# Association of the interleukin-18 receptor 1 and interleukin-18 receptor accessory protein polymorphisms with the risk of esophageal cancer

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**Abstract.** Esophageal cancer is the fourth leading cause of cancer-associated fatalities and the fourth most commonly diagnosed cancer. In addition to environmental risk factors, genetic factors may have a significant role in esophageal cancer carcinogenesis. A hospital-based case-control study was conducted to evaluate the genetic effects of functional single-nucleotide polymorphisms in the interleukin-18 (*IL-18*), IL-18 receptor 1 protein (*IL-18R1*), IL-18 receptor accessory protein (*IL-18RAP*) and *IL-28B* on the development of esophageal cancer. In total, 380 esophageal squamous cell carcinoma (ESCC) cases and 380 controls were recruited for the present study. The *IL-18* rs360719 A>G, *IL-18R1* rs13015714 G>T, *IL-18RAP* rs917997 C>T and *IL-28B* rs8099917 T>G genotypes were determined. No association was observed between the *IL-18R1* rs13015714 G>T, *IL-18RAP* rs917997 C>T and *IL-28B* rs8099917 T>G polymorphisms and the risk of ESCC. However, in stratification analyses, a significantly decreased risk of ESCC associated with the *IL-18R1* rs13015714 G>T polymorphism and a significantly increased risk of ESCC associated with the *IL-18RAP* rs917997 C>T polymorphism was evident among male patients and patients who smoked or consumed alcohol. These findings highlighted that functional polymorphisms *IL-18R1* rs13015714 G>T and *IL-18RAP* rs917997 C>T may contribute to ESCC susceptibility among these subgroups. However, the present results were obtained with a limited sample size and further epidemiological studies are warranted to clarify the role of *IL-18R1* and *IL-18RAP* variants in the development of ESCC.

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*Abbreviations:* CI, confidence interval; IL-18, interleukin-18; OR, odds ratio; SNP, single-nucleotide polymorphism

*Key words:* interleukin-18, interleukin-18 receptor 1, interleukin-18 receptor accessory protein, polymorphisms, esophageal cancer, molecular epidemiology

## Introduction

Esophageal carcinoma is the fourth leading cause of cancer-associated fatalities and the fourth most commonly diagnosed cancer in China in 2010 (1). For esophageal cancer patients, the prior study reported that the 5-year survival rate is extremely poor and accounts for only 12.3% (2). In the highest-risk area, esophageal squamous cell carcinoma (ESCC) accounts for >90% of esophageal cancers (3,4). ESCC carcinogenesis is multifactorial, and in addition to the established environmental risk factors, such as heavy drinking and smoking (5), genetic aberrations, such as single-nucleotide polymorphisms (SNPs), may have significant roles (6).

Interleukin-18 (IL-18), an interferon-γ (IFN-γ)-inducing factor, upregulates several cytokines, including IL-1β and tumor necrosis factor-α, and IFN-γ promotes T helper cell type 1 (Th1) differentiation (7). IL-18 is also one of the main

cytokines of the inflammasomes and has been confirmed to effect carcinogenesis and tumor progression significantly (8). The IL-18 receptor is comprised of IL-18 receptor accessory protein (IL-18RAP) and IL-18 receptor 1 (IL-18R1) protein (9,10). Upon binding to its receptor, IL-18R1 protein, IL-18 triggers the recruitment of IL-18RAP, which initiates signaling. IL-18 has a critical role in MyD88-mediated signaling to prevent colon adenocarcinoma development (11). IL-18RAP forms the signaling chain of this receptor complex and has been shown to be crucial for signaling of IL-18, resulting in the production of IFN-γ (12). These two subunits of the IL-18R are mainly expressed on Th1 cells in response to IL-12 and/or IFN-α (13). IL-18RAP was also correlated with inflammatory bowel disease (14). A previous study reported that the *IL-18R1* and *IL-18RAP* genes were associated with atherosclerosis and its cardiovascular complications (15).

*IL-18* is located at the 11q22.2-22.3 chromosome and its promoter region is relatively unique and is comprised of several transcription initiation sites. The *IL-18* rs360719 polymorphism (A→G mutation) leads to loss of the octamer transcription factor-1 (OCT-1) transcription factor binding site. OCT-1 is identified as a ubiquitously expressed factor and has an important role in the regulation of certain genes. OCT-1 can also downregulate the expression of specific cytokines (16). Allele A of rs917997, an SNP that is 1.5 kb downstream of *IL-18RAP*, was strongly associated with coeliac disease susceptibility (17). This allele is also correlated with lower mRNA levels of *IL-18RAP* in whole blood. Furthermore, the *IL-18RAP* GA haplotype of rs13015714 and rs917997 showed the strongest association with coeliac disease (17).

