Investigation of TCF7L2, LEP and LEPR polymorphisms with esophageal squamous cell carcinomas

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ABSTRACT

Single nucleotide polymorphisms (SNPs) in energy metabolism related gene may be key agents in the development of human malignancies. In this study, we aimed to examine the association of transcription factor 7-like 2, Leptin (LEP) and LEP receptor (LEPR) polymorphisms with esophageal squamous cell carcinoma (ESCC). A total of 507 ESCC cases and 1,496 controls were enrolled. We found that LEPR rs6588147 AA genotype was associated with ESCC risk (AA vs. GG/GA: adjusted OR=1.90, 95%CI=1.00–3.61, P=0.049). In the stratified analyses, LEPR rs6588147 G>A polymorphism increased the risk of ESCC (<63 years subgroup: AA vs. GG: adjusted OR=2.58, 95%CI=1.00–6.62, P=0.049 and AA vs. GA/GG: adjusted OR=2.71, 95%CI=1.06–6.91, P=0.038; male subgroup: AA vs. GG: adjusted OR=2.19, 95%CI=1.02–4.67, P=0.044 and AA vs. GA/GG: adjusted OR=2.26, 95%CI=1.06–4.80, P=0.035). However, LEP rs7799039 G>A polymorphism increased the risk of ESCC (<63 years subgroup: GA vs. GG: adjusted OR=2.58, 95%CI=1.00–6.62, P=0.049 and AA vs. GA/GG: adjusted OR=2.71, 95%CI=1.06–6.91, P=0.038; male subgroup: AA vs. GG: adjusted OR=2.19, 95%CI=1.02–4.67, P=0.044 and AA vs. GA/GG: adjusted OR=2.26, 95%CI=1.06–4.80, P=0.035). In addition, LEPR rs1137101 G>A polymorphism decreased ESCC risk in some subgroups (ever smoking subgroup: GA vs. GG: adjusted OR=0.66, 95%CI=0.45–0.99, P=0.044). Our findings suggest that LEPR rs6588147 G>A polymorphism is associated with the increased risk of ESCC; however, LEP rs7799039 A>G and LEPR rs1137101 G>A polymorphisms may be protective factors for ESCC.
INTRODUCTION

In China, esophageal cancer (EC) is the fourth most commonly diagnosed cancer in males and the fifth in females, with an estimated 477,900 new patients and 375,000 related deaths occurring in 2015 [1]. Esophageal squamous cell carcinoma (ESCC) is the main form of EC in China and Eastern Asia. The contributing risk factors for ESCC are not fully known, but are thought to involve low intake of vegetables and fruits, poor nutritional status, smoking and eating and/or drinking at high temperatures. However, these primarily identified risk factors could not account for all the etiology of ESCC. Nowadays, there are convincing evidences that obesity increases the susceptibility of many malignancies, including EC, postmenopausal breast cancer, endometrial cancer, colorectal cancer, pancreatic cancer and liver cancer [2]. A recent study indicated that preoperative metabolic syndrome might be an effective predictor of ESCC mortality [3]. These accumulating evidences suggested that obesity and diabetes related gene might play vital roles in the development of EC.

The transcription factor 7-like 2 (TCF7L2) gene maps to the long arm of chromosome 10q25.3. TCF7L2 belongs to the high mobility group-box (HMGB) family [4] and is a versatile transcription factor. The TCF7L2 protein regulates Wnt/β-catenin signaling pathway [5], therefore it plays important roles in the development and growth of various cells [6, 7]. Ishiguro et al. reported that TCF7L2 expression was associated with a poor prognosis of ESCC [8]. A previous study suggested that TCF7L2 rs7903146 locus might exert its enhancer function by interacting with HMGB1 [9]. TCF7L2 single-nucleotide polymorphisms (SNPs) are proposed susceptibility factors for the development of cancer. Previous studies indicated that TCF7L2 rs7903146 (C/T) polymorphism might influence the risk of breast cancer [10, 11]. TCF7L2 rs290481 T>C polymorphism located on the 3’ end of this gene. Ling et al. reported that this SNP was associated with hepatocellular carcinoma susceptibility with marginal significance [12]. However, the association between these TCF7L2 SNPs and ESCC risk was not explored.

The Leptin (LEP) gene maps to chromosome 7q31.3. LEP is secreted by white adipose tissue and has been identified to be involved in endocrinologic metabolism [13]. It is thought that LEP may regulate the activation and serum levels of insulin. Thus, LEP may involve in the etiology of obesity [14], type 2 diabetes (T2DM) [15] and pathophysiology of carcinoma [16, 17]. LEP receptor (LEPR), also known as CD295 is a single transmembrane protein in human and distributes in various tissues [18]. LEP combines to LEPR and exerts its important roles in the development of metabolic disorders and malignancies. Several studies demonstrated that the elevated LEP levels might affect the onset and progression of many malignancies [19–22]. Thus, LEP and LEPR may be correlated with the development of ESCC.

