The relationship between *IGF2BP2* and *PPARG* polymorphisms and susceptibility to esophageal squamous-cell carcinomas in the eastern Chinese Han population

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Abstract: The aim of this case-control study was to assess whether *PPARG* and *IGF2BP2* polymorphisms confer susceptibility to esophageal squamous-cell carcinoma (ESCC). A total of 507 patients pathologically confirmed for ESCC and 1,496 age-, sex-, and residence-matched healthy individuals were enrolled. The *PPARG* rs1801282 C>G and rs3856806 C>T and *IGF2BP2* rs1470579 A>C and rs4402960 G>T polymorphisms were selected and genotyped by SNPscan genotyping assays. Multivariable logistic analysis suggested that the *PPARG* rs3856806 C>T polymorphism might increase the risk of ESCC. In different stratified analyses, there were significant associations between *PPARG* rs3856806 C>T and risk of ESCC in female, never-smoking, drinking, and never-drinking subgroups. In addition, we also found that *PPARG* rs1801282 C>G increased ESCC risk in the never-smoking subgroup. There was significant difference in C_r1470579G_r4402960C_r1801282C_r3856806 haplotype distribution among ESCC cases and control subjects. In conclusion, our findings highlight that *PPARG* rs1801282 C>G and rs3856806 C>T polymorphisms are candidates for susceptibility to ESCC in the eastern Chinese Han population. The C_r1470579G_r4402960C_r1801282C_r3856806 haplotype is associated with susceptibility to ESCC.

Keywords: *PPARG, IGF2BP2*, polymorphism, risk, ESCC

Introduction

Esophageal cancer (EC) is a complex disease characterized by progressive dysphagia and emaciation. Because of aging and unhealthy lifestyles (eg, low intake of fruit and vegetables, the rising prevalence of smoking and drinking), EC constitutes a burden worldwide. Esophageal squamous-cell carcinoma (ESCC) is the most common subtype of EC in China.1,2 The potential risk factors driving the high incidence of ESCC are not well understood. It is thought that poor nutritional status, insufficient fruit/vegetables intake, smoking, and drinking beverages at very high temperatures may be involved in the development of ESCC, though these potential risk factors cannot explain the total etiology of ESCC. Nowadays, it is considered that genetic variants may influence the risk of ESCC.

PPARs comprise a group of nuclear transcription factors, which are classified into three subtypes: PPARα, PPARβ, and PPARγ.3 PPARγ is also named PPAR. In humans, the *PPARG* gene is located on chromosome 3p25. PPARγ interacts with the retinoid X receptor, constructs a dipolymer, and then regulates its target
genes, which are involved in cellular differentiation and metabolism of carbohydrates and lipids. Polymorphisms in the PPARG gene are assumed to influence the development of malignancies and metabolism-related diseases. Pro12Ala (rs1801282 C>G) and His449His (rs3856806 C>T) polymorphisms are the two most common single-nucleotide polymorphism (SNPs) in the PPARG gene. Recently, a case–control study was conducted to assess the relationship of PPARG rs3856806 C>T with susceptibility to EC. The results indicated that PPARG rs3856806 C>T might be associated with the risk of EC. In addition, the association between PPARG rs1801282 C>G polymorphism and EC risk was unknown.

IGF2BP2 binds to the 5'UTR of IGF2 mRNA and affects its translation. Barghash et al reported that IGF2BP2 expression correlated with poor survival in patients with esophageal adenocarcinoma and ESCC. Case–control studies have indicated that IGF2BP2 rs4402960 G>T polymorphisms might be associated with the risk of breast cancer and colorectal cancer. In addition, it has been reported that IGF2BP2 rs1470579 A>C was associated with the risk of type 2 diabetes. However, the association between IGF2BP2 polymorphisms and EC risk was unclear.

The aim of this case–control study was to explore the potential relationship of genetic variations in PPARG and IGF2BP2 with risk of ESCC in the eastern Chinese Han population. PPARG rs1801282 C>G and rs3856806 C>T and IGF2BP2 rs1470579 A>C and rs4402960 G>T polymorphisms were selected and genotyped by SNPscan genotyping assays in 507 patients with ESCC and 1,496 controls.