The *IL-28B* rs8099917 T>G polymorphism has been demonstrated with the response to IFN-γ-based antiviral therapy in the natural course of hepatitis C and following liver transplantation (18,19).

The biological and pathological significance of *IL-18*, *IL-18R1*, *IL-18RAP* and *IL-28B* suggested that the functional genetic variations in these genes may contribute to the development of ESCC. The objective of the present study was to evaluate the association between *IL-18* rs360719 A>G, *IL-18R1* rs13015714 G>T, *IL-18RAP* rs917997 C>T and *IL-28B* rs8099917 T>G genotypes and ESCC susceptibility in a hospital-based case-control study. Genotyping analyses were performed for these 4 SNPs with 380 ESCC cases and 380 cancer-free controls in a Chinese Han population.

## Patients and methods

*Ethical approval of the study protocol*. The study was approved by the Review Board of Jiangsu University (Zhenjiang, China). The study complied with the World Medical Association Declaration of Helsinki regarding ethical conduct of research involving human subjects and/or animals. All the subjects provided written informed consent for inclusion in the study.

*Study subjects*. A total of 380 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 November 2009. By pathological analysis, all the esophageal cancer cases were diagnosed as ESCC. The exclusion criteria were patients who previously had: Cancer; any metastasized cancer; radiotherapy or chemotherapy. Frequency-matched to the cases with regards to age (±5 years) and gender, the controls were patients without cancer that were recruited from the two hospitals during the same time period. The majority of the control subjects were admitted to these two hospitals for the treatment of trauma.

Using a pre-tested questionnaire, demographic data (such as age and gender) and the associated ESCC risk factors were collected by trained interviewers. The definition of 'smokers' was individuals who smoked one cigarette per day for >1 year. The definition of 'alcohol drinkers' was subjects who consumed ≥3 alcoholic drinks a week for >6 months.

*Isolation of DNA and genotyping by a custom-by-design 48-Plex SNPscan™ kit*. In total, 2-ml blood samples were collected from each subject using vacutainers and transferred to tubes lined with ethylenediamime-N,N,N',N'-tetraacetic acid. Genomic DNA was isolated from whole blood with the QIAamp DNA Blood Mini kit (Qiagen, Berlin, Germany). Sample DNA (10 ng) was amplified by polymerase chain reaction according to the manufacturer's instructions. For *IL-18* rs360719 A>G, *IL-18R1* rs13015714 G>T, *IL-18RAP* rs917997 C>T and *IL-28B* rs8099917 T>G SNPs, genotyping was performed using a custom-by-design 48-Plex SNPscan™ kit (Genesky Biotechnologies Inc., Shanghai, China), as previously described (20). For quality control, repeated analyses were conducted for 4% of randomly selected samples with high DNA quality.

*Statistical analysis*. Differences in the distributions of demographic characteristics, selected variables and genotypes of the *IL-18* rs360719 A>G, *IL-18R1* rs13015714 G>T, *IL-18RAP* rs917997 C>T and *IL-28B* rs8099917 T>G variants between the cases and controls were evaluated using the $\chi^2$ test. The associations between the 4 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 and adjusted ORs when adjusting for age, gender, smoking and drinking status. The Hardy-Weinberg equilibrium (HWE) was tested by a goodness-of-fit $\chi^2$ test to compare the observed genotype frequencies to the expected ones among the control subjects. All the statistical analyses were performed with SAS 9.1.3 (SAS Institute Inc., Cary, NC, USA). $P<0.05$ was considered to indicate a statistically significant difference.

## Results

*Characteristics of the study population*. Characteristics of cases and controls included in the study are provided in Table I. The cases and controls appeared to be adequately matched on age and gender, as suggested by the $\chi^2$ tests (P=0.056 and P=0.346, respectively). As shown in Table I, no significant difference was detected on drinking status between the cases and the controls (P=0.183), however, smoking rate was higher in ESCC patients compared with the control subjects (P=0.014). In Table II, the primary information for

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

| Variables | Cases (n=380), n (%) | Controls (n=380), n (%) | P-value[a] |
|---|---|---|---|
| Age, years | | | |
| <60 | 142 (37.4) | 117 (30.8) | 0.056 |
| ≥60 | 238 (62.6) | 263 (69.2) | |
| Gender | | | |
| Male | 269 (70.8) | 257 (67.6) | 0.346 |
| Female | 111 (29.2) | 123 (32.4) | |
| Tobacco use | | | |
| Never | 220 (57.9) | 253 (66.6) | 0.014 |
| Ever | 160 (42.1) | 127 (33.4) | |
| Alcohol use | | | |
| Never | 253 (66.6) | 270 (71.1) | 0.183 |
| Ever | 127 (33.4) | 110 (28.9) | |