Results of meta-analyses found that both rs7799039 A>G and rs2167270 G>A polymorphisms in LEP gene might influence the risk of cancer [23–25]. In addition, a case-control study found that LEP rs2167270 G>A was associated with the risk of esophageal adenocarcinoma [26]. There are several explanations for the function of these two LEP polymorphisms. It is suggested that rs7799039 A>G polymorphism in the upstream region of LEP gene can affect leptin expression, possibly at the transcriptional level, thereby altering adipose secretion levels of the hormone [27]. Additionally, LEP rs2167270 G>A is a 5’-utr SNP and may play regulatory roles in translation and stability of mRNA. LEPR rs1137100 G>A, rs1137101 G>A polymorphisms are missense SNPs and may alter the structure and the function of LEPR protein. Doecke et al. found LEPR rs1137100 G>A, rs1137101 G>A polymorphisms influence the risk of esophageal adenocarcinoma in Caucasians [26]. LEPR rs6588147 G>A polymorphism locates on the intron region of LEPR gene. Slattery et al. found that LEPR rs6588147 G>A polymorphism affected risk of colon cancer among men [28]. However, the association between LEPR rs1137100 G>A, rs1137101 G>A and rs6588147 G>A polymorphisms and ESCC risk remains unknown in Asians.

In this case-control study, we aimed to examine the potential association of TCF7L2, LEP and LEPR polymorphisms with the risk of ESCC in Eastern Chinese Han populations. The TCF7L2 rs7903146 C>T, rs290481 T>C, LEP rs7799039 A>G, rs2167270 G>A and LEPR rs1137100 G>A, rs1137101 G>A and rs6588147 G>A polymorphisms were genotyped by SNPscan genotyping assays in 507 ESCC cases and 1,496 non-cancer controls.

RESULTS

Baseline characteristics

There were 2,003 participants in the present case-control study including 507 ESCC patients (377 males and 130 females) and 1,496 non-cancer controls (1,084 males and 412 females). The age and sex were well matched in two groups (P = 0.994, P = 0.406, respectively, Table 1). The mean ± SD of weight and body mass index (BMI) was significantly higher in controls compared with ESCC patients (P < 0.05). However, the mean ± SD of height was not significant (P > 0.05). The proportion of smoking and drinking was significantly higher in ESCC patients compared with controls (P < 0.05). Locus information of TCF7L2, LEP and LEPR polymorphisms is listed in Table 2. The genotyping success rates for TCF7L2 rs7903146C>T, rs290481 T>C, LEP rs7799039 A>G, rs2167270 G>A and LEPR rs1137100 G>A, rs1137101 G>A and rs6588147 G>A SNPs were 99.50%, 99.45%, 99.50%, 99.40%, 99.50%, 99.50% and 99.50%, respectively. Minor allele frequency (MAF) in controls is listed in Table 2, which is very similar to the data of
Association of TCF7L2 rs7903146C>T, rs290481 T>C, LEP rs7799039 A>G, rs2167270 G>A and LEPR rs1137100 G>A polymorphisms with ESCC risk

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

| Variable                        | Cases (n=507) | Controls (n=1,496) | P a |
|---------------------------------|---------------|--------------------|-----|
| Age (years)                     | 62.77 (±8.01) | 62.77 (±8.84)      | 0.994|
| Sex                             |               |                    |     |
| Male                            | 377           | 1,084              |     |
| Female                          | 130           | 412                |     |
| Tobacco use                     |               |                    |     |
| Never                           | 247           | 1,090              | <0.001|
| Ever                            | 260           | 406                |     |
| Alcohol use                     |               |                    |     |
| Never                           | 341           | 1,329              | <0.001|
| Ever                            | 166           | 167                |     |
| Height (cm)                     | 166.0 (±7.29) | 166.1 (±7.08)      | 0.743|
| Weight (kg)                     | 61.54 (±9.83) | 66.11 (±9.92)      | <0.001|
| BMI (kg/m²)                     | 22.27 (±2.90) | 23.91 (±3.03)      | <0.001|
| < 24                            | 370           | 779                |     |
| ≥ 24                            | 137           | 717                |     |

Table 4 shows the genotype frequencies of LEP rs7799039 A>G polymorphism in the subgroup analyses.

Chinese population. In addition, the distributions of the TCF7L2 rs7903146C>T, rs290481 T>C, LEP rs7799039 A>G, rs2167270 G>A and LEPR rs1137100 G>A genotypes in controls conform to Hardy-Weinberg equilibrium (HWE).

Association of TCF7L2 rs7903146C>T, rs290481 T>C, LEP rs7799039 A>G, rs2167270 G>A and LEPR rs1137100 G>A polymorphisms with ESCC risk

Table 4 shows the genotype frequencies of LEP rs7799039 A>G polymorphism in the subgroup analyses.

Association of TCF7L2 rs7903146C>T, rs290481 T>C, LEP rs7799039 A>G, rs2167270 G>A and LEPR rs1137100 G>A polymorphisms with ESCC risk in Different Stratification Groups

Table 4 shows the genotype frequencies of LEP rs7799039 A>G polymorphism in the subgroup analyses.
In ≥63 years subgroup, after adjustment for gender, smoking status, BMI and alcohol use, the LEP rs7799039 GG genotype decreased ESCC risk compared with the LEP rs7799039 AA genotype or LEP rs7799039 AA/AG [GG vs. AA: adjusted OR = 0.47, 95% CI 0.23–0.95, \( P = 0.035 \) and GG vs. AA/AG: adjusted OR = 0.48, 95% CI = 0.24–0.96, \( P = 0.038 \) (Table 4)]. In BMI ≥ 24 kg/m\(^2\) subgroup, after adjustment for age, gender, smoking status and alcohol use, we found that LEP rs7799039 AG genotype decreased the risk of ESCC [AG vs. AA: adjusted OR = 0.66, 95% CI 0.45–0.99, \( P = 0.044 \) (Table 4)].

