**N-acetyltransferase 2 Polymorphisms and Risk of Esophageal Cancer in a Chinese Population**

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**Abstract**

Esophageal cancer was the fourth leading cause of cancer death in China in 2009. Genetic factors might play an important role in the carcinogenesis of esophageal squamous cell carcinoma (ESCC). We conducted a hospital-based case-control study to evaluate ten NAT2 tagging single nucleotide polymorphisms (SNPs) on the risk of ESCC. Six hundred and twenty-nine ESCC cases and 686 controls were recruited. Their genotypes were determined using the ligation detection reaction method. In the single locus analyses, there was a borderline statistically significant difference in genotype frequencies of NAT2 rs1565684 T>C SNP between the cases and the controls (p = 0.057). The NAT2 rs1565684 CC genotype was associated with a borderline significantly increased risk for ESCC (CC vs. TT: adjusted OR = 1.77, 95% CI = 0.97–3.21, p = 0.063 and CC vs. TT/TC: adjusted OR = 1.68, 95% CI = 0.93–3.04, p = 0.085). The association was evident among older patients and patients who never drunk. After the Bonferroni correction, in all comparison models, NAT2 rs1565684 T>C SNP was not associated with ESCC risk (p > 0.05). For the other nine NAT2 SNPs, after Bonferroni correction, in all comparison models, the nine SNPs were also not associated with ESCC risk (p > 0.05). Thus, nine NAT2 tagging SNPs were not associated with risk of ESCC. NAT2 rs1565684 T>C SNP might play a slight role in ESCC etiology. Additional, larger studies and tissue-specific biological characterization are required to confirm the current findings.

**Introduction**

Esophageal cancer was the fourth leading cause of cancer death and the fifth most commonly diagnosed cancer in China in 2009 [1]. Genetic factors, such as single nucleotide polymorphisms (SNPs), might play an important role in the carcinogenesis of esophageal squamous cell carcinoma (ESCC) [2].

N-acetyltransferase 2 (NAT2) is an enzyme that plays an essential role in the metabolism of various potential carcinogens. NAT2 is mainly expressed in the human liver and gastrointestinal tract. The NAT2 gene is located on 8p21.3-23.1 and encodes a 290-amino acid protein, NAT2 [3]. NAT2 is polymorphic, and it was thought that NAT2 acetylation status alteration caused by NAT polymorphisms decreased enzymatic activity and result in absence of detoxification efficiency, which could lead to an increase in cancer susceptibility [4]. It has been reported that NAT2 polymorphisms and/or their interaction with smoking is associated with various types of malignancies. Genetic variation of NAT2 may lead to differences in the rate of aryline metabolism and consequently increase cancer risk [5]. The substrates for NAT2 that are involved in carcinogenesis, are represented mainly by heterocyclic amines and polycyclic aromatic hydrocarbon rings found in cooked or smoked meat [6] and cigarette smoke [7].

NAT2 genetic variations may contribute to the development of ESCC. In a hospital-based case-control study, we performed genotyping analyses of ten NAT2 tagging SNPs in 629 ESCC cases and 686 controls in a Chinese population.

**Materials and Methods**

**Ethical approval of the study protocol**

The Review Board of Jiangsu University (Zhenjiang, China) approved this hospital-based case-control study. We have complied with the World Medical Association Declaration of Helsinki regarding ethical conduct of research involving human subjects.
and/or animals. All subjects provided written, informed consent to be included in the study.

Patients and Controls

Six hundred and twenty-nine subjects with esophageal cancer were consecutively recruited from the Affiliated People’s Hospital of Jiangsu University and Affiliated Hospital of Jiangsu University (Zhenjiang, China) between October 2008 and December 2010. All cases of esophageal cancer were diagnosed as ESCC pathologically. The exclusion criteria were patients who had previously had: cancer; any metastasized cancer; radiotherapy or chemotherapy. The 686 controls were patients without cancer and were matched with the cases with regard to age (±5 years) and sex. They were recruited from the two hospitals mentioned above during the same time period. Most of the controls were admitted to the hospitals for the treatment of trauma.

Trained interviewers, using a pre-tested questionnaire, questioned each subject personally to obtain information on demographic data (e.g., age, sex) and related risk factors (including tobacco smoking and alcohol consumption). After the interview, 2-mL samples of venous blood were collected from each subject. Individuals who smoked one cigarette per day for >1 year were defined as “smokers”. Subjects who consumed more than three alcoholic drinks a week for >6 months were considered to be “alcohol drinkers”.

