Validation study of susceptibility loci for esophageal squamous cell carcinoma identified by GWAS in a Han Chinese subgroup from Eastern China

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Abstract

Esophageal squamous cell carcinoma (ESCC) occurs at a relatively high frequency in China and is one of the most prevalent cancers in the world. Genome-wide association studies (GWAS) have identified 24 single-nucleotide polymorphisms (SNPs) that could be associated with ESCC in Chinese patients. This retrospective study aimed to validate the association between these 24 SNPs and ESCC in a Han Chinese subgroup from East China. A total of 2280 and 1900 patients with ESCC (case group) and non-esophageal cancer (control group) were included from a single center. Genotyping of the 24 polymorphisms was performed using the Sequenom MassARRAY system. Unconditional logistic regression analyses were conducted for every polymorphism. It was found that rs12188136 (P=0.027, OR=1.158, 95% CI=1.016-1.319 for AG/AA) was associated with ESCC. Binary logistic regression analyses revealed a significant negative association of rs875339 in RORA (P=0.014, OR=0.762, 95% CI=0.613-0.947 for TT/CC). Under the dominant model, rs6854472 was slightly associated with ESCC risk (P=0.048, OR=1.192, 95% CI=1.002-1.418). Under the recessive model, a significant negative association was observed for rs875339 (P=0.010, OR=0.758, 95% CI=0.615-0.935). In a word, this large-scale replication study validated that rs12188136 and rs6854472 are associated with ESCC in a Han Chinese subgroup from Eastern China, and that rs875339 is negative associated with ESCC.

Key words: esophageal squamous cell carcinoma (ESCC), genome-wide association study (GWAS), single nucleotide polymorphism (SNP), MassARRAY system, Han Chinese population

Introduction

Esophageal cancer is among the most incident malignant tumors worldwide [1] and a serious threat to human health and quality of life [2]. Esophageal squamous cell carcinoma (ESCC) is one of the two main sub-types of esophageal cancer, and ESCC is more common than esophageal adenocarcinoma in the developing world, especially in China [3]. The prognosis of ESCC is poor despite advances in treatment, with 5-year overall survival rate ranging from 15% to 25% [4,5].

Accumulating evidence has demonstrated that genetic factors [6-10], family history of ESCC [11-13], lifestyle habits [14-16], environmental factors [17-23], and HPV infection [24] play important roles in the
development of ESCC. Significant interactions were found between HPV serological status and genetic loci, increasing the risk of ESCC [25,26]. Other risk factors such as exposure to polycyclic aromatic hydrocarbons (PAHs), high-temperature foods, diets, oral health and microbial communities, but they require further research. Esophageal carcinogenesis is the result of the interaction among heredity, environment and living habits [27-29].

In recent years, genome-wide association studies (GWAS) have confirmed the contribution of gene variations to ESCC [30-35]. Six large-scale GWAS of Chinese populations have focused on identifying genetic susceptibility loci for ESCC [31-35]. The earliest ESCC GWAS analysis using 2115 ESCC cases and 3302 controls in a Chinese population revealed that PLCE1 carried cancer susceptibility [31]. Wang et al. identified two new genome-wide significant loci for ESCC: PLCE1 at 10q23 and C20orf54 at 20p13 [32]. Seven loci on chromosomes 5q11, 6p21, 10q23, 12q24, 13q33) had a significant association but only when considering the gene-alcohol interaction. Wu et al. [34] identified nine new ESCC susceptibility loci: rs1050631 in SLC39A6 as being associated with the survival of ESCC patients [35]. Whether those 24 SNPs found by the five GWAS confer an increased risk of ESCC in various Han Chinese populations has not yet been validated. Therefore, we conducted a case-control study to validate the associations of those 24 SNPs with the risk of ESCC in a Han Chinese subgroup from Eastern China.

Material and Methods

Study population

This was a retrospective study. We included 2280 consecutive ESCC subjects and 1900 non-ESCC subjects (control group). The diagnosis of ESCC was confirmed by histopathology or cytology by at least two local pathologists. Histological examination was performed according to the World Health Organization (WHO) criteria [36]. The exclusion criteria for both groups were: 1) psychiatric disorder; 2) any other primary cancer; or 3) a family history of cancer. This study consisted of two ESCC sets: (a) 1900 patients with primary ESCC, and (b) 380 patients with second ESCC. The patients were recruited between January 2012 and December 2014 at Zhejiang Cancer Hospital. Demographic characteristics of the subjects (including gender, age, histological types of esophageal cancer, smoking and drinking status) were obtained from the medical records. Non-ESCC individuals (n=1900) were recruited as control subjects during a routine health check-up (physical examination) at the same hospital during the same time period. The two groups were matched based on the frequency of age and sex. In the present study, all participants were ethnic Han Chinese that lived within the Zhejiang Province of Eastern China.

