Research Article

Investigation of IL-4, IL-10, and HVEM polymorphisms with esophageal squamous cell carcinoma: a case–control study involving 1929 participants

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It is believed that an individual’s hereditary factors may be involved in the development of esophageal cancer (EC). The present study recruited 721 esophageal squamous cell carcinoma (ESCC) cases and 1208 controls and explored the roles of single nucleotide polymorphisms (SNPs) in the interleukin-4 (IL-4), IL-10, and herpesvirus entry mediator (HVEM) genes in contributing to ESCC risk. IL-4, IL-10, and HVEM SNPs were analyzed by employing an SNPscan method. After adjustment for body mass index (BMI), smoking, drinking, age and gender, we identified that the rs2070874 T>C locus in IL-4 gene decreased the risk of ESCC (CC vs. TT: \( P = 0.008 \); CC vs. TT/TC: \( P = 0.010 \)). After a stratified analysis, we suggested that the IL-4 rs2070874 T>C variants might be a protective factor for ESCC in male, ≥63 years old, never smoking, drinking and BMI < 24 kg/m² subgroups. In addition, we identified that the rs2243263 G>C polymorphism in IL-4 gene was a risk factor for ESCC development in the BMI ≥ 24 kg/m² subgroup (GC vs. GG: \( P = 0.030 \) and GC/CC vs. GG: \( P = 0.018 \)). We identified an association of the IL-4 rs2070874 T>C SNP with the decreased susceptibility of ESCC in stage I/II subgroup. Finally, we found an association of the IL-10 rs1800872 T>G SNP with a worse differentiation (TG vs. TT: \( P = 0.048 \) and GG/TG vs. TT: \( P = 0.032 \)). In conclusion, the findings indicate a potential importance of IL-4 rs2070874 T>C, IL-4 rs2243263 G>C and IL-10 rs1800872 T>G SNPs in the development of ESCC.

Introduction

In China, esophageal cancer (EC) is the fourth most frequently diagnosed form of malignant tumor in males and the fifth most commonly diagnosed form in females, approximately 320800 and 157200 cases occurred in 2015, respectively [1]. The incidence of EC in Eastern Asia is in the top five worldwide, including China. Esophageal squamous cell carcinoma (ESCC) is a major histological subtype, accounting for 90% of all EC cases. The complex interaction of economical and environmental conditions with individual’s hereditary factors may lead to EC development [2,3]. The etiology and development of EC is not fully understood, despite many investigations have payed close attention to the importance of immunity [4,5]. Recently, it was hypothesized that some important variants in immune-related genes may influence the susceptibility of ESCC.

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Interleukin-4 (IL-4), coded by the IL-4 gene, is an important regulator of the inflammation pathways. IL-4, a pleiotropic cytokine, may be correlated with survival and growth of lymphocytes [6]. IL-4 is produced by mast cell precursors and by the T-cell thymocyte populations. It is important for B-cell activation, proliferation and differentiation [7]. It is reported that IL-4 is necessary for producing immunoglobulin E and implicated in immune diseases. In the process of innate immune responses, IL-4 may activate M2 macrophage, and then play a specific role. It has anti-inflammatory effect, which is relevant to the development of ESCC. Recently, a number of studies have focused on the relationship of IL-4 with cancer development [8,9]. IL-4 single nucleotide polymorphisms (SNPs) have also been explored for an association with susceptibility to cancer [10–12]. The rs2070874 T>C, located in the 5'-UTR region of the IL-4 gene, is an important SNP in cancer development. Some meta-analyses have indicated that IL-4 rs2070874 may be associated with cancer development in Asian populations [13–15]. Kim et al. reported that IL-4 rs2070874 might affect the role of aspirin in regulating IL-4 expression [16]. Rs2243263 G>C polymorphism is an intron SNP of IL-4 gene. This intron SNP might play a role in splicing. Although the exact role of this intron SNP is unknown, the associations of IL-4 rs2243263 G>C SNP with the human disease have been explored. A previous study suggested that IL-4 rs2243263 was associated with the reverse seroconversion of Hepatitis B virus (HBV) [17]. This SNP was also studied for the relationship of the susceptibility to cancer. A previous report investigated the correlation of the IL-4 rs2243263 locus with colorectal cancer [18]. Although in this study, a null association was identified. However, Lan et al., in a large simple size study, found that the IL-4 rs2243263 G>C SNP might increase the susceptibility to non-Hodgkin lymphoma [19]. Currently, the associations of IL-4 the rs2070874 T>C, and rs2243263 G>C polymorphisms with ESCC development are unknown.

The IL-10 gene is located in chromosome 1q32.2. IL-10, another immune regulator, serves as an inhibitor of dendritic cells and macrophages [20], and inhibits the production of many inflammatory cytokines (e.g. tumor necrosis factor-α, IL-1, IL-6, IL-12, and others) [21]. IL-10 is a vital anti-inflammatory regulator. After IL-10 combines with its receptor (IL-10R), signal transducer and activator of transcription 3 is triggered, which plays a vital role in anti-apoptosis and proliferation [20]. An investigation found that the up-regulated mRNA expression of the IL-10 gene and higher serum levels of IL-10 were found among subjects who carried the rs1800896 G-allele [22]. The rs1800872 SNP, a promoter variant, could influence the level of IL-10 protein [23]. Some investigations have suggested that the IL-10 rs1800896 A>G (−1082) [24] and rs1800872 A>C (−592) [25] variants may influence the susceptibility to ESCC. Of late, a meta-analysis indicated that these IL-10 SNPs increased the risk of EC [26]. However, in this earlier meta-analysis, the sample size was very limited (1883 EC patients and 2857 controls included). The association of the IL-10 rs1800896 A>G and rs1800872 A>C polymorphisms with EC development should be further studied.