Materials and methods

Subjects

A total of 507 patients pathologically confirmed for ESCC from the Affiliated People’s Hospital of Jiangsu University and the Affiliated Union Hospital of Fujian Medical University (mean age 62.77±8.01 years) were recruited in our study. The noncancer controls consisted of 1,496 age-, sex-, and residence-matched healthy individuals (mean age 62.77±8.84 years) without any cancer history or autoimmune diseases. All participants were enrolled between August 2013 and December 2016. EDTA-anticoagulated peripheral blood was collected after written consent had been signed. A questionnaire was used to obtain participants’ risk factors and demographic variables. A body-mass index (BMI) ≥24 kg/m² was accepted as the criterion of obesity and overweight. This study was approved by the institutional review boards of Jiangsu University (Zhenjiang, China) and Fujian Medical University (Fuzhou, China).

DNA extraction and genotyping

Genomic DNA was extracted from whole blood using a DNA kit (Promega, Madison, WI, USA). PPARG rs1801282 C>G and rs3856806 C>T and IGF2BP2 rs1470579 A>C and rs4402960 G>T genotypes were determined by double ligation and multiplex–fluorescence polymerase chain reaction (SNPscan; Genesky Biotechnologies, Shanghai, China). For quality control, 80 samples (4%) were randomly selected from the 2,003 DNA samples and genotyped again by another technician. Genotypes of PPARG rs1801282 C>G and rs3856806 C>T and IGF2BP2 rs1470579 A>C and rs4402960 G>T polymorphisms were confirmed.

Statistical analysis

Continuous variables (age, BMI, height, and weight) are expressed as means ± SD. Comparisons of these continuous variables between two groups were performed using Student’s t-test. The χ² test was used to compare categorical variables (PPARG and IGF2BP2 genotypes, BMI, sex, age, and smoking status and alcohol use). We checked the deviations for Hardy–Weinberg equilibrium in normal controls with an Internet-based calculator (http://ihg.gsf.de/cgi-bin/hwa1.pl). Significance was defined as P<0.05 (two-tailed).

The relationships of PPARG rs1801282 C>G and rs3856806 C>T and IGF2BP2 rs1470579 A>C and rs4402960 G>T polymorphisms with ESCC susceptibility were determined by crude odds ratios (ORs) and 95% CIs. Adjusted for BMI, age, sex, alcohol use, and smoking status, multivariate linear regression was used to assess the potential association further among these polymorphisms and susceptibility to ESCC. SAS 9.4 software for Windows (SAS Institute, Cary, NC, USA) was used to analyze the data. SHEsis software (http://analysis.bio-x.cn/myanalysis.php; Bio-X, Shanghai, China) was used online to construct the haplotypes.

Results

Baseline characteristics

Characteristics of 507 ESCC cases and 1,496 controls included in this case–control study are presented in Table 1. ESCC cases and controls were well matched on age and sex, as shown by χ² tests (P=0.994 and P=0.406, respectively). As shown in Table 1, significant differences were found on smoking status and alcohol use between cases and controls (P<0.001). The primary information for PPARG rs1801282 C>G and rs3856806 C>T and IGF2BP2 rs1470579 A>C and rs4402960 G>T SNPs is shown in Table 2. For these four genotyped SNPs, the successful ratio was 99.45%–99.5% in all 2,003 DNA samples. The concordance rates of
quality-control testing were 100%. Minor allele frequency of PPARG rs1801282 C>G and rs3856806 C>T and IGF2BP2 rs1470579 A>C and rs4402960 G>T SNPs in controls was close to the minor allele-frequency data for Chinese (Table 2). In controls, the genotype frequencies for PPARG rs1801282 C>G and rs3856806 C>T polymorphisms were in Hardy–Weinberg equilibrium (Table 2).