Isolation of DNA, SNPs selection and genotyping by ligation detection reaction

Blood samples were collected from patients using Vacutainers and transferred to tubes lined with ethylenediamine tetra-acetic acid (EDTA). Genomic DNA was isolated from whole blood with the QIAamp DNA Blood Mini Kit (Qiagen, Berlin, Germany) [8]. We used a block-based tagging strategy to find tagging SNPs using Haploview 4.2 software, according to the HapMap database (http://www.hapmap.org/, phase II Nov08, on NCBI B36 Haploview 4.2 software, according the HapMap database). We used a block-based tagging strategy to find tagging SNPs using Haploview 4.2 software, according to the HapMap database [9, 10]. For quality control, repeated analyses were done for 160 (12.17%) randomly selected samples with high DNA quality.

Statistical Analyses

Differences in the distributions of demographic characteristics, selected variables, and genotypes of the NAT2 variants between the cases and controls were evaluated using the χ² test. The associations between the ten SNPs and risk of ESCC were estimated by computing the odds ratios (ORs) and their 95% confidence intervals (CIs) using logistic regression analyses for crude ORs and adjusted ORs when adjusting for age, sex, smoking and drinking status. The Bonferroni correction procedure was applied because of the number of comparisons. The HWE was tested by a goodness-of-fit χ² test to compare the observed genotype frequencies to the expected frequencies among the control subjects. All statistical analyses were performed with SAS 9.1.3 (SAS Institute, Cary, NC, USA).

Results

Characteristics of the study population

Characteristics of cases and controls included in the study are summarized in Table 1. The cases and controls appeared to be adequately matched on age and sex as suggested by the χ² tests. As shown in Table 1, significant difference was detected on smoking status between the cases and the controls, and drinking rate was higher in ESCC patients than in control subjects. The primary information for eight genotyped SNPs was in Table 2. The concordance rates of repeated analyses were 100% except for NAT2 rs11996129 T>C (157/160, 98.13%); rs1565684 T>C (159/160, 99.38%) and rs1799930 G>A (159/160, 99.38%). MAF in our controls was similar to MAF for Chinese in database for all SNPs. The observed genotype frequencies for these ten polymorphisms in the controls were all in HWE except for NAT2 rs4540438 A>C (p = 0.015) (Table 2).

Associations between NAT2 tagging polymorphisms and risk of ESCC

The genotype distributions of NAT2 rs1565684 T>C in the cases and the controls are shown in Table 3. In the single locus analyses, there was a borderline statistically significant difference in genotype frequencies of NAT2 rs1565684 T>C SNP between the cases and the controls (p = 0.057). When the NAT2 rs1565684 TT homozygote genotype was used as the reference group, the TC genotype was not associated with the risk for ESCC (TC vs. TT: OR = 1.14, 95% CI = 0.90–1.44, p = 0.269); the CC genotype was associated with a significantly increased risk for ESCC (CC vs. TT: OR = 1.95, 95% CI = 1.08–3.51, p = 0.026). In the dominant model, the NAT2 rs1565684 TC/CC variants were not associated with the risk of ESCC, compared with the NAT2 rs1565684 TT genotype (TC/CC vs. TT: OR = 1.20, 95% CI = 0.96–1.51, p = 0.107). In the recessive model, when the NAT2 rs1565684 TT/TC genotypes were used as the reference group, the CC homozygote genotype was associated with an 80% increased risk for ESCC.

Table 1. Distribution of selected demographic variables and risk factors in ESCC cases and controls.

| Variable | Cases (n = 629) | Controls (n = 686) | p* |
|----------|----------------|-------------------|----|
| Age (years) | n | % | n | % | χ² |
| <63 | 310 | 49.28 | 365 | 53.21 | 0.541 |
| ≥63 | 319 | 50.72 | 321 | 46.79 | 0.155 |
| Sex | | | | | 0.185 |
| Male | 444 | 70.59 | 461 | 67.20 | | |
| Female | 185 | 29.41 | 225 | 32.80 | | |
| Tobacco use | | <0.001 | | | |
| Never | 355 | 56.44 | 499 | 72.74 | | |
| Ever | 274 | 43.56 | 187 | 27.26 | | |
| Alcohol use | | <0.001 | | | |
| Never | 428 | 68.04 | 526 | 76.68 | | |
| Ever | 201 | 31.96 | 160 | 23.32 | | |