[a]Two-sided $\chi^2$ test. ESCC, esophageal squamous cell carcinoma.

these 4 genotyped SNPs is listed. The genotyping success rate was 96.97% for *IL-18* rs360719 A>G, 96.45% for *IL-18R1* rs13015714 G>T, 96.32% for *IL-18RAP* rs917997 C>T and 97.11% for *IL-28B* rs8099917 T>G in all 760 samples. For all the SNPs, the concordance rates of repeated analyses were 100%. Minor allele frequency (MAF) in the controls was similar to MAF for Chinese subjects in a database for all 4 SNPs (Table II). The observed genotype frequencies for *IL-18* rs360719 A>G, *IL-18R1* rs13015714 G>T, *IL-18RAP* rs917997 C>T and *IL-28B* rs8099917 T>G polymorphisms in the controls were in HWE (P=0.774, P=0.249, P=0.465 and P=0.325, respectively) (Table II).

*Associations between IL-18 rs360719 A>G, IL-18R1 rs13015714 G>T, IL-18RAP rs917997 C>T and IL-28B rs8099917 T>G polymorphisms and the risk of ESCC.* The genotype distributions of *IL-18* rs360719 A>G, *IL-18R1* rs13015714 G>T, *IL-18RAP* rs917997 C>T and *IL-28B* rs8099917 T>G in the cases and the controls are shown in Table III. In the single locus analyses, the genotype frequencies of *IL-18* rs360719 A>G were 72.6 (AA), 26.4 (AG) and 1.1% (GG) in the case patients and 74.8 (AA), 23.6 (AG) and 1.6% (GG) in the control subjects, and the difference was not statistically significant (P=0.579). When the *IL-18* rs360719 AA was used as the reference, the AG genotype was not associated with the risk for ESCC (AG vs. AA: Adjusted OR=1.15; 95% CI, 0.82-1.62; P=0.411); the GG genotype was not associated with the risk for ESCC (GG vs. AA: Adjusted OR=0.68; 95% CI, 0.19-2.46; P=0.553). In the dominant model, the *IL-18* rs360719 AG/GG variants were not associated with the risk for ESCC, compared with the *IL-18* rs360719 AA genotype (adjusted OR=1.12; 95% CI, 0.81-1.56; P=0.498). In the recessive model, when the *IL-18R1* rs13015714 AA/AG genotypes were used as the reference group, the GG homozygote genotype was not associated with the risk for ESCC (adjusted OR=0.65; 95% CI, 0.18-2.37; P=0.517) (Table III).

No association was observed between *IL-18R1* rs13015714 G>T, *IL-18RAP* rs917997 C>T and *IL-28B* rs8099917 T>G polymorphisms and the risk of ESCC (Table III).

*Stratification analyses of IL-18 rs360719 A>G, IL-18R1 rs13015714 G>T, IL-18RAP rs917997 C>T and IL-28B rs8099917 T>G polymorphisms and the risk of ESCC.* To evaluate the effects of *IL-18* rs360719 A>G, *IL-18R1* rs13015714 G>T, *IL-18RAP* rs917997 C>T and *IL-28B* rs8099917 T>G genotypes on ESCC risk according to different age, gender, smoking and alcohol drinking status, the stratification analyses were performed. A significantly decreased risk of ESCC associated with the *IL-18R1* rs13015714 G>T polymorphism was evident among male patients (GT+TT vs. GG:

Table II. Primary information for *IL-18* rs360719 A>G, *IL-18R1* rs13015714 G>T, *IL-18RAP* rs917997 C>T and *IL-28B* rs8099917 T>G polymorphisms.

| Genotyped SNPs | *IL-18* rs360719 A>G | *IL-18R1* rs13015714 G>T | *IL-18RAP* rs917997 C>T | *IL-28B* rs8099917 T>G |
|---|---|---|---|---|
| Chromosome | 11 | 2 | 2 | 19 |
| Location | 5'-Flanking | 5'-Flanking | 3'-Flanking | 5'-Flanking |
| Chr Pos (genome build 36.3) | 111541359 | 102338297 | 102437000 | 44435005 |
| Regulome DB score[a] | 2b | 6 | No data | 4 |
| TFBS[b] | Y | - | - | - |
| MAF[c] for Chinese population | 0.142 | 0.547 | 0.488 | 0.035 |
| MAF in the controls (n=380) | 0.134 | 0.511 | 0.496 | 0.049 |
| P-value for HWE[d] test in our controls | 0.774 | 0.249 | 0.465 | 0.325 |
| Genotyping value, % | 96.97 | 96.45 | 96.32 | 97.11 |