Association of PPARG rs1801282 C>G and rs3856806 C>T and IGF2BP2 rs1470579 A>C and rs4402960 G>T polymorphisms with ESCC risk in different stratification groups

To determine the potential effects of PPARG rs1801282 C>G genotypes on ESCC risk in different subgroups according to BMI, age, sex, and smoking and drinking status, we carried out stratified analyses (Table 4). In the never-smoking subgroup, after adjustment for sex, age, BMI, and alcohol use, we found that the PPARG rs1801282 C>G polymorphism increased ESCC risk in two genetic models (CG vs CC, polymorphisms are summarized in Table 3. In single-locus analyses, the genotype frequencies of PPARG rs3856806 C>T were 54.56% (CC), 39.09% (CT), and 6.35% (TT) in ESCC patients and 59.7% (CC), 36.13% (CT), and 4.16% (TT) in controls. When the PPARG rs3856806 CC homozygote genotype was used as the reference group, the PPARG rs3856806 CT genotype was correlated with a significantly increased risk of ESCC (CT vs CC, adjusted OR 1.28, 95% CI=1.02–1.6; P=0.033). When the PPARG rs3856806 CC homozygote genotype was used as the reference group, the PPARG rs3856806 TT genotype was correlated with a borderline significantly increased risk of ESCC (TT vs CC, adjusted OR 1.55, 95% CI=0.96–2.50; P=0.074). In the recessive model, when the PPARG rs3856806 CC/CT genotypes were used as the reference group, the PPARG rs3856806 TT homozygote genotype was not associated with susceptibility for ESCC (adjusted OR 1.41, 95% CI=0.88–2.26; P=0.153). In the dominant model, PPARG rs3856806 CC/CT genotypes were associated with an increased risk of ESCC compared with the PPARG rs3856806 CC genotype (adjusted OR 1.31, 95% CI=1.06–1.63; P=0.014) (Table 3). Logistic regression analyses showed that PPARG rs1801282 C>G and IGF2BP2 rs1470579 A>C, rs4402960 G>T polymorphisms were not correlated with the susceptibility for ESCC (Table 3).

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

|                        | Cases (n=507) | Controls (n=1,496) | P-value* |
|------------------------|--------------|--------------------|----------|
| **Age (years), mean ± SD** |              |                    |          |
| Age <63                | 271          | 753                | 0.094    |
| Age ≥63                | 236          | 743                | 0.225    |
| **Sex**                |              |                    |          |
| Male                   | 377          | 1,084              | 0.406    |
| Female                 | 130          | 412                | 0.274    |
| **Tobacco use**        |              |                    | <0.001   |
| Never                  | 247          | 1,090              | 0.274    |
| Ever                   | 260          | 406                | 0.274    |
| **Alcohol use**        |              |                    | <0.001   |
| Never                  | 341          | 1,329              | 0.001    |
| Ever                   | 166          | 167                | 0.116    |
| **Height (cm)**        |              |                    | 0.743    |
| Height ≥166±7.29       | 166±7.29     | 166±7.08           |          |
| **Weight (kg)**        |              |                    | <0.001   |
| Weight ≥61.54±9.83     | 61.54±9.83   | 61.11±9.92         |          |
| **BMI (kg/m²), mean ± SD** |          |                    |          |
| BMI ≥22.27±2.90        | 22.27±2.90   | 23.91±3.03         | <0.001   |
| BMI ≥11.6±2.27         | 11.6±2.27    | 11.1±2.38          |          |

Note: *Two-sided *χ*²* test and Student’s t-test.

Abbreviations: ESCC, esophageal squamous-cell carcinoma; BMI, body-mass index.

Table 2 Primary information for PPARG rs1801282 C>G, rs3856806 C>T, and IGF2BP2 rs1470579 A>C, rs4402960 G>T polymorphisms

| Genotyped SNPs       | Chromosome (NCBI build 38) | Chromosome position | MAF for Chinese in database | MAF in our controls (n=1,496) | P-value for HWE test in our controls | Genotyping method | Genotyping value (%) |
|----------------------|---------------------------|---------------------|----------------------------|-------------------------------|--------------------------------------|------------------|----------------------|
| PPARG rs1801282 C>G  | 3                         | 12351626            | 0.07                       | 0.05                          | 0.911                                | SNPscan          | 99.5                 |
| PPARG rs3856806 C>T  | 3                         | 12434058            | 0.25                       | 0.22                          | 0.083                                | SNPscan          | 99.5                 |
| IGF2BP2 rs1470579 A>C| 3                         | 18581129            | 0.27                       | 0.25                          | 0.002                                | SNPscan          | 99.5                 |
| IGF2BP2 rs4402960 G>T| 3                         | 185793899           | 0.26                       | 0.25                          | 0.002                                | SNPscan          | 99.45                |