Herpesvirus entry mediator (HVEM), also known as TNFRSF14, plays a major role in the immune response [27–29]. HVEM has been found to be expressed in lymphoid cells, as well as in other cells. A previous study suggested that the HVEM/B- and lymphocyte attenuator/lymphotoxin/CD160 network in immune reaction to infection and inflammation could play a bidirectional regulatory role [30]. Several investigations have focused on the role of HVEM in cancer survival [31–33]. Zhu et al. reported that higher expression of HVEM may promote apoptosis and herald a good prognosis for bladder cancer patients [34]. Additionally, a previous study has indicated that HVEM is implicated in the development of breast cancer (BC) [35]. A SNP in the HVEM gene, the G to A of rs2234167 in the exon region, was found to influence the development of BC [36]. However, the association of HVEM rs2234167 G>A SNP with the expression of HVEM is unknown. Recently, Migita et al. found that HVEM is critical for both tumor survival and the escape of the host immune system in ESCC cases [37]. Thus, it could be a useful target for ESCC therapy. To date, investigation has not been performed to identify a relationship of the HVEM rs2234167 G>A polymorphism with ESCC susceptibility.

Therefore, in this investigation, the HVEM rs2234167, IL-4 rs2070874 and rs2243263, and IL-10 rs1800896 and rs1800872 polymorphisms were selected and investigated for their effect on ESCC development in a Chinese Han population.

Materials and methods

Subjects

Our case—control study was performed in Fujian Union Hospital (Fuzhou, China) and the No.1 People's Hospital of Zhenjiang City (Zhenjiang, China). This investigation was approved by Jiangsu University (registration ID: K-20160036-Y) and Fujian Medical University (registration ID: 2016-ZQN-25). Participants were recruited between February 2014 and April 2018. Our study included 721 ESCC cases and 1208 controls. These ESCC patients were histopathologically confirmed and were from 41 to 87 years old. Controls were cancer-free individuals from 40 to
87 years old. The controls were not related to any ESCC case. Using a pre-structured questionnaire, we collected epidemiological data from participants. The ESCC patients and normal controls signed consent forms.

**DNA extraction and genotyping of HVEM rs2234167, IL-4 rs2070874, and rs2243263, and IL-10 rs1800896 and rs1800872 loci**

We collected a blood sample (2 ml) from each participant. DNA was extracted carefully as described in a previous study [38]. Using an SNPscan™ assay (Genesky Biotechnologies Inc., Shanghai, China), we determined the genotypes of HVEM rs2234167, IL-4 rs2070874, and rs2243263, and IL-10 rs1800896 and rs1800872 polymorphisms. To confirm the accuracy of genotyping, 77 samples were selected and re-tested. The genotypes of HVEM rs2234167, IL-4 rs2070874, and rs2243263, and IL-10 rs1800896 and rs1800872 loci were re-analyzed by another technician. The genotypes of HVEM rs2234167, IL-4 rs2070874, and rs2243263, and IL-10 rs1800896 and rs1800872 SNPs were unchanged.

**Statistical analysis**

The difference in alcohol consumption, body mass index (BMI), gender, cigarette use, and age were tested by using χ² test. Mean age was calculated by using a Student’s t test. We used a Chi-square test (χ²) or Fisher’s exact test to determine whether the frequencies of HVEM rs2234167, IL-4 rs2070874, and rs2243263, and IL-10 rs1800896 and rs1800872 variants in ESCC cases and controls were different. A multivariate logistic regression analysis method was used to calculate the crude and adjusted odds ratios (ORs) and 95% confidence intervals (CIs) (SAS 9.4 software package; SAS Institute Inc., Cary, NC, U.S.A.). The relationship of HVEM rs2234167, IL-4 rs2070874, and rs2243263, and IL-10 rs1800896 and rs1800872 polymorphisms with ESCC development was determined by ORs and 95% CIs. The statistical significance of all analyses was P < 0.05 (two-sided). An internet-based Hardy–Weinberg equilibrium (HWE) test (http://ihg.gsf.de/cgi-bin/hw/hwa1.pl) was also harnessed to assess whether the distribution of HVEM rs2234167, IL-4 rs2070874, and rs2243263, and IL-10 rs1800896 and rs1800872 genotypes could represent the included population.

**Results**

**Baseline characteristics**

In total, 721 ESCC cases and 1208 controls were recruited (Table 1). Of these ESCC cases, 170 were females and 551 were males, average age was 62.59 ± 8.18 years. In the control group, there were 309 females and 899 males with an average age of 62.92 ± 8.94 years. There was no difference in terms of mean age (P = 0.413). The categorical variables, age and gender, were well-matched (P > 0.05). However, the distribution of other categorical variables (e.g. tobacco use, BMI, and drinking status) were significantly different (all P < 0.001). Among ESCC cases, there were 405 (56.17%) with lymphatic metastasis. The AJCC version 8.0 criteria (2018) was used to determine the ESCC stage; and 328 ESCC cases were stage I/II and 393 were stage III/IV. After genotyping the 1929 participants, the association of tobacco use, BMI, and drinking status) were significantly different (all P < 0.001).

The minor allele frequencies (MAFs) of HVEM rs2234167, IL-4 rs2070874, and rs2243263, and IL-10 rs1800896 and rs1800872 loci are shown in Table 2. They are similar to the data of Chinese population. As presented in Table 2, the HVEM rs2234167, IL-4 rs2070874, and rs2243263, and IL-10 rs1800896 and rs1800872 genotypes could represent the included population.

**Relationship of HVEM rs2234167, IL-4 rs2070874, and rs2243263, and IL-10 rs1800896 and rs1800872 loci with ESCC**

Table 3 shows the HVEM rs2234167, IL-4 rs2070874, and rs2243263, and IL-10 rs1800896 and rs1800872 genotypes. The frequencies of IL-4 rs2070874 TT, TC, and CC genotypes were 486 (67.88%), 214 (29.89%), and 16 (2.23%) in ESCC cases and 780 (64.95%), 371 (30.89%), and 50 (4.16%) in controls. When the reference was IL-4 rs2070874 TT genotype, we found the IL-4 rs2070874 CC genotype significantly decreased the risk of ESCC (P = 0.023). When the reference was IL-4 rs2070874 TT/TC genotype, the IL-4 rs2070874 CC genotype also significantly decreased the risk of ESCC (P = 0.028). Adjustment for BMI, smoking, drinking, age and gender, the decreased susceptibility was also identified (CC vs. TT: P = 0.008; CC vs. TT/TC: P = 0.010).