The genotype frequencies of LEPR rs1137101 G>A polymorphism in the subgroup analyses are showed in Table 5. In ever smoking subgroup, after adjustment for gender, age, BMI and alcohol use, the LEPR rs1137101 GA genotype was associated with the decreased risk of ESCC [GA vs. GG: adjusted OR = 0.66, 95% CI 0.45–0.99, \( P = 0.049 \) (Table 5)]. However, in ever drinking subgroup, after adjustment for gender, smoking status and BMI, the LEPR rs6588147 GA genotype decreased the risk of ESCC [GA vs. GG: adjusted OR = 0.54, 95% CI 0.31–0.92, \( P = 0.024 \) (Table 6)].

### Table 2: Primary information for TCF7L2 rs7903146C>T, rs290481 T>C, LEP rs7799039 A>G, rs2167270 G>A and LEPR rs1137100 G>A, rs1137101 G>A and rs6588147 G>A polymorphisms

| Genotyped SNPs | Chromosome | Chr Pos (NCBI Build 37) | Region | MAF\(^a\) for Chinese in database | MAF in our controls (n = 1, 496) | \( P \) value for HWE\(^b\) test in our controls | Genotyping method | Genotyping value (%) |
|----------------|------------|-------------------------|--------|-----------------------------------|---------------------------------|---------------------------------------------|-----------------|---------------------|
| TCF7L2 rs7903146 C>T | 10 | 114758349 | Intron 4 | 0.026 | 0.031 | 0.733 | SNPscan | 99.50 |
| TCF7L2 rs290481 T>C | 10 | 114923825 | Intron 13 | 0.405 | 0.387 | 0.097 | SNPscan | 99.45 |
| LEP rs7799039 A>G | 7 | 127878783 | Promoter | 0.201 | 0.266 | 0.543 | SNPscan | 99.50 |
| LEP rs2167270 G>A | 7 | 127881349 | 5’ UTR | 0.175 | 0.222 | 0.324 | SNPscan | 99.40 |
| LEPR rs1137100 G>A | 1 | 66036441 | Exon 4 | 0.169 | 0.160 | 0.316 | SNPscan | 99.50 |
| LEPR rs1137101 G>A | 1 | 66058513 | Exon 6 | 0.111 | 0.122 | 0.763 | SNPscan | 99.50 |
| LEPR rs6588147 G>A | 1 | 65935494 | Intron 2 | 0.150 | 0.150 | 0.260 | SNPscan | 99.50 |

\(^a\) MAF: minor allele frequency.

\(^b\) HWE: Hardy–Weinberg equilibrium.
Table 3: Logistic regression analyses of association between TCF7L2 rs7903146C>T, rs290481 T>C, LEP rs7799039 A>G, rs2167270 G>A and LEPR rs1137100 G>A, rs1137101 G>A and rs6588147 G>A polymorphisms and risk of ESCC

| Genotype               | ESCC cases (n=507) | Controls (n=1,496) | Crude OR (95%CI) | P   | Adjusted OR a (95%CI) | P   |
|------------------------|--------------------|--------------------|------------------|-----|------------------------|-----|
| **TCF7L2 rs7903146C>T**|                    |                    |                  |     |                        |     |
| CC                     | 475 94.25          | 1,399 93.96        | 1.00             |     |                        |     |
| CT                     | 29   5.75           | 89    5.98          | 0.96(0.62-1.48)  | 0.847| 1.03(0.65-1.62)         | 0.908|
| TT                     | 0    0              | 1     0.07           | -                | -   |                        | -   |
| CT+TT                  | 29   5.75           | 90    6.04          | 0.95(0.62-1.46)  | 0.814| 1.01(0.64-1.60)         | 0.954|
| CC+CT                  | 504 100.00         | 1488   99.93        | 1.00             | 1.00|                        |     |
| TT                     | 0    0              | 1     0.07           | -                | -   |                        | -   |
| T allele               | 29   2.88           | 91    3.06           |                  |     |                        |     |
| **TCF7L2 rs290481 T>C**|                    |                    |                  |     |                        |     |
| TT                     | 195 38.77           | 575   38.62          | 1.00             |     |                        |     |
| TC                     | 228 45.33           | 676   45.40          | 0.99(0.79-1.23)  | 0.903| 0.96(0.76-1.22)         | 0.748|
| CC                     | 80   15.90           | 238   15.98          | 0.98(0.73-1.33)  | 0.911| 0.99(0.71-1.36)         | 0.927|
| TC+CC                  | 308 61.23           | 914   61.38          | 0.99(0.81-1.22)  | 0.952| 0.98(0.78-1.22)         | 0.830|
| TT+TC                  | 423 84.10           | 1,251 84.02         | 1.00             | 1.00|                        |     |
| CC                     | 80   15.90           | 238   15.98          | 0.99(0.75-1.31)  | 0.967| 1.01(0.75-1.36)         | 0.949|
| C allele               | 388 38.57           | 1,152 38.68         |                  |     |                        |     |
| **LEP rs7799039 A>G**  |                    |                    |                  |     |                        |     |
| AA                     | 291  57.74           | 797   53.53          | 1.00             | 1.00|                        |     |
| AG                     | 184  36.51           | 591   39.69          | 0.85(0.69-1.05)  | 0.138| 0.85(0.67-1.06)         | 0.144|
| GG                     | 29   5.75            | 101   6.78           | 0.79(0.51-1.21)  | 0.275| 0.73(0.46-1.17)         | 0.191|
| AG+GG                  | 213  42.26           | 692   46.47          | 0.84(0.69-1.03)  | 0.101| 0.83(0.67-1.03)         | 0.091|
| AA+AG                  | 475  94.25           | 1,388 93.22         | 1.00             | 1.00|                        |     |
| GG                     | 29   5.75            | 101   6.78           | 0.84(0.55-1.28)  | 0.419| 0.79(0.50-1.24)         | 0.300|
| G allele               | 242  24.01           | 793   26.63          |                  |     |                        |     |
| **LEP rs2167270 G>A**  |                    |                    |                  |     |                        |     |
| GG                     | 318  63.35           | 894   60.04          | 1.00             | 1.00|                        |     |
| GA                     | 165  32.87           | 528   35.46          | 0.87(0.70-1.08)  | 0.213| 0.87(0.69-1.09)         | 0.220|
| AA                     | 19   3.78            | 67    4.50           | 0.79(0.47-1.34)  | 0.382| 0.81(0.47-1.42)         | 0.469|