SNP selection

We selected the 24 top SNPs (rs4478858, rs10881372, rs10801638, rs10173378, rs888103, rs3815501, rs6717108, rs10934685, rs6768588, rs9824873, rs6854472, rs12188136, rs2294693, rs9364414, rs7916519, rs11225815, rs10895458, rs4578395, rs11059556, rs2025245, rs9584006, rs347940, rs875339, and rs12922317) from the reports focusing on ESCC susceptibility loci identified by five GWAS projects in Han Chinese (PubMed search) [31-35].

SNP genotyping assays

Venous blood (2 mL) was sampled in citrate glass tubes and kept at -40°C. Leukocyte total genomic DNA was extracted from 1 mL of peripheral blood using the Whole Blood DNA Extraction Kit (QIAamp® DNA Blood Mini Kit), according to the manufacturer’s instructions. The extracted genomic DNA was dissolved in 0.1× TE buffer (10 mM Tris and 1 mM EDTA, pH 8.0) to 0.4-0.6 mg/mL and stored at -20°C.

The SNPs were determined using iPLEX chemistry on a matrix-assisted laser desorption/ionization time-of-flight mass spectrometer (MALDI-TOF-MS, MassARRAY system, Sequenom, Inc.), as previously published [37]. PCR reactions (5 μL each) were carried out in 384-well plates using 10 ng of genomic DNA, 0.5 units of Taq polymerase (HotStarTaq, Qiagen), 500 μmol of each of the four deoxy-nucleotides triphosphate (dNTP), and 100 nmol of each primer. An ABI-9700 thermocycler was used with the following program: 1) 15 min at 94°C; 2) 45 cycles of 20 s at 94°C, 30 s at 56°C, and 60 s at 72°C. The reaction products were separated on 2.0% agarose. After PCR, 0.3 units of shrimp alkaline phosphatase was added and incubated at 37°C for 20 min followed by inactivation for 5 min at 85°C. The concentration of the extension primers was adjusted to optimize the signal-to-noise ratio. The iPLEX Gold Kits (Sequenom, Inc.) was used to prepare the samples with 0.2 μL (100 μmol) of termination mix, 0.05 units of DNA polymerase (Sequenom, Inc.), and 625 to 1250 nmol/L extension primers. The iPLEX reaction was performed using the following program:
1) initial denaturation for 30 s at 94°C; 2) 5 s at 94°C and five cycles of 5 s at 52°C and 5 s at 80°C; 3) 40 annealing and extension cycles; 4) 5 s at 94°C; 5) five cycles of 5 s at 52°C and 5 s at 80°C; and 6) 72°C for 3 min and the sample. The products were analyzed by MALDI-TOF-MS. The samples were desalted using 6 mg of resin and transferred to a 384-well SpectroCHIP (Sequenom, Inc.). The mass spectra were acquired and analyzed using the MassARRAYTyper 4.0 Software (Sequenom, Inc.). Controls were performed without template DNA. All laboratory technicians were unaware of patient status.

Statistical analyses

Values were expressed as means ± standard deviation (SD) or numbers. Continuous variables were analyzed using the unpaired Student’s t-test. Differences in frequencies of the alleles and genotypes between case group and control group were evaluated using the χ²-test. Genotype distribution and allele frequencies were compared using the chi-square test. The chi-square test was also used to examine the Hardy-Weinberg Equilibrium (HWE) in the control group (P-value of <0.05 was considered to be statistically significant). Akaike’s information criteria were used to select the most parsimonious genetic model for each SNP [38]. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated by unconditional logistic regression analysis. All analyses were conducted with Stata statistical package (version 10.0; Stata Corp LP, College Station, TX, USA). The P value of allele difference was conducted with chi-square test between esophageal cancer and control group. P-value < 0.05 was considered statistically significant.

Results

Characteristics of the subjects

The demographic characteristics of the subjects are shown in Table 1. There were no differences in age (57.0±8.8 vs. 56.4±9.3 years) or gender (male, 63.1% vs. 64.5%) between the two groups (both P > 0.05).