HVEM rs2234167, IL-4 rs2243263 and IL-10 rs1800896 and rs1800872 genotypes are shown in Table 3. Both crude and adjusted comparisons indicated that HVEM rs2234167, IL-4 rs2243263, and IL-10 rs1800896 and rs1800872 loci were not associated with the risk of ESCC (Table 4).
Table 1 Distribution of selected demographic variables and risk factors in ESCC cases and controls

| Variable                        | Cases (n=721) | Controls (n=1208) | P^1 |
|---------------------------------|--------------|------------------|-----|
| Age (years)                     | 62.59 ± 8.18 | 62.92 ± 8.94     | 0.413 |
| Age (years)                     |              |                  |     |
| <63                             | 337          | 579              |     |
| ≥63                             | 384          | 629              | 0.613 |
| Sex                             |              |                  | 0.325 |
| Male                            | 551          | 899              |     |
| Female                          | 170          | 309              |     |
| Tobacco use                     |              |                  | <0.001 |
| Never                           | 342          | 881              |     |
| Ever                            | 379          | 327              |     |
| Alcohol use                     |              |                  | <0.001 |
| Never                           | 502          | 1,046            |     |
| Ever                            | 219          | 162              |     |
| BMI (kg/m²)                     |              |                  | <0.001 |
| <24                             | 527          | 651              |     |
| ≥24                             | 194          | 557              |     |
| Lymph node status               |              |                  |     |
| Positive                        | 405          |                  |     |
| Negative                        | 316          |                  |     |
| TMN stage                       |              |                  |     |
| I                               | 143          |                  |     |
| II                              | 185          |                  |     |
| III                             | 307          |                  |     |
| IV                              | 86           |                  |     |
| Grade                           |              |                  |     |
| G1                              | 142          |                  |     |
| G2                              | 405          |                  |     |
| G3                              | 174          |                  |     |

Bold values are statistically significant (P<0.05). Abbreviation: TMN, tumor-lymph node-metastasis.

^1Two-sided χ^2 test and Student’s t test.

Table 2 Primary information for the included SNPs

| Genotyped polymorphisms | HVEM rs2234167 G>A | IL-4 rs2070874 T>C | IL-4 rs2243263 G>C | IL-10 rs1800872 T>G | IL-10 rs1800896 T>C |
|-------------------------|---------------------|---------------------|---------------------|---------------------|---------------------|
| Chromosome              | 1                   | 5                   | 5                   | 1                   | 1                   |
| Position_28             | 2562891             | 132674018           | 132677607           | 206773062           | 206773552           |
| Region                  | 3'-UTR              | 5'-UTR              | intron,variant      | 5'-flanking         | 5'-flanking         |
| MAF^1 in database (1000g- Chinese Han populotons) | 0.058 | 0.228 | 0.072 | 0.286 | 0.048 |
| MAF in our controls (n=1208) | 0.036 | 0.196 | 0.065 | 0.324 | 0.060 |
| P-value for HWE^2 test in our controls | 0.239 | 0.484 | 0.593 | 0.825 | 0.871 |
| % Genotyping value      | 99.38%              | 99.38%              | 99.27%              | 99.33%              | 99.22%              |

^1MAF.

^2HWE.

Additionally, a subgroup analysis was conducted by ESCC stage. We identified an association between IL-4 rs2070874 T>C SNP and the decreased susceptibility of ESCC in stage I/II subgroup (CC vs. TT: P=0.022; CC vs. TT/TC: P=0.025, Table 4). However, this association could not been identified for other SNPs.
### Table 3 The frequencies of HVEM rs2234167, IL-4 rs2070874, rs2243263, and IL-10 rs1800896 and rs1800872 polymorphisms in different ESCC subgroups

| Genotype          | Overall cases (n=721) | Stage I/II patients (n=328) | Stage III/IV patients (n=393) | Controls (n=1208) |
|-------------------|-----------------------|-----------------------------|-------------------------------|-------------------|
|                   | n  | %  | n  | %  | n  | %  | n  | %  | n  | %  |
| HVEM rs2234167 G>A |     |    |     |    |     |    |     |    |     |    |
| GG                | 668| 93.30 | 302| 92.92 | 366| 93.61 | 1,117| 93.01 |
| GA                | 47 | 6.56  | 23 | 7.08  | 24 | 6.14  | 81 | 6.74  |
| AA                | 1 | 0.14  | 0 | 0.0   | 1 | 0.26  | 3 | 0.25  |
| A allele          | 49 | 3.42  | 23 | 3.54  | 26 | 3.32  | 87 | 3.62  |
| IL-4 rs2070874 T>C|     |    |     |    |     |    |     |    |     |    |
| TT                | 486| 67.88 | 223| 68.62 | 263| 67.26  | 780 | 64.95 |
| TC                | 214| 29.89 | 96 | 29.54 | 118| 29.19  | 371 | 30.89 |
| CC                | 16 | 2.23  | 6  | 1.85  | 10 | 2.56  | 50 | 4.16  |
| C allele          | 246| 17.18 | 108| 16.62 | 138| 17.65 | 471 | 19.61 |
| IL-4 rs2243263 G>C|     |    |     |    |     |    |     |    |     |    |
| GG                | 615| 86.13 | 282| 87.04 | 333| 85.38 | 1,048| 87.26 |
| GC                | 96 | 13.45 | 41 | 12.65 | 55 | 14.10 | 149 | 12.41 |
| CC                | 3  | 0.42  | 1  | 0.31  | 2  | 0.51  | 4  | 0.33  |
| C allele          | 102| 7.14  | 43 | 6.64  | 59 | 7.56  | 157 | 6.54  |
| IL-10 rs1800872 T>G|     |    |     |    |     |    |     |    |     |    |
| TT                | 349| 48.81 | 161| 49.54 | 188| 48.21 | 550 | 45.80 |
| TG                | 301| 42.10 | 136| 41.85 | 165| 42.31 | 523 | 43.55 |
| GG                | 65 | 9.09  | 28 | 8.62  | 37 | 9.44  | 128 | 10.65 |
| G allele          | 431| 30.14 | 192| 29.54 | 239| 30.64 | 779 | 32.43 |
| IL-10 rs1800896 T>C|     |    |     |    |     |    |     |    |     |    |
| TT                | 625| 87.66 | 280| 86.42 | 345| 88.69 | 1,061| 88.34 |
| TC                | 84 | 11.78 | 42 | 12.96 | 42 | 10.80 | 136 | 11.32 |
| CC                | 4  | 0.56  | 2 | 0.62  | 2  | 0.51  | 4  | 0.34  |
| C allele          | 92 | 6.45  | 46 | 7.10  | 46 | 9.81  | 144 | 6.00  |