*pTwo-sided χ² test and student t test; Bold values are statistically significant (p<0.05). doi:10.1371/journal.pone.0087783.t001
### Table 2. Primary information for NAT2 rs1041983 C>T, rs11780884 A>G, rs11996129 T>C, rs12674710 C>A, rs1390359 C>A, rs1390360 G>A, rs1565684 T>C, rs1799930 G>A, rs1799931 G>A and rs4540438 A>C polymorphisms.

| Genotyped SNPs | NAT2 rs1041983 C>T | NAT2 rs11780884 A>G | NAT2 rs11996129 T>C | NAT2 rs12674710 C>A | NAT2 rs1390359 C>A | NAT2 rs1390360 G>A | NAT2 rs1565684 T>C | NAT2 rs1799930 G>A | NAT2 rs1799931 G>A | NAT2 rs4540438 A>C |
|----------------|---------------------|---------------------|---------------------|---------------------|---------------------|---------------------|---------------------|---------------------|---------------------|---------------------|
| Chromosome     | 8                   | 8                   | 8                   | 8                   | 8                   | 8                   | 8                   | 8                   | 8                   | 8                   |
| Gene Official Symbol | NAT2 | NAT2 | NAT2 | NAT2 | NAT2 | NAT2 | NAT2 | NAT2 | NAT2 | NAT2 |
| Function       | cds-synon           | No Data             | intron region       | No Data             | No Data             | No Data             | No Data             | No Data             | missense           | missense           |
| Chr Pos (Genome Build 36.3) | 18302075 | 18290333 | 18298855 | 18307943 | 18305609 | 18305773 | 18290944 | 18302383 | 18302650 | 18307883 |
| Regulome DB Score | 5 | No Data | No Data | No Data | No Data | No Data | No Data | 4 | 6 | No Data |
| TFBSb          | Y                   | Y                   | Y                   | Y                   | Y                   | Y                   | Y                   | Y                   | Y                   | Y                   |
| Splicing (ESE or ESS) | — | — | — | — | — | — | — | — | — | — |
| miRNA (miRanda) | — | — | — | — | — | — | — | — | — | — |
| miRNA (Sanger) | — | — | — | — | — | — | — | — | — | — |
| rsSNP | — | — | — | — | — | — | — | Y | Y | — |
| MAF<sup>c</sup> for Chinese in database | 0.366 | 0.453 | 0.276 | 0.471 | 0.163 | 0.244 | 0.188 | 0.207 | 0.159 | 0.058 |
| MAF in our controls (n = 686) | 0.387 | 0.452 | 0.249 | 0.460 | 0.188 | 0.257 | 0.193 | 0.227 | 0.153 | 0.067 |
| p value for HWE<sup>d</sup> test in our controls | 0.432 | 0.317 | 0.073 | 0.544 | 0.841 | 0.484 | 0.124 | 0.328 | 0.201 | 0.015 |
| Genotyping method<sup>e</sup> | LDR | LDR | LDR | LDR | LDR | LDR | LDR | LDR | LDR | LDR |
| % Genotyping value | 98.48% | 96.43% | 96.35% | 98.18% | 96.43% | 98.48% | 98.18% | 96.81% | 95.29% | 98.18% |

<sup>a</sup>http://www.regulomedb.org/;  
<sup>b</sup>TFBS: Transcription Factor Binding Site (http://snpinfo.niehs.nih.gov/snpinfo/snpfunc.htm);  
<sup>c</sup>MAF: minor allele frequency, NAT2 rs4540438 A>C MAF is in CHB+JPT population;  
<sup>d</sup>HWE: Hardy-Weinberg equilibrium;  
<sup>e</sup>LDR: ligation detection reaction; Bold values are statistically significant (p < 0.05).  