Abbreviations: SNPs, single-nucleotide polymorphisms; MAF, minor allele frequency; HWE, Hardy–Weinberg equilibrium.
Table 3 Logistic regression analyses of association between PPARG rs1801282 C>G, rs3856806 C>T and IGF2BP2 1470579 A>C, rs4402960 G>T polymorphisms and risk of ESCC

| Genotype        | ESCC cases (n=507) | Controls (n=1,496) | Crude OR (95% CI) | P-value | Adjusted OR* (95% CI) | P-value |
|-----------------|--------------------|--------------------|-------------------|---------|-----------------------|---------|
|                 | n                  | %                  | n                 | %       |                       |         |
| PPARG rs1801282 C>G |                    |                    |                   |         |                       |         |
| CC              | 440                | 87.3               | 1,334             | 89.59   | 1                     |         |
| GC              | 63                 | 12.5               | 151               | 10.14   | 1.26 (0.92–1.73)       | 0.144   |
| GG              | 1                  | 0.20               | 4                 | 0.27    | 0.76 (0.08–6.79)       | 0.004   |
| GC/GG           | 64                 | 12.7               | 155               | 10.41   | 1.25 (0.92–1.71)       | 0.156   |
| CC/GC           | 503                | 99.8               | 1,485             | 99.73   | 1                     |         |
| GG              | 1                  | 0.20               | 4                 | 0.27    | 0.74 (0.08–6.62)       | 0.786   |
| G allele        | 65                 | 6.45               | 159               | 5.34    | 1.05 (0.11–10.26)      | 0.966   |
| IGF2BP2 1470579 A>C |                    |                    |                   |         |                       |         |
| CC              | 275                | 54.56              | 889               | 59.7    | 1                     |         |
| CT              | 197                | 39.09              | 538               | 36.13   | 1.18 (0.96–1.46)       | 0.125   |
| TT              | 32                 | 6.35               | 62                | 4.16    | 1.66 (1.06–2.6)        | 0.026   |
| CT+TT           | 229                | 45.44              | 600               | 40.30   | 1.23 (1.01–1.51)       | 0.043   |
| CC+CT           | 472                | 93.65              | 1,427             | 95.84   | 1                     |         |
| TT              | 32                 | 6.35               | 62                | 4.16    | 1.56 (1.01–2.42)       | 0.047   |
| T allele        | 261                | 25.89              | 662               | 22.23   | 1.41 (0.88–2.26)       | 0.153   |
| IGF2BP2 rs4402960 G>T |                |                    |                   |         |                       |         |
| AA              | 280                | 55.56              | 855               | 57.42   | 1                     |         |
| AC              | 194                | 38.49              | 517               | 34.72   | 1.14 (0.92–1.41)       | 0.218   |
| CC              | 30                 | 5.95               | 117               | 7.86    | 0.78 (0.51–1.19)       | 0.252   |
| AC+CC           | 224                | 44.44              | 634               | 42.58   | 1.08 (0.88–1.32)       | 0.465   |
| AA+AC           | 474                | 94.05              | 1,372             | 92.14   | 1.04 (0.83–1.29)       | 0.748   |
| CC              | 30                 | 5.95               | 117               | 7.86    | 0.74 (0.49–1.12)       | 0.159   |
| C allele        | 254                | 25.20              | 751               | 25.22   | 0.76 (0.49–1.17)       | 0.213   |

Note: Adjusted for age, sex, BMI, alcohol use, and smoking status.
Abbreviations: ESCC, esophageal squamous-cell carcinoma; BMI, body-mass index; OR, odds ratio.

Adjusted OR 1.54, 95% CI 1.01–2.35, P=0.047; CG/GG vs CC, adjusted OR 1.54, 95% CI 1.01–2.34, P=0.044 [Table 4].