### Relationship of HVEM rs2234167, IL-4 rs2070874, rs2243263, and IL-10 rs1800896 and rs1800872 loci with ESCC in stratified analyses

In a stratified analysis, the IL-4 rs2070874 genotypes are listed in Table 5. After an adjustment, we suggested that IL-4 rs2070874 C allele was a protective factor for ESCC in five subgroups (male subgroup: CC vs. TT: \( P = 0.028 \); CC vs. TT/TC: \( P = 0.031 \); ≥63 years old subgroup: CC vs. TT: \( P = 0.026 \); CC vs. TT/TC: \( P = 0.029 \); never smoking subgroup: CC vs. TT: \( P = 0.041 \); CC/TC vs. TT: \( P = 0.013 \) and TC vs. TT: \( P = 0.042 \); drinking subgroup: CC vs. TT: \( P = 0.025 \); CC vs. TT/TC: \( P = 0.024 \) and BMI < 24 kg/m² subgroup: CC vs. TT: \( P = 0.010 \); CC vs. TT/TC: \( P = 0.012 \)). In other subgroups, no association of IL-4 rs2070874 with ESCC risk was found (Table 5).

The IL-4 rs2243263 G>C genotypes in the stratified analysis are listed in Table 6. After adjustment, we identified that IL-4 rs2243263 G>C polymorphism was a risk factor for ESCC development in the BMI ≥ 24 kg/m² subgroup (GC vs. GG: \( P = 0.030 \) and GC/CC vs. GG: \( P = 0.018 \), Table 6).

In other stratified analyses, adjustment comparisons suggested that HVEM rs2234167, and IL-10 rs1800872 and rs1800896 loci did not confer a risk of ESCC (data not shown).

### Association of HVEM rs2234167, IL-4 rs2070874, and rs2243263, and IL-10 rs1800896 and rs1800872 loci with lymphatic metastasis in ESCC cases

Among the 721 ESCC patients, 405 patients had lymphatic metastasis. As presented in Table 7, we found a null association of HVEM rs2234167, IL-4 rs2070874, rs2243263 and IL-10 rs1800896 and rs1800872 SNPs with different lymph node status.
Table 4 Logistic regression analyses of association of HVEM rs2234167, IL-4 rs2070874, rs2243263 and IL-10 rs1800896 and rs1800872 polymorphisms with risk of ESCC

| Genotype          | Overall patients (n=721) vs. controls | Stage I/II patients (n=328) vs. controls | Stage III/IV patients (n=393) vs. controls |
|-------------------|----------------------------------------|------------------------------------------|------------------------------------------|
|                   | Crude OR (95% CI)                        | Adjusted OR1 (95% CI)                     | Crude OR (95% CI)                        | Adjusted OR1 (95% CI) | Crude OR (95% CI)                        | Adjusted OR1 (95% CI) |
| HVEM rs2234167 G>A |                                         |                                          |                                          |                        |                                          |                        |
| GA vs. GG         | 0.97 (0.67–1.41)                         | 1.05 (0.65–1.70)                         | 0.90 (0.57–1.45)                         | 1.03 (0.63–1.69)       | 0.86 (0.57–1.34)                         | 0.90 (0.57–1.45)     |
| AA vs. GG         | 0.96 (0.66–1.38)                         | 1.01 (0.63–1.63)                         | 1.04 (0.61–1.63)                         | 0.99 (0.57–1.44)       | 0.91 (0.57–1.44)                         | 0.91 (0.57–1.44)     |
| AA vs. GG/GA      | 0.56 (0.06–5.37)                         | 0.56 (0.04–4.49)                         | 0.56 (0.04–4.49)                         | 0.56 (0.04–4.49)       | 0.56 (0.04–4.49)                         | 0.56 (0.04–4.49)     |
| IL-4 rs2070874 T>C |                                         |                                          |                                          |                        |                                          |                        |
| TC vs. TT         | 0.93 (0.76–1.13)                         | 0.93 (0.70–1.22)                         | 0.94 (0.74–1.21)                         | 0.95 (0.72–1.24)       | 0.95 (0.72–1.24)                         | 0.95 (0.72–1.24)     |
| CC vs. TT         | 0.51 (0.29–0.91)                         | 0.42 (0.18–0.89)                         | 0.59 (0.30–1.19)                         | 0.52 (0.25–1.09)       | 0.52 (0.25–1.09)                         | 0.52 (0.25–1.09)     |
| TC/CC vs. TT      | 0.88 (0.72–1.07)                         | 0.85 (0.65–1.10)                         | 0.90 (0.71–1.15)                         | 0.89 (0.69–1.16)       | 0.89 (0.69–1.16)                         | 0.89 (0.69–1.16)     |
| CC vs. TT/TC      | 0.53 (0.30–0.93)                         | 0.43 (0.18–1.02)                         | 0.60 (0.30–1.20)                         | 0.53 (0.26–1.10)       | 0.53 (0.26–1.10)                         | 0.53 (0.26–1.10)     |
| IL-4 rs2243263 G>C |                                         |                                          |                                          |                        |                                          |                        |
| GC vs. CC         | 1.10 (0.83–1.45)                         | 1.02 (0.71–1.48)                         | 1.16 (0.83–1.62)                         | 1.03 (0.70–1.51)       | 1.03 (0.70–1.51)                         | 1.03 (0.70–1.51)     |
| CC vs. GG         | 1.28 (0.29–5.73)                         | 0.93 (0.10–8.35)                         | 1.57 (0.29–8.63)                         | 0.84 (0.09–8.01)       | 0.84 (0.09–8.01)                         | 0.84 (0.09–8.01)     |
| GC/CC vs. GG      | 1.10 (0.84–1.45)                         | 1.02 (0.71–1.47)                         | 1.17 (0.84–1.63)                         | 1.02 (0.70–1.49)       | 1.02 (0.70–1.49)                         | 1.02 (0.70–1.49)     |
| CC vs. GG/GC      | 1.26 (0.28–6.68)                         | 0.93 (0.10–8.32)                         | 1.54 (0.28–8.46)                         | 0.83 (0.09–7.98)       | 0.83 (0.09–7.98)                         | 0.83 (0.09–7.98)     |
| IL-10 rs1800872 T>G |                                         |                                          |                                          |                        |                                          |                        |
| TG vs. TT         | 0.91 (0.75–1.10)                         | 0.89 (0.69–1.15)                         | 0.92 (0.73–1.17)                         | 0.90 (0.69–1.18)       | 0.90 (0.69–1.18)                         | 0.90 (0.69–1.18)     |
| GG vs. TT         | 0.80 (0.58–1.11)                         | 0.75 (0.48–1.17)                         | 0.92 (0.73–1.17)                         | 0.80 (0.58–1.11)       | 0.80 (0.58–1.11)                         | 0.80 (0.58–1.11)     |
| GG/TG vs. TT      | 0.89 (0.74–1.07)                         | 0.86 (0.67–1.10)                         | 0.90 (0.72–1.14)                         | 0.88 (0.68–1.13)       | 0.88 (0.68–1.13)                         | 0.88 (0.68–1.13)     |
| GC vs. TT/GC      | 0.84 (0.61–1.15)                         | 0.79 (0.52–1.21)                         | 0.88 (0.60–1.29)                         | 0.79 (0.51–1.23)       | 0.79 (0.51–1.23)                         | 0.79 (0.51–1.23)     |
| IL-10 rs1800896 T>C |                                         |                                          |                                          |                        |                                          |                        |
| TC vs. TT         | 1.05 (0.79–1.40)                         | 1.17 (0.81–1.70)                         | 0.95 (0.66–1.37)                         | 0.90 (0.66–1.37)       | 0.90 (0.66–1.37)                         | 0.90 (0.66–1.37)     |
| CC vs. TT         | 1.70 (0.42–6.81)                         | 1.71 (0.40–7.33)                         | 1.63 (0.27–9.70)                         | 1.71 (0.40–7.33)       | 1.71 (0.40–7.33)                         | 1.71 (0.40–7.33)     |
| TC/CC vs. TT      | 1.07 (0.80–1.42)                         | 1.19 (0.83–1.71)                         | 0.90 (0.61–1.33)                         | 1.19 (0.82–1.73)       | 1.19 (0.82–1.73)                         | 1.19 (0.82–1.73)     |
| CC vs. TT/TC      | 1.69 (0.42–6.77)                         | 1.68 (0.42–6.77)                         | 1.65 (0.28–8.48)                         | 1.68 (0.42–6.77)       | 1.68 (0.42–6.77)                         | 1.68 (0.42–6.77)     |