(Continued)
| Genotype      | ESCC cases (n=507) | Controls (n=1,496) | Crude OR (95%CI) | P     | Adjusted OR * (95%CI) | P     |
|---------------|-------------------|-------------------|-----------------|-------|----------------------|-------|
|               | n     | %     | n     | %     |                   |       |
| GA+AA         | 184   | 36.65 | 595   | 39.96 | 0.87(0.71-1.07)    | 0.190 | 0.86(0.69-1.08)      | 0.198 |
| GG+GA         | 483   | 96.22 | 1,422 | 95.50 | 1.00               |       | 1.00                 |       |
| AA            | 19    | 3.78  | 67    | 4.50  | 0.84(0.50-1.40)    | 0.496 | 0.86(0.49-1.50)      | 0.591 |
| A allele      | 203   | 20.22 | 662   | 22.23 |                     |       |                     |       |
| LEPR rs1137100 G>A |     |      |       |       |                     |       |                     |       |
| GG            | 342   | 67.86 | 1,045 | 70.18 | 1.00               |       | 1.00                 |       |
| GA            | 147   | 29.17 | 411   | 27.60 | 1.09(0.87-1.37)    | 0.448 | 1.08(0.85-1.38)      | 0.517 |
| AA            | 15    | 2.98  | 33    | 2.22  | 1.39(0.74-2.58)    | 0.304 | 1.30(0.67-2.52)      | 0.436 |
| GA+AA         | 162   | 32.14 | 444   | 29.82 | 1.12(0.90-1.39)    | 0.327 | 1.10(0.87-1.39)      | 0.417 |
| GG+GA         | 489   | 97.02 | 1,456 | 97.78 | 1.00               |       | 1.00                 |       |
| AA            | 15    | 2.98  | 33    | 2.22  |                     |       | 1.35(0.73-2.51)      | 0.338 | 1.27(0.66-2.46)      | 0.472 |
| A allele      | 177   | 17.56 | 477   | 16.02 |                     |       |                     |       |
| LEPR rs1137101 G>A |     |      |       |       |                     |       |                     |       |
| GG            | 390   | 77.38 | 1,146 | 76.96 | 1.00               |       | 1.00                 |       |
| GA            | 108   | 21.43 | 322   | 21.63 | 0.98(0.77-1.26)    | 0.898 | 0.91(0.70-1.18)      | 0.473 |
| AA            | 6     | 1.19  | 21    | 1.41  | 0.84(0.34-2.09)    | 0.705 | 0.91(0.35-2.37)      | 0.848 |
| GA+AA         | 114   | 22.62 | 343   | 23.04 | 0.98(0.77-1.24)    | 0.848 | 0.91(0.70-1.18)      | 0.468 |
| GG+GA         | 498   | 98.81 | 1,468 | 98.59 | 1.00               |       | 1.00                 |       |
| AA            | 6     | 1.19  | 21    | 1.41  | 0.84(0.34-2.10)    | 0.712 | 0.93(0.36-2.42)      | 0.884 |
| A allele      | 120   | 11.90 | 364   | 12.22 |                     |       |                     |       |
| LEPR rs6588147 G>A |     |      |       |       |                     |       |                     |       |
| GG            | 367   | 72.82 | 1,070 | 71.86 | 1.00               |       | 1.00                 |       |
| GA            | 119   | 23.61 | 391   | 26.26 | 0.89(0.70-1.12)    | 0.316 | 0.85(0.66-1.09)      | 0.199 |
| AA            | 18    | 3.57  | 28    | 1.88  |                     |       |                     |       |
| GA+AA         | 137   | 27.18 | 419   | 28.14 | 0.95(0.76-1.20)    | 0.680 | 0.91(0.72-1.16)      | 0.465 |
| GG+GA         | 486   | 96.43 | 1,461 | 98.12 | 1.00               |       | 1.00                 |       |
| AA            | 18    | 3.57  | 28    | 1.88  |                     |       |                     |       |
| A allele      | 155   | 15.38 | 447   | 15.01 |                     |       |                     |       |