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Table 3. Logistic regression analyses of associations between NAT2 rs1041983 C>T, rs11780884 A>G, rs11996129 T>C, rs12674710 C>A, rs1390359 C>A, rs1390360 G>A, rs1565684 T>C, rs1799930 G>A, rs1799931 G>A and rs4540438 A>C polymorphisms and risk of ESCC.

| Genotype | Cases (n = 629) | Controls (n = 686) | Crude OR (95%CI) | p | Adjusted OR* (95%CI) | p |
|----------|----------------|-------------------|-----------------|---|---------------------|---|
| NAT2 rs1041983 C>T | | | | | | |
| CC | 209 | 33.8 | 259 | 38.3 | 1.00 | 1.00 |
| CT | 315 | 50.9 | 311 | 46.0 | 1.26 (0.99–1.60) | 0.064 | 1.23 (0.96–1.57) | 0.097 |
| TT | 95 | 15.3 | 106 | 15.7 | 1.11 (0.80–1.55) | 0.535 | 1.13 (0.81–1.59) | 0.478 |
| CT+TT | 410 | 66.2 | 417 | 61.7 | 1.22 (0.97–1.53) | 0.089 | 1.21 (0.96–1.52) | 0.114 |
| CC+CT | 524 | 84.7 | 570 | 84.3 | 1.00 | 1.00 |
| TT | 95 | 15.3 | 106 | 15.7 | 0.98 (0.72–1.32) | 0.069 | 1.00 (0.74–1.37) | 0.079 |
| NAT2 rs11780884 A>G | | | | | | |
| AA | 165 | 27.3 | 206 | 31.0 | 1.00 | 1.00 |
| AG | 303 | 50.2 | 316 | 47.6 | 1.20 (0.93–1.55) | 0.172 | 1.18 (0.91–1.53) | 0.219 |
| GG | 136 | 22.5 | 142 | 21.4 | 1.20 (0.88–1.53) | 0.148 | 1.22 (0.93–1.53) | 0.163 |
| AG+GG | 439 | 72.7 | 458 | 69.0 | 1.20 (0.94–1.53) | 0.148 | 1.21 (0.96–1.52) | 0.114 |
| AA+AG | 468 | 77.5 | 522 | 78.6 | 1.00 | 1.00 |
| GG | 136 | 22.5 | 142 | 21.4 | 0.98 (0.72–1.32) | 0.069 | 1.00 (0.74–1.37) | 0.079 |
| NAT2 rs11996129 T>C | | | | | | |
| TT | 345 | 56.2 | 377 | 57.7 | 1.00 | 1.00 |
| TC | 236 | 38.4 | 227 | 34.8 | 1.14 (0.90–1.44) | 0.284 | 1.16 (0.92–1.47) | 0.220 |
| CC | 33 | 5.4 | 49 | 7.5 | 0.74 (0.46–1.17) | 0.196 | 0.80 (0.50–1.29) | 0.366 |
| TC+CC | 269 | 43.8 | 276 | 42.3 | 1.07 (0.85–1.33) | 0.579 | 1.10 (0.88–1.38) | 0.411 |
| TT+TC | 581 | 94.6 | 604 | 92.5 | 1.00 | 1.00 |
| CC | 33 | 5.4 | 49 | 7.5 | 0.70 (0.44–1.10) | 0.125 | 0.76 (0.47–1.21) | 0.243 |
| NAT2 rs12674710 C>A | | | | | | |
| CC | 166 | 27.2 | 202 | 29.7 | 1.00 | 1.00 |
| CA | 309 | 50.6 | 330 | 48.5 | 1.14 (0.88–1.47) | 0.321 | 1.11 (0.86–1.45) | 0.421 |
| AA | 136 | 22.3 | 148 | 21.8 | 1.19 (0.82–1.53) | 0.482 | 1.15 (0.84–1.58) | 0.388 |
| CA+AA | 445 | 72.8 | 478 | 70.3 | 1.13 (0.89–1.44) | 0.314 | 1.13 (0.88–1.44) | 0.351 |
| CC+CA | 475 | 77.7 | 532 | 78.2 | 1.00 | 1.00 |
| AA | 136 | 22.3 | 148 | 21.8 | 1.03 (0.79–1.34) | 0.831 | 1.07 (0.82–1.41) | 0.604 |
| NAT2 rs1390359 C>A | | | | | | |
| CC | 412 | 67.4 | 434 | 66.1 | 1.00 | 1.00 |
| CA | 179 | 29.3 | 199 | 30.3 | 0.95 (0.74–1.21) | 0.664 | 0.96 (0.75–1.23) | 0.735 |
| AA | 20 | 3.3 | 24 | 3.7 | 0.88 (0.48–1.61) | 0.676 | 1.03 (0.55–1.91) | 0.930 |
| CA+AA | 199 | 32.6 | 223 | 33.9 | 0.94 (0.74–1.19) | 0.605 | 0.97 (0.76–1.23) | 0.771 |
| CC+CA | 591 | 96.7 | 633 | 96.3 | 1.00 | 1.00 |
| AA | 20 | 3.3 | 24 | 3.7 | 0.89 (0.49–1.63) | 0.713 | 1.04 (0.56–1.93) | 0.896 |
| NAT2 rs1390360 G>A | | | | | | |
| GG | 343 | 55.4 | 377 | 55.8 | 1.00 | 1.00 |
| GA | 242 | 39.1 | 251 | 37.1 | 1.06 (0.84–1.33) | 0.620 | 1.07 (0.85–1.36) | 0.555 |
| AA | 34 | 5.5 | 48 | 7.1 | 0.78 (0.49–1.24) | 0.289 | 0.88 (0.55–1.42) | 0.609 |
| GA+AA | 276 | 44.6 | 299 | 44.2 | 1.02 (0.82–1.26) | 0.897 | 1.05 (0.84–1.31) | 0.703 |
| GG+GA | 585 | 94.5 | 628 | 92.9 | 1.00 | 1.00 |
| AA | 34 | 5.5 | 48 | 7.1 | 0.76 (0.48–1.20) | 0.237 | 0.86 (0.54–1.37) | 0.519 |
| NAT2 rs1565684 T>C | | | | | | |
| TT | 366 | 59.9 | 437 | 64.3 | 1.00 | 1.00 |
| TC | 214 | 35.0 | 224 | 32.9 | 1.14 (0.90–1.44) | 0.269 | 1.14 (0.90–1.45) | 0.270 |
| CC | 31 | 5.1 | 19 | 2.8 | 1.95 (1.08–3.51) | 0.026 | 1.77 (0.97–3.21) | 0.063 |
of ESCC (CC vs. TT/TC: OR = 1.86, 95% CI = 1.04–3.33, \(p = 0.037\)) (Table 3). After adjusted for age, sex, smoking and drinking status, the CC genotype was associated with a borderline significantly increased risk for ESCC (CC vs. TT: adjusted OR = 1.77, 95% CI = 0.97–3.21, \(p = 0.063\) and CC vs. TT/TC: adjusted OR = 1.68, 95% CI = 0.93–3.04, \(p = 0.085\)). After the Bonferroni correction, in all comparison models, NAT2 rs1565684 T>C SNP was not associated with ESCC risk (\(p_{\text{correct}} > 0.05\)).