[a]http://www.regulomedb.org/; [b]TFBS, transcription factor binding site (http://snpinfo.niehs.nih.gov/snpinfo/snpfunc.htm); [c]MAF, minor allele frequency; *IL-18* rs360719 A>G MAF information was available for the Chinese Han and Japanese populations; [d]HWE, Hardy-Weinberg equilibrium. SNPs, single-nucleotide polymorphisms; *IL-18*, interleukin-18; *IL-18R1*, IL-18 receptor 1; *IL-18RAP*, IL-18 receptor accessory protein; Chr Pos, chromosome position; Y, yes.

Table III. Logistic regression analyses of the associations between *IL-18* rs360719 A>G, *IL-18R1* rs13015714 G>T, *IL-18RAP* rs917997 C>T and *IL-28B* rs8099917 T>G polymorphisms and the risk of ESCC.

| Genotype | Cases (n=380), n (%) | Controls (n=380), n (%) | Crude OR (95% CI) | P-value | Adjusted OR[a] (95% CI) | P-value |
|---|---|---|---|---|---|---|
| *IL-18* rs360719 A>G | | | | | | |
| AA | 267 (72.6) | 276 (74.8) | 1.00 | | 1.00 | |
| AG | 97 (26.4) | 87 (23.6) | 1.15 (0.83-1.61) | 0.406 | 1.15 (0.82-1.62) | 0.411 |
| GG | 4 (1.1) | 6 (1.6) | 0.69 (0.19-2.47) | 0.568 | 0.68 (0.19-2.46) | 0.553 |
| GG vs. AG vs. AA | | | | | | 0.579 |
| AG+GG | 101 (27.4) | 93 (25.2) | 1.12 (0.81-1.56) | 0.490 | 1.12 (0.81-1.56) | 0.498 |
| AA+AG | 364 (98.9) | 363 (98.4) | 1.00 | | 1.00 | |
| GG | 4 (1.1) | 6 (1.6) | 0.67 (0.19-2.38) | 0.530 | 0.65 (0.18-2.37) | 0.517 |
| G allele | 105 (14.3) | 99 (13.4) | | | | |
| *IL-18R1* rs13015714 G>T | | | | | | |
| GG | 100 (27.5) | 83 (22.4) | 1.00 | | 1.00 | |
| GT | 173 (47.7) | 196 (53.0) | 0.73 (0.51-1.05) | 0.086 | 0.71 (0.49-1.02) | 0.060 |
| TT | 90 (24.8) | 91 (24.6) | 0.82 (0.54-1.24) | 0.348 | 0.83 (0.54-1.25) | 0.364 |
| TT vs. GT vs. GG | | | | | | 0.229 |
| GT+TT | 263 (72.5) | 287 (77.6) | 0.76 (0.54-1.06) | 0.110 | 0.75 (0.53-1.05) | 0.089 |
| GG+GT | 273 (75.2) | 279 (75.4) | 1.00 | | 1.00 | |
| TT | 90 (24.8) | 91 (24.6) | 1.01 (0.72-1.41) | 0.950 | 1.04 (0.74-1.46) | 0.829 |
| T allele | 353 (48.6) | 378 (51.1) | | | | |
| *IL-18RAP* rs917997 C>T | | | | | | |
| CC | 91 (25.0) | 90 (24.5) | 1.00 | | 1.00 | |
| CT | 167 (45.9) | 191 (51.9) | 0.87 (0.61-1.24) | 0.426 | 0.84 (0.58-1.20) | 0.334 |
| TT | 106 (29.1) | 87 (23.6) | 1.21 (0.80-1.81) | 0.369 | 1.19 (0.79-1.80) | 0.404 |
| TT vs. CT vs. CC | | | | | | 0.177 |
| CT+TT | 273 (75.0) | 278 (75.5) | 0.97 (0.69-1.36) | 0.865 | 0.95 (0.67-1.33) | 0.753 |
| CC+CT | 258 (70.9) | 281 (76.4) | 1.00 | | 1.00 | |
| TT | 106 (29.1) | 87 (23.6) | 1.33 (0.95-1.85) | 0.093 | 1.34 (0.96-1.87) | 0.085 |
| T allele | 379 (52.1) | 365 (49.6) | | | | |
| *IL-28B* rs8099917 T>G | | | | | | |
| TT | 335 (91.0) | 334 (90.3) | 1.00 | | 1.00 | |
| TG | 31 (8.4) | 36 (9.7) | 0.86 (0.52-1.42) | 0.553 | 0.87 (0.52-1.44) | 0.585 |
| GG | 2 (0.5) | 0 (0.0) | - | 0.980 | - | 0.980 |
| GG vs. TG vs. TT | | | | | | 0.306 |
| TG+GG | 33 (9.0) | 36 (9.7) | 0.91 (0.56-1.50) | 0.722 | 0.92 (0.56-1.52) | 0.741 |
| TT+TG | 366 (99.5) | 370 (100) | 1.00 | | 1.00 | |
| GG | 2 (0.5) | 0 (0.0) | - | 0.980 | - | 0.980 |
| G allele | 35 (4.8) | 36 (4.9) | | | | |