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

Bold values are statistically significant (P <0.05).
| Variable                  | LEP rs7799039 A>G (case/control)<sup>a</sup> | Adjusted OR<sup>b</sup> (95% CI); P  | GG vs. (AG/AA) |
|--------------------------|-----------------------------------------------|--------------------------------------|----------------|
| AA                       | 222/581                                      | 0.81 (0.62-1.06); P: 0.117           |                |
| AG                       | 134/425                                      | 0.60 (0.33-1.06); P: 0.079           | 0.65 (0.37-1.15); P: 0.136 |
| GG                       | 19/72                                        | 0.77 (0.60-1.00); P: 0.052           |                |
| AG/GG                    | 1.00                                         |                                      |                |
| Male                     | 222/581                                      | 0.81 (0.62-1.06); P: 0.117           |                |
| Sex                      | 134/425                                      | 1.32 (0.60-2.97); P: 0.475           | 1.36 (0.62-2.95); P: 0.442 |
| Female                   | 19/72                                        | 1.03 (0.68-1.55); P: 0.897           |                |
| Age                      | 0.81 (0.62-1.06); P: 0.079                   |                                      |                |
| <63                      | 139/395                                      | 1.31 (0.69-2.50); P: 0.409           | 1.43 (0.76-2.69); P: 0.263 |
| ≥63                      | 152/402                                      | 0.47 (0.23-0.95); P: 0.035           | 0.48 (0.24-0.96); P: 0.038 |
| Smoking status           |                                              |                                      |                |
| Never                    | 146/589                                      | 0.79 (0.59-1.08); P: 0.135           | 1.09 (0.61-1.93); P: 0.779 |
| Ever                     | 145/208                                      | 0.92 (0.65-1.31); P: 0.637           | 0.50 (0.24-1.04); P: 0.063 |
| Alcohol consumption      |                                              |                                      |                |
| Never                    | 198/706                                      | 0.82 (0.63-1.06); P: 0.135           | 0.78 (0.46-1.33); P: 0.359 |
| Ever                     | 93/91                                        | 1.06 (0.64-1.77); P: 0.820           | 0.72 (0.28-1.85); P: 0.492 |
| BMI (kg/m²)              |                                              |                                      |                |
| <24                      | 210/436                                      | 0.96 (0.72-1.26); P: 0.744           | 0.64 (0.36-1.13); P: 0.126 |
| ≥24                      | 81/361                                       | 0.66 (0.45-0.99); P: 0.044           | 1.09 (0.52-2.31); P: 0.816 |

<sup>a</sup> For LEP rs7799039 A>G, the genotyping was successful in 507 (99.41%) ESCC cases, and 1,496 (99.53%) controls.

<sup>b</sup> Adjusted for multiple comparisons [age, sex, BMI, smoking status and alcohol consumption (besides stratified factors accordingly)] in a logistic regression model.
Table 5: Stratified analyses between LEPR rs1137101 G>A polymorphism and ESCC risk by sex, age, BMI, smoking status and alcohol consumption

| Variable               | LEPR rs1137101 G>A (case/control) | Adjusted OR* (95% CI); P    |
|------------------------|----------------------------------|----------------------------|
|                        | GG     | GA    | AA    | GG     | GA    | AA    | GA/AA | AA vs. (GA/GG) |
| Sex                    |        |       |       |        |       |       |       |                |
| Male                   | 292/832| 78/235| 5/11  | 1.00   | 0.84(0.61-1.15); P: 0.275 | 1.52(0.49-4.75); P: 0.473 | 0.87(0.64-1.18); P: 0.353 | 1.57(0.50-4.91); P: 0.435 |
| Female                 | 98/314 | 30/87 | 1/10  | 1.00   | 1.11(0.68-1.81); P: 0.686 | 0.27(0.03-2.24); P: 0.226 | 1.02(0.63-1.65); P: 0.943 | 0.27(0.03-2.20); P: 0.220 |
| Age                    |        |       |       |        |       |       |       |                |
| <63                    | 177/579| 55/157| 4/11  | 1.00   | 1.06(0.72-1.57); P: 0.772 | 1.77(0.51-6.13); P: 0.370 | 1.09(0.74-1.59); P: 0.666 | 1.73(0.50-5.98); P: 0.387 |
| ≥63                    | 213/567| 53/165| 2/10  | 1.00   | 0.75(0.52-1.08); P: 0.123 | 0.43(0.09-2.02); P: 0.283 | 0.74(0.52-1.06); P: 0.097 | 0.46(0.10-2.16); P: 0.323 |
| Smoking status         |        |       |       |        |       |       |       |                |
| Never                  | 186/848| 56/221| 3/17  | 1.00   | 1.15(0.82-1.61); P: 0.432 | 0.72(0.20-2.56); P: 0.613 | 1.12(0.80-1.57); P: 0.504 | 0.70(0.20-2.49); P: 0.585 |
| Ever                   | 204/298| 52/101| 3/4   | 1.00   | 0.66(0.44-1.00); P: 0.049 | 1.51(0.30-7.58); P: 0.616 | 0.68(0.46-1.02); P: 0.063 | 1.65(0.33-8.24); P: 0.543 |
| Alcohol consumption    |        |       |       |        |       |       |       |                |
| Never                  | 260/1,028| 73/276| 5/19  | 1.00   | 1.00(0.74-1.35); P: 0.999 | 1.04(0.37-2.89); P: 0.943 | 1.01(0.75-1.35); P: 0.953 | 1.04(0.38-2.89); P: 0.939 |
| Ever                   | 130/118| 35/46 | 1/2   | 1.00   | 0.54(0.31-0.95); P: 0.031 | 0.56(0.04-8.70); P: 0.679 | 0.54(0.31-0.93); P: 0.027 | 0.64(0.04-9.68); P: 0.750 |
| BMI (kg/m²)            |        |       |       |        |       |       |       |                |
| <24                    | 279/600| 83/165| 5/9   | 1.00   | 0.99(0.72-1.36); P: 0.930 | 1.32(0.42-4.18); P: 0.633 | 1.01(0.74-1.38); P: 0.972 | 1.33(0.42-4.20); P: 0.623 |
| ≥24                    | 111/546| 25/157| 1/12  | 1.00   | 0.76(0.47-1.22); P: 0.250 | 0.39(0.05-3.12); P: 0.376 | 0.73(0.45-1.16); P: 0.183 | 0.41(0.05-3.29); P: 0.405 |