Table 1. Demographic characteristics of ESCC cases and controls used in the study

| Study       | N   | Age, mean (s.d.) | Sex, male (%) |
|-------------|-----|-----------------|---------------|
| Cases       | 2280| 57.0 (8.8)      | 64.6          |
| First ESCC  | 1900| 57.0 (9.4)      | 64.5          |
| Second ESCC | 380 | 56.8 (9.0)      | 64.7          |
| Controls    | 1900| 56.4 (9.3)      | 64.5          |

Individual SNP association analysis

The genomic characteristics of 24 SNPs are given in Table 2. There was no deviation from the Hardy-Weinberg equilibrium in the control group (all P > 0.01). In the single-locus analyses, the allelic frequencies of rs10173378: A>G (0.241 vs. 0.221, P = 0.0409) and rs6854472: G>T (0.072 vs. 0.084, P = 0.0477) were slightly different between the ESCC and control group, but 100,000 permutations showed that there were no significant differences between the two groups. The genotype distributions of the 24 SNPs in the two groups are summarized in Table 3. The distribution of the rs12188136 (47.4% vs. 50.2%, P = 0.0493) and rs875339 (49.4% vs. 48.4%, P = 0.0341) genotypes showed significant differences between the cases and controls.

Logistic regression analyses revealed that in the codominant-effect model, the ESCC risk was associated with rs12188136 (P = 0.027, OR = 1.158, 95% CI = 1.016-1.319 for AG/AA). Binary logistic regression analyses revealed a slight negative association of rs10895458 (P = 0.044, OR = 0.547, 95% CI = 0.304-0.983 for CC/AA) and a significant negative association of rs875339 (P = 0.014, OR = 0.762, 95% CI = 0.613-0.947 for TT/CC), but because of the rarity of the homozygous mutant genotype (<3%), the results were invalid for rs10895458. In addition, marginal esophageal cancer risk was found for rs6854472 (P = 0.056, OR = 1.187, 95% CI = 0.995-1.417 for GT/GG) (Table 3).

Using the dominant model, significant ESCC risk was observed for rs6854472 (P = 0.048, OR = 1.192, 95% CI = 1.002-1.418). Using the recessive model, a significant negative association was observed for rs875339 (P = 0.010, OR = 0.758, 95% CI = 0.615-0.935) (Table 4).

Discussion

ESCC is one of the most prevalent cancers worldwide and occurs at a relatively high frequency in China. Some recent genome-wide association studies have identified 24 single-nucleotide polymorphisms that may be associated with ESCC. This study aimed to validate the association between these 24 polymorphisms and ESCC in a Han subgroup from Eastern China. The results suggest that rs12188136 and rs6854472 are associated with ESCC in this Han Chinese subgroup, and that rs875339 is negative associated with ESCC.

This study was a large-scale study in Han Chinese patients from Eastern China that describes the association between ESCC and 24 genome-wide SNPs. Besides rs12188136 and rs6854472 localizing in intergenic areas, RORA could play a role in the development of ESCC [31-35]. Abnet et al. [31] conducted the first large-scale genome-wide association studies for ESCC using 2115 ESCC cases and 3302 controls in Chinese, and identified PLCE1 at...
10q23 for ESCC susceptibility. Then, Wang et al. [32] performed a GWAS of ESCC by genotyping 1077 individuals with ESCC and 1733 control subjects of Han Chinese descent, and found that PLCE1 and C20orf54 play important roles for ESCC carcinogenesis. Wu et al. [33] performed a GWAS on 2031 ESCC individuals and 2044 controls of Chinese descent, and evaluated promising associations in an additional 6276 cases and 6165 controls from different areas of China. They identified five chromosomal regions (5q11, 6p21, 10q23, 12q24 and 21q22) that carried seven susceptibility loci for ESCC in the Chinese population, of which three (5q11, 6p21 and 21q22) were newly discovered [33]. Wu et al. [34] reported a multistage GWAS of ESCC in 10,123 ESCC cases and 10,664 controls. This GWAS identified nine new susceptibility loci for ESCC, of which seven (4q23, 16q12.1, 17q21, 22q12, 3q27, 17p13 and 18p11) had a significant marginal effect and two of which (2q22 and 13q33) had a significant association in the gene-alcohol interaction only [34]. Among 5337 Chinese with ESCC and 5787 controls (replication in 9654 Chinese with ESCC and 10,058 controls), Wu et al. [34] showed that rs7447927 at 5q32.1 and rs1642764 at 17p13.1 were associated with ESCC susceptibility [34]. Furthermore, Hu et al. [39] showed that rs2274223 was associated with reduced PLCE1 expression and increased risk of ESCC. Another replication study by Wang et al. [40] showed that the ADH1B-ADH1C-ADH7 axis was modulated by the rs1042026, rs17033, rs1614972, rs1789903 and rs17028973 SNPs. In the present study, the identified polymorphisms matched those found by the previous studies, and included rs2294693 in 6p21.1, rs11059556 in 12q24, rs6854472 in 4q22, rs12922317 in 16p13.12, and rs9824873 in 3q28. The discrepancies among studies regarding the identified loci can be due to the genetic diversity among different regions of China and of the world. Additional studies are necessary to better understand the risk of ESCC.