1 Adjusted for age, sex, smoking status, alcohol use and BMI status. Bold values are statistically significant (P<0.05).
Table 5 Stratified analyses between IL-4 rs2070874 T>C polymorphism and CRC risk by sex, age, BMI, smoking status, and alcohol consumption

| Variable          | IL-4 rs2070874 T>C (case/control) | Adjusted OR\(^2\) (95% CI); \(P\) |
|-------------------|-----------------------------------|------------------------------------|
| Sex               |                                   |                                    |
| Male              | 366/578                           | 0.93 (0.73–1.20); 0.88 (0.69–1.22); 0.46 (0.23–0.93); |
|                  | 12/35                             | 0.593                              | 0.281                              |
| Female            | 120/202                           | 0.94 (0.61–1.45); 0.86 (0.57–1.31); 0.44 (0.14–1.38); |
|                  | 4/15                              | 0.785                              | 0.489                              |
| Age (years)       |                                   |                                    |
| <63               | 164/292                           | 0.94 (0.68–1.29); 0.89 (0.65–1.22); 0.51 (0.20–1.34); |
|                  | 84/143                            | 0.681                              | 0.465                              |
| ≥63               | 322/488                           | 0.91 (0.68–1.23); 0.84 (0.63–1.11); 0.42 (0.20–0.92); |
|                  | 130/228                           | 0.546                              | 0.220                              |
| Smoking status    |                                   |                                    |
| Never             | 246/564                           | 0.74 (0.55–0.99); 0.70 (0.53–0.93); 0.45 (0.20–1.05); |
|                  | 87/276                            | 0.41 (0.18–0.96); 0.40 (0.20–1.09); 0.46 (0.20–1.09); |
|                  | 7/34                              | 0.177                              | 0.404                              |
| Ever              | 240/216                           | 1.26 (0.90–1.76); 1.15 (0.83–1.58); 0.46 (0.20–1.09); |
|                  | 127/95                            | 0.116                              | 0.404                              |
| Alcohol consumption|                                 |                                    |
| Never             | 341/675                           | 0.91 (0.71–1.16); 0.86 (0.68–1.09); 0.55 (0.29–1.06); |
|                  | 146/323                           | 0.54 (0.28–1.103); 0.80 (0.209); 0.074 |
|                  | 0.428                             | 0.20 (0.05–0.82); 0.80 (0.55–1.39); 0.20 (0.05–0.81); |
|                  | 0.21 (1.19); 0.404                 | 0.025                              | 0.024                              |
| Ever              | 145/105                           | 1.01 (0.62–1.63); 0.88 (0.55–1.39); 0.20 (0.05–0.81); |
|                  | 68/48                            | 0.979                              | 0.570                              |
|                  | 3/8                               | 0.025                              | 0.024                              |
| BMI (kg/m\(^2\)) |                                   |                                    |
| <24               | 356/417                           | 0.92 (0.70–1.20); 0.84 (0.65–1.08); 0.41 (0.20–0.82); |
|                  | 154/196                           | 0.517                              | 0.179                              |
|                  | 12/32                             | 0.80 (0.65–1.35); 0.90 (0.63–1.29); 0.60 (0.20–1.85); |
|                  | 0.719                             | 0.40 (0.20–0.81); 0.573             | 0.576                              |
| ≥24               | 130/363                           | 0.94 (0.65–1.35); 0.80 (0.63–1.29); 0.60 (0.20–1.85); |
|                  | 60/175                            | 0.59 (0.19–1.822); 0.573            | 0.576                              |

\(^1\) For IL-4 rs2070874 T>C, the genotyping was successful in 716 (99.31%) CRC cases and 1201 (99.42%) controls.