*a For LEPR rs1137101 G>A, the genotyping was successful in 507 (99.41%) ESCC cases, and 1,496 (99.53%) controls.
*b Adjusted for multiple comparisons [age, sex, BMI, smoking status and alcohol consumption (besides stratified factors accordingly)] in a logistic regression model.
Table 6: Stratified analyses between *LEPR* rs6588147 G>A polymorphism and ESCC risk by sex, age, BMI, smoking status and alcohol consumption

| Variable                  | LEPR rs6588147 G>A (case/control)<sup>a</sup> | Adjusted OR<sup>b</sup> (95% CI); <i>P</i> | AA vs. (GA/GG) |
|---------------------------|-----------------------------------------------|-------------------------------------------|----------------|
|                           | GG    | GA   | AA   | 1.00 | 0.89 (0.67-1.20); <i>P</i>: 0.449 | 2.19 (1.02-4.67); <i>P</i>: 0.044 | 0.97 (0.73-1.29); <i>P</i>: 0.834 | 2.26 (1.06-4.80); <i>P</i>: 0.035 |
| Sex                       |       |      |      |      |                                 |                               |                               |                                |
| Male                      | 267/769 | 94/290 | 14/19 | 1.00 | 0.72 (0.43-1.20); <i>P</i>: 0.204 | 1.19 (0.34-4.22); <i>P</i>: 0.785 | 0.76 (0.47-1.24); <i>P</i>: 0.274 | 1.29 (0.37-4.55); <i>P</i>: 0.688 |
| Female                    | 100/301 | 25/101 | 4/9  | 1.00 | 0.84 (0.59-1.20); <i>P</i>: 0.339 | 1.40 (0.58-3.39); <i>P</i>: 0.458 | 0.90 (0.64-1.26); <i>P</i>: 0.534 | 1.48 (0.61-3.56); <i>P</i>: 0.386 |
| Age                       |       |      |      |      |                                 |                               |                               |                                |
| <63                        | 168/527 | 59/206 | 9/14 | 1.00 | 0.80 (0.55-1.16); <i>P</i>: 0.233 | 2.58 (1.00-6.62); <i>P</i>: 0.049 | 0.88 (0.62-1.26); <i>P</i>: 0.484 | 2.71 (1.06-6.91); <i>P</i>: 0.038 |
| ≥63                       | 199/543 | 60/185 | 9/14 | 1.00 | 0.84 (0.59-1.20); <i>P</i>: 0.339 | 1.40 (0.58-3.39); <i>P</i>: 0.458 | 0.90 (0.64-1.26); <i>P</i>: 0.534 | 1.48 (0.61-3.56); <i>P</i>: 0.386 |
| Smoking status             |       |      |      |      |                                 |                               |                               |                                |
| Never                     | 180/787 | 56/279 | 9/20 | 1.00 | 0.89 (0.63-1.24); <i>P</i>: 0.486 | 1.88 (0.82-4.31); <i>P</i>: 0.139 | 0.96 (0.70-1.32); <i>P</i>: 0.807 | 1.94 (0.85-4.44); <i>P</i>: 0.117 |
| Ever                      | 187/283 | 63/112 | 9/8  | 1.00 | 0.80 (0.54-1.17); <i>P</i>: 0.248 | 2.00 (0.71-5.66); <i>P</i>: 0.191 | 0.86 (0.59-1.25); <i>P</i>: 0.438 | 2.12 (0.75-5.97); <i>P</i>: 0.155 |
| Alcohol consumption       |       |      |      |      |                                 |                               |                               |                                |
| Never                     | 245/961 | 80/335 | 13/27 | 1.00 | 0.92 (0.69-1.23); <i>P</i>: 0.590 | 1.69 (0.84-3.40); <i>P</i>: 0.145 | 0.99 (0.75-1.30); <i>P</i>: 0.944 | 1.73 (0.86-3.47); <i>P</i>: 0.124 |
| Ever                      | 122/109 | 39/56  | 5/1  | 1.00 | 0.54 (0.31-0.92); <i>P</i>: 0.024 | 5.03 (0.48-52.46); <i>P</i>: 0.177 | 0.60 (0.35-1.01); <i>P</i>: 0.056 | 5.79 (0.56-59.52); <i>P</i>: 0.139 |
| BMI (kg/m<sup>2</sup>)     |       |      |      |      |                                 |                               |                               |                                |
| <24                       | 261/552 | 92/204 | 14/18 | 1.00 | 0.94 (0.69-1.28); <i>P</i>: 0.700 | 1.79 (0.84-3.82); <i>P</i>: 0.130 | 1.01 (0.76-1.36); <i>P</i>: 0.936 | 1.83 (0.86-3.89); <i>P</i>: 0.115 |
| ≥24                       | 106/518 | 27/187 | 4/10 | 1.00 | 0.67 (0.42-1.07); <i>P</i>: 0.093 | 1.96 (0.59-6.59); <i>P</i>: 0.275 | 0.73 (0.47-1.14); <i>P</i>: 0.168 | 2.14 (0.64-7.17); <i>P</i>: 0.215 |