Table 2: Information about 24 validated SNPs.

| Gene: locus and OMIM No. | SNP_ID | Chromosome Position | Reference allele | Effect allele | MAF<sup>a</sup> | Ps | P value for HWE<sup>b</sup> test | Genotyping call Rate (%)<sup>c</sup> |
|-------------------------|--------|---------------------|-----------------|--------------|----------------|----|---------------------------------|----------------------------------|
| SERINC2: 1p35.1 OMIM: 614549 | rs4478858 | 31411078 | G | A | 0.200 | 0.196 | 0.213 | 0.0592 | 0.351 | 96.75 |
| 1p13 | rs10881372 | 106210655 | C | T | 0.163 | 0.196 | 0.187 | 0.2664 | 0.224 | 97.37 |
| 1q31 | rs10810638 | 19802090 | C | T | 0.349 | 0.302 | 0.304 | 0.8751 | 0.795 | 97.13 |
| 2p22 | rs10137387 | 43196650 | A | G | 0.198 | 0.241 | 0.221 | 0.0409 | 0.600 | 97.18 |
| LYPD6: 2q22.2 OMIM: 613359 | rs888103 | 149370922 | C | T | 0.128 | 0.115 | 0.119 | 0.6138 | 0.464 | 97.32 |
| BZW1: 2q33 OMIM: N.A. | rs3815501 | 200821399 | G | A | 0.488 | 0.464 | 0.467 | 0.8099 | 0.448 | 97.15 |
| 2q36 | rs6717108 | 224696318 | C | T | 0.444 | 0.444 | 0.449 | 0.6156 | 0.265 | 98.82 |
| UMP3: 3q21.2 OMIM: 613891 | rs10934685 | 124747673 | C | T | 0.389 | 0.341 | 0.335 | 0.5626 | 0.677 | 96.65 |
| ITGB5: 3q21.2 OMIM: 147561 | rs6768588 | 12476488 | A | G | 0.244 | 0.278 | 0.282 | 0.7215 | 0.475 | 96.60 |
| 3q28 | rs9824873 | 183583986 | T | C | 0.291 | 0.329 | 0.325 | 0.7073 | 0.928 | 96.17 |
| 4q22 | rs6854472 | 89513521 | G | T | 0.085 | 0.072 | 0.084 | 0.0477 | 0.871 | 79.75 |
| 5p35 | rs12188136 | 174407635 | A | G | 0.256 | 0.296 | 0.305 | 0.3698 | 0.222 | 96.77 |
| UNCSCL: 6p21.1 OMIM: N.A. | rs2294693 | 41037763 | T | C | 0.267 | 0.253 | 0.245 | 0.4418 | 0.074 | 97.13 |
| 6q27 | rs9364414 | 168171267 | G | A | 0.360 | 0.376 | 0.387 | 0.3088 | 0.439 | 96.79 |
| 10p12 | rs7916519 | 23177805 | G | A | 0.140 | 0.230 | 0.234 | 0.6363 | 0.929 | 97.01 |
| DYNCH21H: 11q22.3 OMIM: 603297 | rs11225815 | 103469085 | T | C | 0.233 | 0.255 | 0.248 | 0.4848 | 0.606 | 96.56 |
| 11q22 | rs10895458 | 103457356 | A | C | 0.133 | 0.113 | 0.101 | 0.0938 | 0.266 | 97.30 |
| OPCML: 11q25 OMIM: 600032 | rs4578395 | 133242688 | T | C | 0.105 | 0.091 | 0.088 | 0.7086 | 0.252 | 96.82 |
| 12q24.3 | rs11059556 | 128161518 | C | T | 0.279 | 0.336 | 0.338 | 0.8632 | 0.826 | 96.41 |
| 13q13 | rs2025245 | 37529440 | A | G | 0.354 | 0.381 | 0.364 | 0.1169 | 0.401 | 97.22 |
| GPC3: 13q31.3 OMIM: 602446 | rs9584016 | 92249673 | T | G | 0.372 | 0.422 | 0.406 | 0.1536 | 0.746 | 96.39 |
| FMN1: 15q13.3 OMIM: 136335 | rs347940 | 32885469 | A | G | 0.442 | 0.357 | 0.357 | 0.9613 | 0.553 | 95.96 |
| RORA: 15q22.2 OMIM: 608025 | rs875339 | 60803856 | C | T | 0.314 | 0.313 | 0.295 | 0.0881 | 0.024 | 97.01 |
| SNA29: 16p13.13-p13.12 OMIM: N.A. | rs12922317 | 11983775 | G | A | 0.256 | 0.318 | 0.300 | 0.0916 | 0.369 | 96.82 |