[a]Adjusted for age, gender, smoking status and alcohol consumption. *IL-18*, interleukin-18; *IL-18R1*, IL-18 receptor 1; *IL-18RAP*, IL-18 receptor accessory protein; ESCC, esophageal squamous cell carcinoma; OR, odds ratio; CI, confidence interval.

Adjusted OR=0.65; 95% CI, 0.43-0.98; P=0.040), patients who smoked (GT+TT vs. GG: Adjusted OR=0.43; 95% CI, 0.23-0.79; P=0.007) or were alcohol drinkers (GT+TT vs. GG: Adjusted OR=0.38; 95% CI, 0.19-0.76; P=0.006) (Table IV). A significantly increased risk of ESCC associated with the *IL-18RAP* rs917997 C>T polymorphism was evident among male patients (TT vs. CT+CC: Adjusted OR=1.58; 95% CI, 1.05-2.38; P=0.029), patients who smoked (TT vs. CT+CC: Adjusted OR=2.36; 95% CI, 1.29-4.30; P=0.005) or were alcohol drinkers (TT vs. CT+CC: Adjusted OR=3.01; 95% CI, 1.52-5.97; P=0.002) (Table V). For *IL-18* rs360719 A>G and *IL-28B* rs8099917 T>G polymorphisms, no association was observed following stratification (data not shown).

Table IV. Stratified analyses between the *IL-18R1* rs13015714 G>T polymorphism and ESCC risk by gender, age, smoking status and alcohol consumption.

| | *IL18R1* rs13015714 G>T, case/control[a] | | | | Adjusted OR[b] (95% CI); P-value | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Variables | GG | GT | TT | GT+TT | GG | GT | TT | GT+TT | TT vs. (GT+GG) |
| Gender | | | | | | | | | |
| Male | 72/52 | 124/136 | 62/65 | 186/201 | 1.00 | 0.63 (0.40-0.97); 0.037[c] | 0.69 (0.42-1.15); 0.157 | 0.65 (0.43-0.98); 0.040[c] | 0.95 (0.63-1.43); 0.816 |
| Female | 28/31 | 49/60 | 28/26 | 77/86 | 1.00 | 0.90 (0.48-1.71); 0.753 | 1.26 (0.60-2.65); 0.546 | 1.01 (0.55-1.83); 0.983 | 1.34 (0.72-2.50); 0.350 |
| Age, years | | | | | | | | | |
| <60 | 34/22 | 65/65 | 37/26 | 102/91 | 1.00 | 0.57 (0.29-1.11); 0.349 | 0.91 (0.42-1.96); 0.204 | 0.66 (0.35-1.25); 0.803 | 1.34 (0.73-2.47); 0.098 |
| ≥60 | 66/61 | 108/131 | 53/65 | 161/196 | 1.00 | 0.77 (0.50-1.19); 0.245 | 0.75 (0.45-1.24); 0.261 | 0.77 (0.51-1.15); 0.198 | 0.88 (0.58-1.35); 0.566 |
| Smoking status | | | | | | | | | |
| Never | 53/62 | 96/122 | 58/62 | 154/184 | 1.00 | 0.98 (0.62-1.56); 0.938 | 1.16 (0.69-1.95); 0.583 | 1.04 (0.68-1.61); 0.857 | 1.17 (0.77-1.79); 0.464 |
| Ever | 47/21 | 77/74 | 32/29 | 109/103 | 1.00 | 0.42 (0.22-0.80); 0.008[c] | 0.45 (0.21-0.95); 0.036[c] | 0.43 (0.23-0.79); 0.007[c] | 0.81 (0.45-1.48); 0.499 |
| Alcohol consumption | | | | | | | | | |
| Never | 62/66 | 113/130 | 63/66 | 176/196 | 1.00 | 0.96 (0.62-1.48); 0.846 | 1.08 (0.66-1.78); 0.753 | 1.00 (0.66-1.51); 0.998 | 1.16 (0.74-1.68); 0.601 |
| Ever | 38/17 | 60/66 | 27/25 | 87/91 | 1.00 | 0.38 (0.18-0.78); 0.008[c] | 0.39 (0.17-0.93); 0.033[c] | 0.38 (0.19-0.76); 0.006[c] | 0.79 (0.40-1.54); 0.485 |