<sup>a</sup> For *LEPR* rs1137101 G>A, the genotyping was successful in 507 (99.41%) ESCC cases, and 1,496 (99.53%) controls.

<sup>b</sup> Adjusted for multiple comparisons [age, sex, BMI, smoking status and alcohol consumption (besides stratified factors accordingly)] in a logistic regression model.
In addition, after a logistic regression analysis, we found that TCF7L2 rs7903146C>T, rs290481 T>C, LEP rs2167270 G>A and LEPR rs1137100 G>A polymorphisms were not associated with the risk of ESCC in any subgroup (data not shown).

DISCUSSION

The pathogenesis of ESCC was very complex. Multiple factors (e.g. a number of genetic and environmental factors) may contribute to the etiology of ESCC. Understanding of the individual’s heredity background may be helpful for the prevention and treatment of ESCC. In this study, we selected energy metabolism and insulin-sensitivity relative gene (TCF7L2, LEP and LEPR) polymorphisms and focused on their susceptibility to ESCC. The association between LEPR rs6588147 G>A polymorphism and the increased risk of overall ESCC was identified. We also found that LEPR rs6588147 G>A polymorphism increased the risk of ESCC in <63 years and male subgroups. LEP rs7799039 A>G was associated with the risk of ESCC in ≥63 years and BMI ≥ 24 kg/m² subgroups. In addition, LEPR rs1137101 G>A polymorphism decreased the risk of ESCC in ever smoking and ever drinking subgroups.

There was a difference in the LEPR rs6588147 G>A polymorphism between overall ESCC patients and non-cancer controls. The LEPR rs6588147 AA genotype were higher in ESCC patients compared with controls, indicating that LEPR rs6588147 AA genotype may contribute to esophageal carcinogenesis. The LEPR rs6588147 G>A polymorphism is located on intron of LEPR gene. It may be difficult to interpret the exact function of intronic polymorphism. However, the possible interpretations may be as follows. The intronic polymorphism rs6588147 G>A is located near the regulatory components or splice acceptor site, where any slight variant may lead to the disruption of the splice site and induce aberrant splicing [29]. This SNP probably influences the expression of the LEPR protein by altering mRNA splicing. However, we found that LEPR rs6588147 AA genotype may decrease the risk of ESCC in ever drinking subgroup. These findings seemed to be controversial. The probable reason might be due to the limited sample size in this subgroup, which could generate an unauthentic results.

LEP is mainly secreted by adipose tissue, and has been suggested to promote tumor growth [30]. Some studies indicated that the serum LEP level was significantly higher in breast cancer patients compared with which in controls both pre-menopausal and post-menopausal [31, 32]. A number of studies have found that LEP may play vital roles in cell proliferation, apoptosis, cell migration and angiogenesis [33, 34]. Results of several meta-analyses suggested that LEP rs7799039 G allele might decrease the risk of multiple cancers [24, 35–37]. However, there was only one study focused on the relationship between LEP rs7799039 A>G polymorphism and cancer risk in Asian populations. Thus, the association of this polymorphism with cancer risk might be unclear in Asians. In this study, we conducted a case-control study focused on the association between LEP rs7799039 A>G polymorphism and ESCC risk with a relatively large sample size. We found LEP rs7799039 A>G was associated with the decreased risk of ESCC in ≥63 years and BMI ≥ 24 kg/m² subgroups. These findings were very similar to the results of previous studies. Hofsted et al. reported that individuals carried the LEP rs7799039 AA genotype had higher serum LEP levels than those who carried the LEP rs7799039AG or GG genotypes [27]. In this study, we found that LEP rs7799039 A>G polymorphism was a protective factor for ESCC, suggesting the presence of the LEP rs7799039 G allele, which is associated with the decreased level of LEP, might decrease the risk of ESCC.

