow if the ratio of the number of cases to the number of controls exceeds the preset value T, as low risk cells by light shadow if not more than the threshold and empty cells by no shadow which means no cases and controls. The multifactor cells labeled as “high risk” or “low risk” are then used to assess the classification and predication accuracy,

thus identifying the best model in the subsequent steps (drink 1: now, 2: ever, 3: never; smoke 1: now, 2: ever, 3: never).