[a]Genotyping was successful in 363 (95.5%) ESCC cases and 370 (97.4%) controls for *IL-18R1* rs13015714 G>T; [b]adjusted for age, gender, smoking status and alcohol consumption (in addition to stratified factors accordingly) in a logistic regression model; [c]statistically significant (P<0.05). ESCC, esophageal squamous cell carcinoma; *IL-18R1*, interleukin-18 receptor 1; OR, odds ratio.

Table V. Stratified analyses between the *IL-18RAP* rs917997 C>T polymorphism and ESCC risk by gender, age, smoking status and alcohol consumption.

| | *IL18RAP* rs917997 C>T , case/control[a] | | | | Adjusted OR[b] (95% CI); P-value | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Variables | CC | CT | TT | CT+TT | CC | CT | TT | CT+TT | TT vs. (CT+CC) |
| Gender | | | | | | | | | |
| Male | 61/65 | 120/132 | 77/54 | 197/186 | 1.00 | 0.92 (0.59-1.42); 0.703 | 1.49 (0.90-2.46); 0.119 | 1.08 (0.72-1.63); 0.701 | 1.58 (1.05-2.38); 0.029[c] |
| Female | 30/25 | 47/59 | 29/33 | 76/92 | 1.00 | 0.63 (0.32-1.21); 0.166 | 0.69 (0.33-1.44); 0.327 | 0.65 (0.35-1.21); 0.172 | 0.94 (0.52-1.70); 0.848 |
| Age, years | | | | | | | | | |
| <60 | 37/25 | 62/62 | 37/24 | 99/86 | 1.00 | 0.59 (0.31-1.15); 0.122 | 1.01 (0.47-2.16); 0.984 | 0.71 (0.38-1.32); 0.277 | 1.43 (0.77-2.65); 0.262 |
| ≥60 | 54/65 | 105/129 | 69/63 | 174/192 | 1.00 | 1.01 (0.64-1.57); 0.978 | 1.34 (0.81-2.20); 0.257 | 1.12 (0.73-1.70); 0.609 | 1.33 (0.89-1.99); 0.166 |
| Smoking status | | | | | | | | | |
| Never | 59/61 | 94/120 | 55/65 | 149/185 | 1.00 | 0.80 (0.51-1.25); 0.326 | 0.82 (0.49-1.37); 0.441 | 0.80 (0.53-1.23); 0.312 | 0.94 (0.62-1.45); 0.791 |
| Ever | 32/29 | 73/71 | 51/22 | 124/93 | 1.00 | 0.94 (0.50-1.76); 0.835 | 2.25 (1.07-4.74); 0.033[c] | 1.24 (0.68-2.26); 0.479 | 2.36 (1.29-4.30); 0.005[c] |
| Alcohol consumption | | | | | | | | | |
| Never | 65/65 | 110/126 | 64/70 | 174/196 | 1.00 | 0.85 (0.55-1.31); 0.462 | 0.85 (0.52-1.39); 0.523 | 0.85 (0.57-1.28); 0.434 | 0.95 (0.63-1.42); 0.790 |
| Ever | 26/25 | 57/65 | 42/17 | 99/82 | 1.00 | 0.90 (0.44-1.83); 0.766 | 2.79 (1.19-6.54); 0.019[c] | 1.27 (0.64-2.50); 0.493 | 3.01 (1.52-5.97); 0.002[c] |

[a]Genotyping was successful in 364 (95.8%) ESCC cases and 368 (96.8%) controls for *IL-18RAP* rs917997 C>T; [b]adjusted for age, gender, smoking status and alcohol consumption (in addition to stratified factors accordingly) in a logistic regression model; [c]statistically significant (P<0.05). ESCC, esophageal squamous cell carcinoma; *IL-18RAP*, interleukin-18 receptor accessory protein; OR, odds ratio.