Table 5 shows genotype frequencies of PPARG rs3856806 C>T in different subgroups. Significantly increased susceptibility to ESCC associated with the PPARG rs3856806 C>T polymorphism was found among several subgroups (Table 5). In the female subgroup after adjustment for BMI, age, and smoking and drinking status, the PPARG rs3856806 CT/TT genotypes were associated with increased ESCC risk compared with the PPARG rs3856806 CC genotype (CT/TT vs CC, adjusted OR 1.37, 95% CI 1.03–1.82; P=0.032 [Table 5]). In the drinking subgroup after adjustment for BMI, age, sex, and smoking status, significantly increased risk of ESCC associated with the PPARG rs3856806 C>T polymorphism was also found (TT vs CC, adjusted OR 3.36, 95% CI 1.05–12.74, P=0.041; TT vs CT/CC, adjusted OR 3.58, 95% CI 1.04–12.29, P=0.043 [Table 5]). In the never-drinking subgroup after adjustment for BMI, age, sex, and smoking status, significantly increased risk of ESCC associated with the PPARG rs3856806 C>T polymorphism was also found (CT vs CC, adjusted OR 1.37, 95% CI 1.06–1.77, P=0.015; CT/TT vs CC, adjusted OR 1.37, 95% CI 1.07–1.75, P=0.013.
Table 4 Stratified analyses between PPARG rs1801282 C>G polymorphism and ESCC risk by sex, age, BMI, smoking status, and alcohol consumption

|                  | PPARG rs1801282 C>G (case/control) | Adjusted OR* (95% CI); P-value |
|------------------|------------------------------------|---------------------------------|
|                  | CC       | CG   | GG   | CC       | CG   | GG   | CG/GG | GG vs (CG/CC) |
| **Sex**          |          |      |      |          |      |      |        |               |
| Male             | 328/963  | 47/112| 0/3  | 1.21 (0.81–1.79); P=0.351 | –     | –     | 1.18 (0.8–1.75); P=0.407 | –               |
| Female           | 112/371  | 16/39 | 1/1  | 1.55 (0.81–2.99); P=0.188 | 3.96 (0.25–63.97); P=0.332 | 1.62 (0.86–3.08); P=0.138 | 3.77 (0.23–60.91); P=0.349 |
| **Age, years**   |          |      |      |          |      |      |        |               |
| <63              | 207/679  | 28/66 | 1/2  | 1.43 (0.85–2.41); P=0.179 | 1.78 (0.13–24.51); P=0.665 | 1.43 (0.86–2.39); P=0.172 | 1.71 (0.13–23.44); P=0.687 |
| ≥63              | 233/655  | 35/85 | 0/2  | 1.14 (0.73–1.78); P=0.555 | –     | –     | 1.14 (0.73–1.77); P=0.571 | –               |
| **Smoking status**|          |      |      |          |      |      |        |               |
| Never            | 210/976  | 34/106| 1/4  | 1.54 (1.01–2.35); P=0.047 | 1.34 (0.14–12.59); P=0.796 | 1.54 (1.01–2.34); P=0.044 | 1.28 (014–11.97); P=0.830 |
| Ever             | 230/358  | 29/45 | 0/0  | 0.92 (0.54–1.57); P=0.757 | –     | –     | 0.92 (0.54–1.56); P=0.746 | –               |
| **Alcohol consumption**|      |      |      |          |      |      |        |               |
| Never            | 296/1,186| 41/134| 1/3  | 1.20 (0.82–1.76); P=0.348 | 1.82 (0.18–18.63); P=0.614 | 1.22 (0.84–1.78); P=0.305 | 1.78 (0.17–18.25); P=0.626 |
| Ever             | 144/148  | 22/17 | 0/1  | 1.41 (0.67–3); P=0.369 | –     | –     | 1.32 (0.63–2.77); P=0.457 | –               |
| **BMI (kg/m²)**  |          |      |      |          |      |      |        |               |
| <24              | 319/695  | 47/78 | 1/1  | 1.32 (0.87–1.99); P=0.193 | 2.3 (0.14–37.56); P=0.558 | 1.34 (0.89–2.02); P=0.165 | 2.23 (0.14–36.38); P=0.574 |
| ≥24              | 121/639  | 16/73 | 0/3  | 1.13 (0.63–2.03); P=0.691 | –     | –     | 1.09 (0.61–1.96); P=0.775 | –               |

Notes: For PPARG rs1801282 C>G, genotyping was successful in 507 (99.41%) ESCC cases and 1,496 (99.53%) controls; *adjusted for multiple comparisons (age, sex, BMI, smoking status, and alcohol consumption [besides stratified factors accordingly]) in a logistic regression model.