For the other nine SNPs, in the single locus analyses, there was no statistically significant difference in genotype frequencies of these nine SNPs between the cases and the controls (\(p > 0.05\)). Logistic regression analyses revealed that none of these nine polymorphic sites was associated with the susceptibility to ESCC. In all comparison models, the nine SNPs were not associated with ESCC risk (\(p > 0.05\)) before and after the Bonferroni correction (Table 3).

Stratification analyses of NAT2 rs1565684 T>C polymorphisms and risk of ESCC

To evaluate the effects of NAT2 rs1565684 T>C genotypes on ESCC risk according to different age, sex, smoking and alcohol drinking status; we performed the stratification analyses (Table 4). A significantly increased risk of ESCC associated with the NAT2 rs1565684 T>C polymorphism was evident among older patients and patients who never drunk.

Discussion

In this hospital-based case-control study of ESCC, we found that ten selected NAT2 tagging SNPs were not associated with the risk of ESCC after the Bonferroni correction. NAT2 rs1565684 CC genotype was associated with a borderline significantly increased risk for ESCC. A significantly increased risk of ESCC associated with the NAT2 rs1565684 T>C polymorphism was evident among older patients and patients who never drank. To the best of our knowledge, it's the first positive finding of NAT2 rs1565684 T>C polymorphism and ESCC risk.

NAT2 is involved in the metabolism of a major class of tobacco smoke carcinogens (the aromatic amines) and NAT2 variant alleles result in slow clearance of aromatic amines. In humans, the NAT2 gene encodes a phase II enzyme that plays an essential role in aromatic, heterocyclic amines and hydrazines metabolism [11]. NAT2 influences the detoxification of aromatic and heterocyclic amine carcinogens (which are present in tobacco smoke) by two pathways: the metabolism reaction may result in the detoxification by N-acetylation, or bioactivation by O-acetylation often preceded by CYP450 hydroxylation [11].