Several case-control study focused on the relationship of LEPR rs1137101 G>A polymorphism and the risk of cancer. Recently, results of two meta-analyses indicated that this SNP was not associated with the risk of overall cancer [37, 38]. In addition, most of these studies conducted on Caucasian population. The evidence of the association between LEPR rs1137101 G>A polymorphism and cancer risk was insufficient in Asians. A previous study suggested that LEPR rs1137101 G>A polymorphism might be associated with variation in binding with LEP and, as such, inter-individual differences in serum LEP levels [39]. Just as we mentioned above, LEP may affect cell proliferation, apoptosis, cell migration and angiogenesis. LEPR rs1137101 G>A polymorphism may alter the susceptibility of cancer by influencing the ability of binding with LEP. Thus, we aimed to examine the potential association of this polymorphism with the risk of ESCC in Eastern Chinese Han subjects. We found that the LEPR rs1137101 G>A polymorphism decreased ESCC risk in ever drinking and ever smoking subgroups. In the future, function of LEPR rs1137101 G>A polymorphism should be further explored to confirm these primary findings in ESCC.

Our study had several limitations. Firstly, ESCC patients and controls were enrolled from two hospitals of Jiangsu University and Fujian Medical University and might therefore not be full-representative of the general Eastern Chinese Han population; the possible bias might lead to spurious findings. Secondly, for the limited ESCC patients recruited in this study, this study might have insufficient power to observe the potential relationships. Thirdly, because we only selected some functional polymorphisms in TCF7L2, LEP and LEPR gene, a fine-mapping case-control studies should be conducted in the future. Finally, for lack of some important risk factors, the interactive effect between gene-gene and gene-environment was not further analyzed.
In summary, our findings suggest that \textit{LEPR} rs6588147 G>A polymorphism is associated with the increased risk of ESCC in Eastern Chinese Han population. However, the results of this case-control study highlight that \textit{LEP} rs7799039 A>G and \textit{LEPR} rs1137101 G>A polymorphisms may decrease the risk of ESCC. A fine-mapping study with large sample size and functional exploration is needed to confirm our findings.

\textbf{MATERIALS AND METHODS}

\textbf{Subjects}

A total number of 507 ESCC patients and 1,496 non-cancer controls were enrolled in this study. The ESCC patients were from the Affiliated People’s Hospital, Jiangsu University and the Affiliated Union Hospital, Fujian Medical University between August 2013 and December 2016. The diagnosis of ESCC was confirmed based on pathological examination. At the same time, the controls were recruited from physical examination center in these hospitals with sex and age matched. Each subject signed an informed written consent. This study was approved by the Institutional Review Board of Jiangsu University and Fujian Medical University for human subjects (No. SQ20140030, K201408, respectively). When each subject was interviewed, a questionnaire was used to obtain demographic variables and risk factors. And weight and height were also measured. In this study, a BMI ≥ 24 was considered as the criteria for obesity and overweight [40, 41].

\textbf{DNA extraction and genotyping}

Genomic DNA was carefully isolated from EDTA-anticoagulated blood of recipients by using a Promega DNA blood mini kit (Promega, Madison, USA). \textit{TCF7L2} rs7903146C>T, rs290481 T>C, \textit{LEP} rs7799039 A>G, rs2167270 G>A and \textit{LEPR} rs1137100 G>A, rs1137101 G>A and rs6588147 G>A genotypes were assessed by the SNPscan™ kit (Genesky Biotechnologies Inc., Shanghai, China), which is a double ligation and multiplex fluorescence PCR [42]. For quality control, eighty DNA samples (4%) were randomly selected and genotyped by different colleague. The genotypes of \textit{TCF7L2}, \textit{LEP} and \textit{LEPR} polymorphisms were confirmed.

\textbf{Statistical analysis}

Continuous variables (e.g. age, height, weight and BMI) are expressed as mean ±standard deviation (SD). Comparisons between ESCC patients and controls were carried out with Student’s t-test. The categorical variables (e.g. \textit{TCF7L2}, \textit{LEP} and \textit{LEPR} genotypes, sex, age, BMI, smoking and drinking status) were compared with Chi-square test ($\chi^2$). Deviations from the HWE for \textit{TCF7L2}, \textit{LEP} and \textit{LEPR} genotypes distribution in controls were evaluated by an internet-based calculator (http://ihg.gsf.de/cgi-bin/hw/hwa1.pl) [43–49]. The relationships of \textit{TCF7L2} rs7903146C>T, rs290481 T>C, \textit{LEP} rs7799039 A>G, rs2167270 G>A and \textit{LEPR} rs1137100 G>A, rs1137101 G>A and rs6588147 G>A polymorphisms with ESCC susceptibility were evaluated by crude ORs and 95% CIs. Multivariate linear regression adjusted for age, sex, BMI, alcohol use and smoking status was used to determine the relationships between \textit{TCF7L2} rs7903146C>T, rs290481 T>C, \textit{LEP} rs7799039 A>G, rs2167270 G>A and \textit{LEPR} rs1137100 G>A, rs1137101 G>A and rs6588147 G>A polymorphisms and ESCC risk with quantitative traits. Data analysis was conducted with SAS software for windows (Version 9.4, SAS Institute, Cary, NC). A $P < 0.05$ (two-tailed) was accepted as the criterion of statistical significance.

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\textbf{CONFLICTS OF INTEREST}

The authors have no potential financial conflicts of interest.

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