\(^2\) Adjusted for multiple comparisons [age, sex, BMI, smoking status and alcohol consumption (besides stratified factors accordingly)] in a logistic regression model.

Bold values are statistically significant (\(P<0.05\)).

Association of HVEM rs2234167, IL-4 rs2070874, and rs2243263, and IL-10 rs1800896 and rs1800872 loci with tumor grade of ESCC cases

As presented in Table 1, 142 patients had well-differentiated tumors, 405 had moderately differentiated, tumors and 174 has poorly differentiated tumors. We found an association of the IL-10 rs1800872 T>G SNP with a worse differentiation (TG vs. TT: \(P=0.048\) and GG/TG vs. TT: \(P=0.032\), Table 8).

Discussion

Immunotherapy is altering how we comprehend malignancies and offers new methods to treat them. EC is a representative model of immune and inflammation-related cancer [39]. Recently, some studies indicated that the SNPs in inflammation and immune-related genes might influence the risk of EC [40,41]. In this study, we explored the role of immune-related gene SNPs (HVEM rs2234167, IL-4 rs2070874, and rs2243263, and IL-10 rs1800896 and rs1800872) to ESCC development. We observed that IL-4 rs2070874 T>C could decrease a risk to ESCC, even in the stage I/II subgroup. However, in BMI ≥ 24 kg/m\(^2\) subgroup, IL-4 rs2243263 G>C might increase the risk of ESCC. We also found an association of the IL-10 rs1800872 T>G SNP with a worse differentiation.

IL-4 is an important regulator of immune and inflammation pathways. Some reports have suggested that IL-4 levels are higher in untreated ESCC patients than in controls [42–44]. It is considered that IL-4 levels may be implicated in the development of ESCC. The IL-4 rs2070874 T>C polymorphism is a 3′-UTR SNP. In a high-risk gastric cancer (GC) region, a previous study suggested that rs2070874 C allele in the IL-4 gene might decrease the susceptibility to GC in a Chinese population [45]. Lu et al. reported that the rs2070874 C allele increased the risk of HCC in a male subgroup [46]. However, Chang et al. and Wang et al. found that the IL-4 rs2070874 polymorphism might not influence the susceptibility of cancer in Chinese population [47,48]. In this study, we included 1929 subjects and investigated the correlation of this SNP to ESCC susceptibility. We found that IL-4 rs2070874 T>C polymorphism...
seemed to be a protective factor for ESCC development. Our findings were similar to a previous meta-analysis that suggested that the *IL-4* rs2070874 C allele could be associated with a decreased susceptibility of gastrointestinal cancer [14]. A functional study indicated that the *IL-4* rs2070874 allele C could promote a higher level of IL-4 in plasma [49]. *IL-4* has an anti-inflammatory effect and may decrease the risk of ESCC by inhibiting the inflammation. FitzGerald et al. reported that the *IL-4* rs2070874 allele C could decrease the risk of prostate cancer specific mortality [50]. Consistent with that report, we identified an association between the *IL-4* rs2070874 T>C SNP and CRC risk by sex, age, BMI, smoking status and alcohol consumption (besides stratified factors accordingly) in a logistic regression model.

Table 6 Stratified analyses between *IL-4* rs2243263 G>C polymorphism and CRC risk by sex, age, BMI, smoking status, and alcohol consumption

| Variable             | *IL-4* rs2243263 G>C (case/control)\(^1\) | Adjusted OR\(^2\) (95% CI); \(P\) |
|----------------------|------------------------------------------|-----------------------------------|
|                      | GG GC CC                                  | GG GC CC GC/CC CC vs. (GC/GG)     |
| Sex                  |                                          |                                   |
| Male                 | 464/779 79/113 2/3                       | 1.00 (1.03–1.10);  
P: 0.388  |
|                      |                                         | 1.22 (1.08–1.38);  
P: 0.038  |
| Female               | 151/269 17/36 1/1                        | 1.06 (1.00–1.12);  
P: 0.126  |
|                      |                                         | 1.37 (1.09–1.70);  
P: 0.018  |
| Age                  |                                         |                                   |
| <63                  | 215/392 34/59 1/3                       | 1.00 (1.00–1.01);  
P: 0.098  |
|                      |                                         | 0.55 (0.40–0.81);  
P: 0.028  |
| ≥63                  | 400/656 62/90 2/1                       | 1.00 (1.00–1.01);  
P: 0.084  |
|                      |                                         | 1.89 (1.26–2.81);  
P: 0.008  |
| Smoking status       |                                         |                                   |
| Never                | 300/763 38/108 1/2                       | 1.00 (1.00–1.01);  
P: 0.000  |
|                      |                                         | 0.72 (0.53–1.00);  
P: 0.045  |
| Ever                 | 315/285 58/41 2/2                       | 1.00 (1.00–1.01);  
P: 0.052  |
|                      |                                         | 2.36 (1.40–3.96);  
P: 0.003  |
| Alcohol consumption  |                                         |                                   |
| Never                | 433/903 63/134 3/3                       | 1.00 (1.00–1.01);  
P: 0.000  |
|                      |                                         | 0.98 (0.71–1.36);  
P: 0.017  |
| Ever                 | 182/145 33/15 0/1                        | 1.00 (1.00–1.01);  
P: 0.000  |
|                      |                                         | 1.70 (1.16–2.46);  
P: 0.057  |
| BMI (kg/m\(^2\))     |                                         |                                   |
| <24                  | 457/553 62/89 3/3                       | 1.00 (1.00–1.01);  
P: 0.000  |
|                      |                                         | 0.84 (0.59–1.22);  
P: 0.064  |
| ≥24                  | 158/495 34/60 0/1                       | 1.00 (1.00–1.01);  
P: 0.000  |
|                      |                                         | 0.37 (0.24–0.57);  
P: 0.012  |

\(^1\) For *IL-4* rs2243263 G>C, the genotyping was successful in 714 (99.03%) CRC cases and 1201 (99.42%) controls.