Table 3. Cont.

| Genotype | Cases (n = 629) | Controls (n = 686) | Crude OR (95%CI) | \(p\) | Adjusted OR* (95%CI) | \(p\) |
|----------|----------------|-------------------|------------------|-----|---------------------|-----|
| n | % | n | % | | |
| TC+CC | 245 | 40.1 | 243 | 35.7 | 1.20 (0.96–1.51) | 0.107 | 1.19 (0.95–1.50) | 0.130 |
| TT+TC | 580 | 94.9 | 661 | 97.2 | 1.00 | | | |
| CC | 31 | 5.1 | 19 | 2.8 | 1.86 (1.04–3.33) | 0.037 | 1.68 (0.93–3.04) | 0.085 |

NAT2 rs1799930 G>A

| Genotype | Cases (n = 629) | Controls (n = 686) | Crude OR (95%CI) | \(p\) | Adjusted OR* (95%CI) | \(p\) |
|----------|----------------|-------------------|------------------|-----|---------------------|-----|
| n | % | n | % | | |
| GG | 375 | 62.5 | 407 | 60.5 | 1.00 | | | | | |
| GA | 200 | 33.3 | 227 | 33.7 | 0.96 (0.76–1.21) | 0.711 | 0.97 (0.76–1.23) | 0.804 |
| AA | 25 | 4.2 | 39 | 5.8 | 0.70 (0.41–1.17) | 0.173 | 0.78 (0.45–1.33) | 0.353 |

NAT2 rs1799931 G>A

| Genotype | Cases (n = 629) | Controls (n = 686) | Crude OR (95%CI) | \(p\) | Adjusted OR* (95%CI) | \(p\) |
|----------|----------------|-------------------|------------------|-----|---------------------|-----|
| n | % | n | % | | |
| GG | 406 | 67.3 | 462 | 67.1 | 1.00 | | | | | |
| GA | 181 | 30.0 | 177 | 27.2 | 1.16 (0.91–1.49) | 0.228 | 1.15 (0.89–1.47) | 0.289 |
| AA | 16 | 2.7 | 11 | 1.7 | 1.66 (0.76–3.61) | 0.205 | 1.35 (0.61–2.99) | 0.465 |

NAT2 rs4540438 A>C

| Genotype | Cases (n = 629) | Controls (n = 686) | Crude OR (95%CI) | \(p\) | Adjusted OR* (95%CI) | \(p\) |
|----------|----------------|-------------------|------------------|-----|---------------------|-----|
| n | % | n | % | | |
| AA | 522 | 85.4 | 596 | 87.6 | 1.00 | | | | | |
| AC | 86 | 14.1 | 77 | 11.3 | 1.28 (0.92–1.77) | 0.148 | 1.31 (0.93–1.83) | 0.118 |
| CC | 3 | 0.5 | 7 | 1.0 | 0.49 (0.13–1.60) | 0.302 | 0.52 (0.13–2.04) | 0.344 |

NAT2 rs1565684 T>C polymorphism was evident among older patients and patients who never drank (Table 4).

*Adjusted for age, sex, smoking status and alcohol consumption; Bonferroni correction was performed to correct the \(p\) value (\(p_{\text{correct}}\)); For the 10 NAT2 SNPs, \(p_{\text{correct}} > 0.05\) in all comparison models; Bold values are statistically significant (\(p < 0.05\)).

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Polymorphisms of NAT2 and Esophageal Cancer Risk

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| Variable            | NAT2 rs1565684 T>C (case/control)* | Adjusted OR\(^b\) (95% CI); p\(^c\) p\(^h\) | \(\chi^2\) p-value |
|---------------------|------------------------------------|---------------------------------------------|-------------------|
| Sex                 |                                    |                                             |                   |
| Male                | 263/290                            | 1.04 (0.78–1.38); p: 0.815; p\(^h\):0.621 | 1.86 (0.94–3.68); p: 0.077; p\(^h\):0.621 |
| Female              | 103/147                            | 1.41 (0.93–2.13); p: 0.106; p\(^h\):0.621 | 1.53 (0.43–5.45); p: 0.516; p\(^h\):0.621 |
| Age                 |                                    |                                             |                   |
| <63                 | 187/231                            | 0.92 (0.66–1.30); p: 0.645; p\(^h\):0.591 | 1.73 (0.70–4.31); p: 0.239; p\(^h\):0.591 |
| ≥63                 | 179/206                            | 1.38 (0.99–1.94); p: 0.059; p\(^h\):0.591 | 1.73 (0.78–3.87); p: 0.178; p\(^h\):0.591 |
| Smoking status      |                                    |                                             |                   |
| Never               | 205/323                            | 1.22 (0.91–1.64); p: 0.190; p\(^h\):0.378 | 2.03 (0.90–4.59); p: 0.088; p\(^h\):0.636 |
| Ever                | 161/114                            | 1.05 (0.70–1.58); p: 0.821; p\(^h\):0.378 | 1.51 (0.62–3.69); p: 0.368; p\(^h\):0.636 |
| Alcohol consumption |                                    |                                             |                   |
| Never               | 252/341                            | 1.17 (0.88–1.56); p: 0.273; p\(^h\):0.908 | 2.38 (1.10–5.14); p: 0.028; p\(^h\):0.150 |
| Ever                | 114/96                             | 1.12 (0.71–1.77); p: 0.615; p\(^h\):0.908 | 1.14 (0.43–3.02); p: 0.799; p\(^h\):0.150 |