a. OMIM, Online Mendelian Inheritance in Man (http://www.ncbi.nlm.nih.gov/Omim); b. SNP position in the NCBI dbSNP Build 38 database (http://www.ncbi.nlm.nih.gov/SNP/); c. MAF, minor allele frequency, representing the frequency of effect allele; d. MAF for Chinese in the NCBI dbSNPs database; e. MAF for control group; f. MAF for esophageal cancer group; g. P value, which was conducted with t-test, for difference in allele distributions between esophageal cancer and control group; h. HWE, Hardy–Weinberg equilibrium in control group; i. The percentage of successful genotype calls.
Table 3. Genotype frequencies of 24 validated SNPs among cases and control and their associations with esophageal cancer risk under co-dominant genetic model.

| Gene     | SNP ID   | Genotype | Case No. | Control No. | P (2 df) | Logistic regression OR (95%CI) | Pvalue |
|----------|----------|----------|----------|-------------|----------|-------------------------------|--------|
| SERINC2  | rs4478858| GG       | 1380     | 1185        | 0.1732   | 1.000 (reference)             | 0.061  |
|          |          | GA       | 732      | 567         | 0.133    | 1.109 (0.969-1.268)           |        |
|          | rs100881372| AA      | 106      | 74          | 0.186    | 1.200 (0.905-1.627)           |        |
|          |          | CC       | 1481     | 1172        | 0.282    | 1.000 (reference)             |        |
|          | rs10801638| CT      | 688      | 592         | 0.221    | 0.920 (0.804-1.052)           |        |
|          |          | TT      | 75       | 62          | 0.876    | 0.979 (0.858-1.114)           |        |
|          | rs10173378| AA      | 1341     | 1053        | 0.840    | 1.053 (0.863-1.132)           |        |
|          |          | AG      | 789      | 678         | 0.039    | 0.914 (0.802-1.041)           |        |
|          |          | GG      | 99       | 102         | 0.065    | 0.762 (0.571-1.017)           |        |
| LYPD6    | rs888103 | CC      | 1743     | 1432        | 0.615    | 1.000 (reference)             |        |
|          |          | CT      | 456      | 379         | 0.882    | 0.988 (0.848-1.152)           |        |
|          | rs3815501| TT      | 37       | 21          | 0.179    | 1.448 (0.844-2.484)           |        |
| BZW1     | rs6717108| CC      | 689      | 575         | 0.620    | 1.000 (reference)             |        |
|          |          | CT      | 1075     | 875         | 0.731    | 1.025 (0.889-1.182)           |        |
|          | rs6768588| TT      | 463      | 370         | 0.089    | 0.8648 (0.850-1.136)          |        |
|          |          | AA      | 486      | 386         | 0.179    | 1.026 (0.860-1.225)           |        |
| UMP2     | rs10934665| CC      | 972      | 787         | 0.560    | 0.898 (0.850-1.136)           |        |
|          |          | CT      | 1066     | 827         | 0.821    | 0.985 (0.864-1.123)           |        |
|          | rs6854472| TT      | 240      | 208         | 0.522    | 0.934 (0.759-1.150)           |        |
| ITGB5    | rs12188136| AA     | 1139     | 939         | 0.719    | 1.000 (reference)             |        |
|          |          | AG      | 919      | 740         | 0.367    | 1.024 (0.899-1.166)           |        |
|          | rs9624873| GG      | 167      | 134         | 0.827    | 1.027 (0.836-1.310)           |        |
|          |          | TT      | 1014     | 816         | 0.708    | 1.000 (reference)             |        |
|          | rs664414 | TC      | 960      | 796         | 0.566    | 0.977 (0.851-1.110)           |        |
|          | rs7916159| CC      | 238      | 196         | 0.830    | 0.997 (0.792-1.206)           |        |
|          |          | GG      | 1884     | 1586        | 0.047    | 0.999 (0.850-1.136)           |        |
|          | rs516864 | GT      | 347      | 246         | 0.047    | 0.999 (0.850-1.136)           |        |
| UNC5CL   | rs2294693| AA      | 1050     | 917         | 0.522    | 1.000 (reference)             |        |
|          |          | AG      | 981      | 740         | 0.376    | 1.024 (0.899-1.166)           |        |
|          | rs1048572| GG      | 186      | 171         | 0.656    | 0.950 (0.758-1.191)           |        |
|          |          | CT      | 1268     | 1035        | 0.445    | 1.000 (reference)             |        |
| DYNC2H1  | rs1122851| TC      | 824      | 694         | 0.334    | 1.000 (reference)             |        |
|          | rs10895458| CC     | 141      | 113         | 0.912    | 0.985 (0.759-1.279)           |        |
|          |          | AA      | 1800     | 1450        | 0.094    | 1.000 (reference)             |        |
|          | rs4578935| TT      | 1845     | 1501        | 0.705    | 0.9295 (0.821-1.144)          |        |
|          | rs11059556| TC     | 368      | 309         | 0.705    | 0.9295 (0.821-1.144)          |        |
|          |          | CC      | 13       | 11          | 0.014    | 0.916 (0.823-1.069)           |        |
|          | rs1025245| GC      | 907      | 708         | 0.154    | 0.938 (0.823-1.069)           |        |
|          |          | GG      | 1020     | 845         | 0.154    | 0.938 (0.823-1.069)           |        |
|          | rs9584006| AA      | 30       | 274         | 0.154    | 0.938 (0.823-1.069)           |        |
|          |          | TT      | 780      | 609         | 0.154    | 0.938 (0.823-1.069)           |        |
| GPC5     | rs4578935| TC      | 1077     | 876         | 0.154    | 0.938 (0.823-1.069)           |        |
|          | rs437940 | TT      | 362      | 325         | 0.154    | 0.938 (0.823-1.069)           |        |
| FMN1     | rs347940 | AG      | 911      | 745         | 0.154    | 0.938 (0.823-1.069)           |        |
|          |          | GT      | 1037     | 810         | 0.154    | 0.938 (0.823-1.069)           |        |
| RORA     | rs875339 | CC      | 1104     | 881         | 0.154    | 0.938 (0.823-1.069)           |        |