Table VI. Logistic regression analyses of associations between *IL-18* rs360719 A>G, *IL-18R1* rs13015714 G>T, *IL-18RAP* rs917997 C>T and *IL-28B* rs8099917 T>G polymorphisms and risk of esophageal cancer lymph node metastasis.

| Genotype | LN meta (+) (n=85), n (%) | LN meta (-) (n=270), n (%) | Crude OR (95% CI) | P-value | Adjusted OR[a] (95% CI) | P-value |
|---|---|---|---|---|---|---|
| *IL-18* rs360719 A>G | | | | | | |
| AA | 63 (75.9) | 185 (70.9) | 1.00 | | 1.00 | |
| AG | 18 (21.7) | 74 (28.4) | 0.71 (0.40-1.29) | 0.263 | 0.70 (0.39-1.27) | 0.241 |
| GG | 2 (2.4) | 2 (0.8) | 2.94 (0.41-21.28) | 0.287 | 2.66 (0.35-20.11) | 0.343 |
| GG vs. AG vs. AA | | | | | | 0.255 |
| AG+GG | 20 (24.1) | 76 (29.1) | 0.77 (0.44-1.37) | 0.375 | 0.76 (0.43-1.34) | 0.339 |
| AA+AG | 81 (97.6) | 259 (99.2) | 1.00 | | 1.00 | |
| GG | 2 (2.4) | 2 (0.8) | 3.20 (0.44-23.06) | 0.249 | 2.91 (0.39-21.86) | 0.299 |
| G allele | 22 (13.3) | 78 (14.9) | | | | |
| *IL-18R1* rs13015714 G>T | | | | | | |
| GG | 22 (26.8) | 73 (28.4) | 1.00 | | 1.00 | |
| GT | 41 (50.0) | 123 (47.9) | 1.11 (0.61-2.00) | 0.739 | 1.09 (0.60-1.98) | 0.782 |
| TT | 19 (23.2) | 61 (23.7) | 1.03 (0.51-2.09) | 0.927 | 1.04 (0.51-2.11) | 0.917 |
| TT vs. GT vs. GG | | | | | | 0.941 |
| GT+TT | 60 (73.2) | 184 (71.6) | 1.08 (0.62-1.89) | 0.782 | 1.07 (0.61-1.88) | 0.809 |
| GG+GT | 63 (76.8) | 196 (76.3) | 1.00 | | 1.00 | |
| TT | 19 (23.2) | 61 (23.7) | 0.97 (0.54-1.75) | 0.917 | 0.98 (0.54-1.78) | 0.957 |
| T allele | 79 (48.2) | 245 (47.7) | | | | |
| *IL-18RAP* rs917997 C>T | | | | | | |
| CC | 20 (24.4) | 61 (23.6) | 1.00 | | 1.00 | |
| CT | 40 (48.8) | 120 (46.3) | 1.02 (0.55-1.89) | 0.958 | 0.99 (0.53-1.86) | 0.984 |
| TT | 22 (26.8) | 78 (30.1) | 0.86 (0.43-1.72) | 0.670 | 0.84 (0.42-1.70) | 0.635 |
| TT vs. CT vs. CC | | | | | | 0.849 |
| CT+TT | 62 (75.6) | 198 (76.4) | 0.96 (0.54-1.71) | 0.876 | 0.94 (0.52-1.68) | 0.824 |
| CC+CT | 60 (73.2) | 181 (69.9) | 1.00 | | 1.00 | |
| TT | 22 (26.8) | 78 (30.1) | 0.85 (0.49-1.48) | 0.569 | 0.85 (0.48-1.49) | 0.563 |
| T allele | 84 (51.2) | 276 (53.3) | | | | |
| *IL-28B* rs8099917 T>G | | | | | | |
| TT | 75 (90.4) | 238 (91.2) | 1.00 | | 1.00 | |
| TG | 8 (9.6) | 21 (8.0) | 1.21 (0.51-2.84) | 0.664 | 1.20 (0.51-2.84) | 0.680 |
| GG | 0 (0.0) | 2 (0.8) | — | 0.990 | — | 0.990 |
| GG vs. TG vs. TT | | | | | | 0.660 |
| TG+GG | 8 (9.6) | 23 (8.8) | 1.10 (0.47-2.57) | 0.819 | 1.07 (0.46-2.51) | 0.872 |
| TT+TG | 83 (100) | 259 (99.2) | 1.00 | | 1.00 | |
| GG | 0 (0.0) | 2 (0.8) | — | 0.990 | — | 0.990 |
| G allele | 8 (4.8) | 25 (4.8) | | | | |

[a]Adjusted for age, gender, smoking status and alcohol consumption. LN meta, lymph node metastasis; *IL-18*, interleukin-18; *IL-18R1*, IL-18 receptor 1; *IL-18RAP*, IL-18 receptor accessory protein; OR, odds ratio; CI, confidence interval.