Abbreviations: ESCC, esophageal squamous-cell carcinoma; BMI, body-mass index; OR, odds ratio.

[Table 5]). In addition, there was no significant risk of ESCC correlated with the IGF2BP2 rs1470579 A>C and rs4402960 G>T polymorphisms evident among any subgroup (data not shown).

SNP haplotypes

Using the SHESis software,[22] we constructed eight haplotypes (Table 6). There were significant differences in the CGCC haplotype of the order rs1470579 A>C, rs4402960 G>T, rs1801282 C>G and rs3856806 C>T polymorphism distribution among ESCC cases and the control subjects (OR 2.23, 95% CI=1.09–4.59; P=0.025 [Table 6]).

Discussion

In this case–control study, we explored the associations between the PPARG rs1801282 C>G and rs3856806 C>T and IGF2BP2 rs1470579 A>C and rs4402960 G>T SNPs and risk of ESCC in the eastern Chinese Han population. Multivariable logistic analysis suggested that PPARG rs3856806 C>T might be associated with an increased risk of ESCC. In different stratified analyses, there were significant associations between this polymorphism and risk of ESCC in the female, never-smoking, drinking, and never-drinking subgroups. In addition, we also found that PPARG rs1801282 C>G increased ESCC risk in the never-smoking subgroup. To the best of our knowledge, this is the first study to identify a potential association between PPARG rs1801282 C>G and rs3856806 C>T polymorphisms and increased risk of ESCC in Asians.

PPARG is a member of the nuclear hormone-receptor superfamily and may possess anti-inflammatory properties.[23] PPARG also plays an important role in cell proliferation/differentiation, which affects the development and progression of cancer.[26,27] The PPARG rs1801282 C>G polymorphism is located in the exon B region of the PPARG gene. Deeb et al reported that this SNP was associated with decreased transactivation activity and lower BMI and promoted insulin sensitivity.[24] A recent meta-analysis suggested that PPARG rs1801282 C>G polymorphism is a candidate for susceptibility to Asians.[29] The association
Table 5: Stratified analyses between PPARG rs3856806 C>T polymorphism and ESCC risk by sex, age, BMI, smoking status, and alcohol consumption