\(^2\) Adjusted for multiple comparisons [age, sex, BMI, smoking status and alcohol consumption (besides stratified factors accordingly)] in a logistic regression model.

Bold values are statistically significant \((P<0.05)\).

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Table 7 Logistic regression analyses of association between HVEM rs2234167, IL-4 rs2070874, rs2243263 and IL-10 rs1800896 and rs1800872 polymorphisms and lymph node status in ESCC patients

| Genotype       | Positive (n=405) | Negative (n=316) | Crude OR (95% CI) | P       | Adjusted OR¹ (95% CI) | P       |
|---------------|-----------------|-----------------|------------------|--------|----------------------|--------|
|               | n   | %    | n   | %    | P               | P       |
| HVE/M rs2234167 G>A |     |      |     |      |                  |        |
| GG            | 378 | 93.80 | 290 | 92.65 | 1.00             | 1.00   |
| GA            | 24  | 5.96  | 23  | 7.35  | 0.80 (0.44–1.45) | 0.461  |
| AA            | 1   | 0.25  | 0   | 0     | -                | -      |
| GA + AA       | 25  | 6.20  | 23  | 7.35  | 0.83 (0.46–1.50) | 0.544  |
| GG+GA         | 402 | 99.75 | 313 | 100.00| 1.00             | 1.00   |
| AA            | 1   | 0.25  | 0   | 0     | -                | -      |
| IL-4 rs2070874 T>C |   |      |     |      |                  |        |
| TT            | 275 | 68.24 | 211 | 67.41 | 1.00             | 1.00   |
| TC            | 118 | 29.28 | 96  | 30.67 | 0.94 (0.68–1.30) | 0.723  |
| CC            | 10  | 2.48  | 6   | 1.92  | 1.28 (0.46–3.57) | 0.639  |
| CC+TC         | 128 | 31.76 | 102 | 32.59 | 0.96 (0.70–1.32) | 0.814  |
| TT+TC         | 393 | 97.52 | 307 | 98.08 | 1.00             | 1.00   |
| CC            | 10  | 2.48  | 6   | 1.92  | 1.30 (0.47–3.62) | 0.613  |
| A allele      | 26  | 3.23  | 23  | 3.67  |                  |        |
| IL-4 rs2243263 G>C |   |      |     |      |                  |        |
| GG            | 346 | 86.07 | 269 | 86.22 | 1.00             | 1.00   |
| GC            | 54  | 13.42 | 42  | 13.64 | 1.00 (0.65–1.54) | 0.999  |
| CC            | 2   | 0.50  | 1   | 0.32  | 1.56 (0.14–17.24)| 0.719  |
| CC+TC         | 56  | 13.93 | 43  | 13.78 | 1.01 (0.68–1.55) | 0.955  |
| TT+TC         | 400 | 99.50 | 311 | 99.68 | 1.00             | 1.00   |
| CC            | 2   | 0.50  | 1   | 0.32  | 1.56 (0.14–17.23)| 0.719  |
| C allele      | 58  | 17.12 | 108 | 17.25 |                  |        |
| IL-10 rs1800872 T>G |  |      |     |      |                  |        |
| TT            | 195 | 48.51 | 154 | 49.20 | 1.00             | 1.00   |
| TG            | 169 | 42.04 | 132 | 42.17 | 1.01 (0.74–1.38) | 0.944  |
| GG            | 38  | 9.45  | 27  | 8.63  | 1.11 (0.65–1.90) | 0.700  |
| GG+TG         | 207 | 51.49 | 159 | 50.80 | 1.03 (0.77–1.38) | 0.854  |
| TT+TG         | 364 | 90.55 | 286 | 88.54 | 1.00             | 1.00   |
| GG            | 38  | 9.45  | 27  | 8.63  | 1.11 (0.66–1.86) | 0.703  |
| G allele      | 245 | 30.47 | 186 | 29.71 |                  |        |
| IL-10 rs1800896 T>C |  |      |     |      |                  |        |
| TT            | 356 | 88.78 | 269 | 86.22 | 1.00             | 1.00   |
| TC            | 43  | 10.72 | 41  | 13.14 | 0.79 (0.50–1.25) | 0.318  |
| CC            | 2   | 0.50  | 2   | 0.64  | 0.76 (0.11–5.40) | 0.780  |
| CC+TC         | 45  | 11.22 | 43  | 13.78 | 0.79 (0.51–1.24) | 0.303  |
| TT+TC         | 399 | 99.50 | 310 | 99.36 | 1.00             | 1.00   |
| CC            | 2   | 0.50  | 2   | 0.64  | 0.78 (0.11–5.55) | 0.801  |
| C allele      | 47  | 5.86  | 45  | 7.21  |                  |        |

¹Adjusted for age, sex, smoking, alcohol use, and BMI status.

that the rs2243263 G>C polymorphism influences the level of IL-4 by regulating gene transcription. In the future, a functional study should be considered to explore the potential mechanism.

The IL-10 rs1800872 T>G is a promotor SNP. Torres-Poveda et al. reported that the expression of IL-10 mRNA and the level of serum IL-10 were significantly higher in subjects with the IL-10 rs1800872 T allele [54]. A recent study found that IL-10 rs1800872 T>G SNP promoted the risk of EC [25]. A meta-analysis also confirmed this association [55]. In our case–control study, we did not find the association of IL-10 rs1800872 T>G SNP with the development of EC, even in stratified analyses and reviewing different lymph node status. Additionally, Liu et al. reported that IL-10 rs1800872 GG genotypes predicted the worse survival of diffuse large B-cell lymphoma patients treated with rituximab-CHOP (cyclophosphamide, doxorubicin, vincristine, and prednisone) [56]. In this study, we found that
Table 8 Logistic regression analyses of association between HVEM rs2234167, IL-4 rs2070874, rs2243263 and IL-10 rs1800896 and rs1800872 polymorphisms and grades of ESCC