*Associations between IL-18 rs360719 A>G, IL-18R1 rs13015714 G>T, IL-18RAP rs917997 C>T and IL-28B rs8099917 T>G polymorphisms and the risk of esophageal cancer lymph node metastasis*. Analyses between *IL-18* rs360719 A>G, *IL-18R1* rs13015714 G>T, *IL-18RAP* rs917997 C>T and *IL-28B* rs8099917 T>G polymorphisms and risk of esophageal cancer lymph node metastasis were further conducted. No association was observed between the 4 polymorphisms and lymph node metastasis (Table VI).

## Discussion

In the present hospital-based case-control study of ESCC, the associations of *IL-18* rs360719 A>G, *IL-18R1* rs13015714

G>T, *IL-18RAP* rs917997 C>T and *IL-28B* rs8099917 T>G were associated with the risk of ESCC in a high-risk Chinese population. The multivariable logistic analysis revealed that a significantly decreased risk of ESCC associated with the *IL-18R1* rs13015714 G>T polymorphism was evident among male patients and patients who were smokers or alcohol drinkers. In addition, a significantly increased risk of ESCC associated with the *IL-18RAP* rs917997 C>T polymorphism was evident among male patients and patients who were smokers or alcohol drinkers.

IL-18R is comprised of IL-18RAP and IL-18R1. Allele A of *IL-18RAP* rs917997 was strongly correlated with coeliac disease susceptibility (17). The *IL-18RAP* rs917997 A allele has a significant effect on the level of *IL-18RAP* mRNA expression. The *IL-18RAP* rs917997 C allele is strongly associated with a protective effect in Barrett's esophagus and esophageal adenocarcinoma (21). The CC genotype at the *IL-18RAP* locus rs917997 was associated with a protective effect against esophageal disease, which is in accordance with the present findings in the stratification analyses (21).

The frequencies of genetic polymorphisms often vary between different ethnic groups (22). In the present study, the MAF of *IL-18R1* rs13015714 T was 0.511 among 380 control subjects, which is consistent with that of the Chinese Han population (0.547); however, this was significantly lower than that of the European population (0.796) and sub-Saharan African population (0.894) in the SNP database (http://www.ncbi.nlm.nih.gov/SNP). Similarly, the MAF of *IL-18RAP* rs917997 T was 0.496 among 380 control subjects, which is consistent with that of the Chinese Han population (0.488); however, this was significantly higher than that of the European population (0.208) and sub-Saharan African population (0.049) in the SNP database (http://www.ncbi.nlm.nih.gov/SNP).

Considering *IL-18R1* rs13015714 G>T mutant alleles in the control group, OR, ESCC samples and control samples, the power of the present analysis ($\alpha$=0.05) was 0.679 in 258 ESCC cases and 253 controls with adjusted OR=0.65 for *IL-18R1* rs13015714 G>T in the male subgroup. The power of the analysis ($\alpha$=0.05) was 0.932 in 156 ESCC cases and 124 controls with adjusted OR=0.43 in the smoking subgroup and 0.950 in 125 ESCC cases and 108 controls with adjusted OR=0.38 in the drinking subgroup.

For *IL-18RAP* rs917997 C>T, the power of the analysis ($\alpha$=0.05) was 0.728 in 258 ESCC cases and 251 controls with adjusted OR=1.58 in the male subgroup, 0.937 in 156 ESCC cases and 122 controls with adjusted OR=2.36 in the smoking subgroup and 0.983 in 125 ESCC cases and 107 controls with adjusted OR=3.01 in the drinking subgroup.

Several weaknesses should be addressed in the case-control study. Firstly, all the subjects were selected from hospitals, which may have led to a bias and consequently affected the validity of the findings. Secondly, the relatively small sample size restricted the statistical power of the study, particularly in the stratification analyses. Further larger sample size studies with a well-designed two-stage fine-mapping strategy are warranted to confirm the present findings. Thirdly, due to the lack of detailed information on cancer metastasis and survival, further investigations on the potential role of *IL-18R1* rs13015714 G>T and *IL-18RAP* rs917997 C>T polymorphisms in ESCC progression and prognosis were not performed.

In conclusion, the present study provides evidence that functional *IL-18R1* rs13015714 G>T and *IL-18RAP* rs917997 C>T polymorphisms may contribute to the development of ESCC. However, the power of the present analysis was relatively low with a limited sample size, particularly in the subgroup analyses. Therefore, only preliminary conclusions were drawn. Larger studies with other ethnic populations are required to confirm the present findings.

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