| PPARG rs3856806 C>T (case/control)* | Adjusted OR* (95% CI); P-value |
|-------------------------------------|---------------------------------|
|                                     | CC CT TT                        | CC CT TT CT/TT TT vs (CT/CC) |
| Sex                                 |                                |
| Male                                | 206/632 144/403 25/43           | 1.22 (0.93–1.59); 1.57 (0.89–2.77); 1.26 (0.98–1.63); 1.46 (0.84–2.54); |
|                                     | P=0.146  P=0.116  P=0.078  P=0.185 |
| Female                              | 69/257 53/135 7/19              | 1.53 (0.99–2.36); 1.57 (0.61–4.03); 1.55 (1.02–2.35); 1.33 (0.53–3.37); |
|                                     | P=0.054  P=0.351  P=0.041  P=0.543 |
| Age, years                          |                                |
| <63                                 | 131/457 89/259 16/31            | 1.35 (0.96–1.9); 1.6 (0.78–3.28); 1.37 (0.99–1.9); 1.42 (0.7–2.87); |
|                                     | P=0.081  P=0.199  P=0.061  P=0.331 |
| ≥63                                 | 144/432 108/279 16/31           | 1.26 (0.93–1.72); 1.53 (0.79–2.96); 1.31 (0.98–1.77); 1.4 (0.73–2.68); |
|                                     | P=0.136  P=0.209  P=0.073  P=0.306 |
| Smoking status                      |                                |
| Never                               | 129/645 103/396 13/45           | 1.35 (1.01–1.82); 1.35 (0.7–2.6); 1.37 (1.03–1.82); 1.2 (0.63–2.29); |
|                                     | P=0.044  P=0.37  P=0.032  P=0.578 |
| Ever                                | 146/244 94/142 19/17            | 1.21 (0.84–1.73); 1.82 (0.86–3.84); 1.27 (0.9–1.79); 1.69 (0.81–3.52); |
|                                     | P=0.302  P=0.117  P=0.171  P=0.162 |
| Alcohol consumption                 |                                |
| Never                               | 179/793 142/472 17/58           | 1.37 (1.06–1.77); 1.24 (0.7–2.21); 1.37 (1.07–1.75); 1.1 (0.63–1.94); |
|                                     | P=0.015  P=0.458  P=0.013  P=0.740 |
| Ever                                | 96/96 55/66 15/4               | 1.03 (0.62–1.72); 3.66 (1.05–12.74); 1.19 (0.73–1.94); 3.58 (1.04–12.29); |
|                                     | P=0.901  P=0.041  P=0.48  P=0.043 |
| BMI (kg/m²)                         |                                |
| <24                                 | 207/469 135/268 25/37           | 1.23 (0.93–1.62); 1.53 (0.86–2.72); 1.27 (0.97–1.67); 1.42 (0.81–2.51); |
|                                     | P=0.156  P=0.146  P=0.08  P=0.222 |
| ≥24                                 | 68/420 62/270 7/25             | 1.41 (0.96–2.07); 1.66 (0.68–4.05); 1.43 (0.98–2.07); 1.43 (0.6–3.44); |
|                                     | P=0.080  P=0.268  P=0.063  P=0.423 |

Notes: *For PPARG rs3856806 C>T, genotyping was successful in 507 (99.41%) ESCC cases and 1,4% (99.53%) controls; *adjusted for multiple comparisons (age, sex, BMI, smoking status, and alcohol consumption [besides stratified factors accordingly]) in a logistic regression model.

Abbreviations: ESCC, esophageal squamous-cell carcinoma; OR, odds ratio.

between PPARG rs1801282 C>G and risk of ESCC has not been studied before. In this study, we found that the PPARG rs1801282 CG genotype was more frequent in ESCC patients in the never-smoking subgroup, which was in accordance with the results of the meta-analysis just mentioned. The function of the PPARG rs1801282 C>G SNP remains to be investigated in ESCC patients.

There was a difference in genotype distribution of the PPARG rs3856806 C>T polymorphism between ESCC patients and controls. The PPARG rs3856806 CT and TT/CT genotypes were more frequent in ESCC patients compared with healthy controls, suggesting that the PPARG rs3856806 TT/CT and CT genotypes might contribute to the development of ESCC. The PPARG rs3856806 C>T polymorphism is located in the exon of the PPARG gene. It is difficult to illustrate the exact function of a synonymous SNP. It is proposed that PPARG rs3856806 C→T substitution may disrupt the splice site, and then affect the expression of PPARG. A meta-analysis suggested that PPARG rs3856806 C>T is marginally associated with cancer susceptibility, and our results were similar.

In the present investigation, we constructed eight haplotypes to study inherited patterns. We found that the C→T haplotype was associated

Table 6: Haplotype frequencies (%) in cases and controls and risk of ESCC

| Haplotype | Cases (n=1,006) | Controls (n=2,978) | Crude OR (95% CI) | P-value |
|-----------|----------------|--------------------|-------------------|---------|
| AGCC      | 543/53.98      | 1,679/56.38        | Reference         |         |
| CTCC      | 174/17.30      | 565/18.97          | 0.95 (0.78–1.16)  | 0.624   |
| AGCT      | 164/16.30      | 430/14.44          | 1.18 (0.96–1.45)  | 0.113   |
| CTCT      | 45/4.47        | 107/3.59           | 1.3 (0.91–1.87)   | 0.153   |
| AGGT      | 36/3.68        | 79/2.65            | 1.41 (0.94–2.12)  | 0.096   |
| CTGT      | 13/1.29        | 34/1.14            | 1.18 (0.62–2.26)  | 0.611   |
| CGCC      | 13/1.29        | 18/0.60            | 2.23 (1.09–4.59)  | 0.025   |
| AGGC      | 9/0.89         | 30/1.01            | 0.93 (0.44–1.97)  | 0.845   |
| Others    | 9/0.89         | 36/1.21            | 0.77 (0.37–1.62)  | 0.492   |