Note: P values < 0.05 were considered statistically significant.
The present study is not without limitations. Statistical correction was used to adjust for multiple testing for a specific gene, but this is controversial. The Bonferroni correction and Bayesian techniques are frequently used, but they are problematic when correcting multiple comparisons [41] and such corrections might not be needed when different associations are of interest on a purely one-at-a-time basis [42,43]. Secondly, our study included patients with first ESCC and second ESCC. First ESCC is more relevant to genetic factors than second ESCC. Thirdly, although our study suggested that some loci may be involved in the prevalence of acquired ESCC, only selected SNPs based on the literature were examined and they might not be enough to describe the entire genetic variation of Han Chinese. Finally, this was a
retrospective study and data about lifestyle habits (especially smoking and drinking) were not available or reliable for all patients, preventing subgroup and interaction analyses. Beyond the association studies, the literature is currently limited by the lack of mechanistic studies about the involvement of these SNPs in the development of ESCC and the present study was not designed to determine those mechanisms. Additional studies will have to be carried out on this issue.

Conclusion

This large-scale replication study showed that rs12188136 and rs6854472 are associated with ESCC in a Han Chinese subgroup from Eastern China, and that rs875339 is negative associated with ESCC. This study underlines the genetic complexity of ESCC development.

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Author contributions

Wang KL, Chen XL, Lei L, Li P and Ling ZQ contributed to the design, execution, and analysis of this paper. Wang KL, Chen XL, Lei L, Li P and Ling ZQ drafted the manuscript. Hong LL and Huang XC provided some help for data analysis. All the authors (including Mao WM) were involved in the critical revision of the manuscript.

Ethics statement

The study was performed in accordance with the Declaration of Helsinki and approved by the Ethics Committees of Zhejiang Cancer Hospital. Written informed consent was obtained for the recruitment of each participant. Each participant was then interviewed to collect detailed information on demographic characteristics.

Competing Interests

The authors have declared that no competing interest exists.

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