*The genotyping was successful in 611 (97.1%) ESCC cases, and 680 (99.1%) controls for NAT2 rs1565684 T>C.

\(^b\)Adjusted for age, sex, smoking status and alcohol consumption (besides stratified factors accordingly) in a logistic regression model;

\(^c\)p\(^h\) for heterogeneity.

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Previous case-control reports have yielded inconsistent results regarding the association of \( \text{NAT2} \) SNPs with cancers, possibly because of the small number of subjects, which would compromise the power of the statistical analyses in these studies. In the esophagus, the slow \( \text{NAT2} \) acetylator genotype was more susceptible to esophageal cancer in Japan [12]. However, in another study in Taiwan, \( \text{NAT2} \) polymorphisms did not affect the risk of esophageal cancer, irrespective of environmental factors [13]. In a more recent study in India, \( \text{NAT2} \) acetylator genotypes did not influence susceptibility to esophageal cancer. \( \text{NAT2} \) polymorphisms did not significantly modulate the cancer risk after interaction with environmental factors, such as tobacco, alcohol or occupational exposure [14]. In another study in the Kashmir Valley, none of the three \( \text{NAT2} \) polymorphic alleles (rs1799929, rs1799930 and rs1799931) was found to be independently associated with risk of esophageal and gastric cancers [15], which was also in accordance with our results. Meta-analysis also suggested that \( \text{NAT2} \) genotypes are not associated with lung cancer [16], gastric cancer [17], breast cancer [18], prostate cancer [19] and oral cancer [20]. \( \text{NAT2} \) rs1565684 \( >C \) is in linkage disequilibrium with another important SNP \( \text{NAT2} \) rs4354600 \( >A \) (\( r^2 = 0.845 \)) in Chinese Han Beijing population. Although \( \text{NAT2} \) rs1565684 \( >C \) SNP is functional using SNP function prediction websites (http://snpinfo.nichs.nih.gov/snpinfo/snpfunc.htm and http://www.regulomedb.org/). The etiology of \( \text{NAT2} \) rs1565684 \( >C \) SNP is still not well known and need further investigation.

This case-control study had several limitations. First, the patients and controls were enrolled from hospitals; inherent bias may have resulted in spurious findings. Second, the statistical power of our study was limited because of the moderate sample size and absence of a validation cohort; further replication studies are needed. Third, the viral infections and immune parameters information were not available, which restricted the power of our analyses. Finally, we did not obtain detailed information on cancer metastasis and survival, which restricted further analyses of the roles of the \( \text{NAT2} \) polymorphisms in ESCC progression and prognosis.

In conclusion, our study provides evidence that \( \text{NAT2} \) tagging SNPs may not contribute to the risk of ESCC. Larger well-designed studies are required to confirm the current findings.

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**Author Contributions**

Conceived and designed the experiments: HG LW WT YF Y. Shi Y. Sun SC JY. Performed the experiments: Y. Shi Y. Sun JZ XW LZ LW AS. Analyzed the data: HG LW JY YF SC. Contributed reagents/materials/analysis tools: NX LW LZ GD CL RL. Wrote the paper: JY LW WT YF Y. Shi Y. Sun SC. Performed the experiments: HG LW WT YF Y. Shi Y. Sun SC JY. Contributed reagents/materials/analysis tools: HG LW WT YF Y. Shi Y. Sun SC JY. Performed the experiments: HG LW WT YF Y. Shi Y. Sun SC JY.

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