| Genotype | G2+G3 (n=579) | G1 (n=142) | Crude OR (95% CI) | P | Adjusted OR\(^1\) (95% CI) | P |
|----------|--------------|------------|------------------|---|---------------------------|---|
| HVEM rs2234167 G>A | | | | | | |
| GG       | 541 93.44 127 89.44 1.00 | | | | | |
| GA       | 33 5.70 14 9.86 0.55 (0.29–1.07) 0.076 0.57 (0.30–1.11) 0.099 |
| AA       | 1 0.17 0 0 - | | | | | |
| GA + AA  | 34 5.87 14 9.86 0.57 (0.30–1.09) 0.091 0.59 (0.31–1.13) 0.112 |
| GG + GA  | 574 99.14 141 99.30 1.00 | | | | | |
| AA       | 1 0.17 0 0 - | | | | | |
| A allele | 35 3.02 14 4.93 | | | | | |
| IL-4 rs2070874 T>C | | | | | | |
| TT       | 391 67.53 95 66.90 1.00 | | | | | |
| TC       | 173 29.88 41 28.87 1.03 (0.68–1.54) 0.905 1.06 (0.70–1.60) 0.785 |
| CC       | 11 1.90 5 3.52 0.54 (0.18–1.58) 0.256 0.56 (0.19–1.66) 0.296 |
| CC + TC  | 184 31.78 46 32.39 0.97 (0.66–1.44) 0.887 1.01 (0.68–1.50) 0.981 |
| TT + TC  | 564 97.41 136 95.77 1.00 | | | | | |
| CC       | 11 1.92 5 3.52 0.53 (0.18–1.55) 0.247 0.55 (0.19–1.62) 0.278 |
| C allele | 196 16.84 51 17.96 | | | | | |
| IL-4 rs2243263 G>C | | | | | | |
| GG       | 493 85.15 122 85.92 1.00 | | | | | |
| GC       | 78 13.47 18 12.68 1.07 (0.62–1.86) 0.803 1.13 (0.65–1.96) 0.674 |
| CC       | 2 0.35 1 0.70 0.70 (0.50–0.50) 0.567 0.62 (0.05–7.19) 0.702 |
| CC + GC  | 80 13.82 19 13.38 1.04 (0.61–1.78) 0.882 1.10 (0.64–1.90) 0.727 |
| GG + GC  | 571 98.62 140 98.59 1.00 | | | | | |
| CC       | 2 0.35 1 0.70 0.49 (0.04–5.45) 0.562 0.61 (0.05–7.03) 0.690 |
| C allele | 82 7.08 20 7.04 | | | | | |
| IL-10 rs1800872 T>G | | | | | | |
| TT       | 269 46.46 80 56.34 1.00 | | | | | |
| TG       | 250 43.18 51 35.92 1.46 (0.99–2.16) 0.059 1.49 (1.00–2.21) 0.048 |
| GG       | 55 9.50 10 7.04 1.64 (0.80–3.36) 0.180 1.59 (0.77–3.27) 0.211 |
| GG + TG  | 305 52.68 61 42.96 1.49 (1.03–2.16) 0.036 1.51 (1.04–2.19) 0.032 |
| TT + TG  | 519 89.64 131 92.25 1.00 | | | | | |
| GG       | 55 9.50 10 7.04 1.39 (0.69–2.80) 0.359 1.34 (0.66–2.71) 0.419 |
| G allele | 360 31.09 71 25.00 | | | | | |
| IL-10 rs1800896 T>C | | | | | | |
| TT       | 501 86.53 124 87.32 1.00 | | | | | |
| TC       | 68 11.74 16 11.27 1.05 (0.59–1.88) 0.864 1.07 (0.60–1.93) 0.809 |
| CC       | 3 0.52 1 0.70 0.74 (0.08–7.20) 0.797 0.88 (0.09–8.64) 0.909 |
| CC + TC  | 71 12.26 17 11.97 1.03 (0.59–1.82) 0.909 1.06 (0.60–1.88) 0.833 |
| TT + TC  | 569 98.27 140 98.59 1.00 | | | | | |
| CC       | 3 0.52 1 0.70 0.74 (0.08–7.15) 0.793 0.87 (0.09–8.55) 0.903 |
| C allele | 74 6.39 18 6.34 | | | | | |

1 Adjusted for age, sex, smoking, alcohol use, and BMI status. Bold values are statistically significant (P<0.05).

the IL-10 rs1800872 G allele was associated with poorly differentiated tumor. Thus, in the future, the association of the IL-10 rs1800872 T>G SNP and the survival of ESCC cases should be further studied.

Limitations in the present study should be acknowledged. First, in the present study, we only included five functional SNPs and explored the association of the risk to ESCC. Second, there were other environmental risk factors (e.g. vegetable and fruit intake, aspirin and NSAIDs use, and physical exercise), which we did not consider for their influence to the development of ESCC. Third, the number of ESCC patients was limited and our study may be
under-powered in some subgroups. Fourth, in this investigation, the protein expression levels of the suspect factors were not measured. Finally, considering the low penetrance of SNP, the other functional polymorphisms in the HVEM, IL-4, and IL-10 genes should not be ignored.

In summary, the present study suggests that the IL-4 rs2070874 T>C polymorphism is a protective factor for ESCC development, while the IL-4 rs2243263 G>C increases a risk to ESCC in obese and overweight subjects. Additionally, it is highlighted that the IL-10 rs1800872 G allele is associated with poorly differentiated tumor.

Competing Interests
The authors declare that there are no competing interests associated with the manuscript.

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Author Contribution
All authors contributed significantly to the present study. Conceived and designed the experiments: W.T. and M.K. Performed the experiments: S.C., R.C. and C.L. Analyzed the data: W.T. and M.K. Contributed reagents/materials/analysis tools: M.K. Wrote the manuscript: S.C. and R.C. Other (please specify): none.

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Abbreviations
AJCC, American Joint Committee on Cancer; BC, breast cancer; BMI, body mass index; CI, confidence interval; EC, esophageal cancer; ESCC, esophageal squamous cell carcinoma; GC, gastric cancer; HBV, Hepatitis B virus; HVEM, herpesvirus entry mediator; HWE, Hardy–Weinberg equilibrium; IL, interleukin; NSAID, nonsteroidal anti-inflammatory drug; OR , odds ratio; SNP, single nucleotide polymorphism.

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