Note: With order of rs1470579 A>C, rs4402960 G>T, rs1801282 C>G and rs3856806 C>T in gene position.

Abbreviations: ESCC, esophageal squamous-cell carcinoma; OR, odds ratio.
with susceptibility for ESCC. Comparing the CGCC with the AGCC haplotype in the order of rs1470579 A>C, rs4402960 G>T, rs1801282 C>G, and rs3856806 C>T polymorphisms, we found that A→C variation in the rs1470579 A>C locus led to susceptibility of the haplotype to ESCC. Several case–control studies have reported that IGF2BP2 rs1470579 A>C was associated with type 2 diabetes mellitus.\textsuperscript{11,32–34} However, a potential association between IGF2BP2 rs1470579 A>C polymorphism and ESCC risk was not found in our case–control study. In the future, more case–control studies with large samples and detailed risk factors should be carried out to confirm or refute our findings.

There were some limitations in our study. Firstly, this case–control study was limited by the moderate sample size of ESCC patients, which might lead to suboptimal power to identify true associations in the stratified analyses. Secondly, the controls were recruited from two local hospitals, and might not represent the general Chinese population well; this possible bias should not be ignored. Thirdly, only some functional SNPs in the PPARG and IGF2BP2 genes were selected. The relationship of PPARG and IGF2BP2 variants was not fully explored. In the future, a fine-mapping study should be conducted. Fourthly, detailed information on metastasis and survival of ESCC was not available at the time of research, which restricted further analysis on the potential role of PPARG and IGF2BP2 variants in ESCC progression and prognosis. Finally, for lack of some environmental risk factors, such as lifestyle and intake of fruit/vegetables, the interaction of gene variants with environmental risk factors was not considered.

**Conclusion**

Our findings highlight that PPARG rs1801282 C>G and rs3856806 C>T polymorphisms are candidates for susceptibility to ESCC in the eastern Chinese Han population. A fine-mapping study is required to confirm these preliminary findings.

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**Disclosure**

The authors report no conflicts of interest in this work.

**References**

1. Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J, Jemal A. Global cancer statistics, 2012. *CA Cancer J Clin*. 2015;65(2):87–108.
2. Torre LA, Siegel RL, Ward EM, Jemal A. Global cancer incidence and mortality rates and trends: an update. *Cancer Epidemiol Biomarkers Prev*. 2016;25(1):16–27.
3. Memisoglu A, Hankinson SE, Manson JE, Colditz GA, Hunter DJ. Lack of association of the codon 12 polymorphism of the peroxisome proliferator-activated receptor γ gene with breast cancer and body mass. *Pharmacogenetics*. 2002;12(8):597–603.
4. Cho MC, Lee K, Paik SG, Yoon DY. Peroxisome proliferators-activated receptor (PPAR) modulators and metabolic disorders. *PPAR Res*. 2008;2008:679137.
5. Doecke JD, Zhao ZZ, Stark MS, et al. Single nucleotide polymorphisms in obesity-related genes and the risk of esophageal cancers. *Cancer Epidemiol Biomarkers Prev*. 2008;17(4):1007–1012.
6. Nielsen J, Christiansen J, Lykke-Andersen J, Johnsen AH, Wewer UM, Nielsen FC. A family of insulin-like growth factor II mRNA-binding proteins represses translation in late development. *Mol Cell Biol*. 1999;19(2):1262–1270.
7. Barghash A, Golob-Schwarzl N, Helms V, Hayback J, Kessler SM. Elevated expression of the IGF2 mRNA binding protein 2 (IGF2BP2/IMP2) is linked to short survival and metastasis in esophageal adenocarcinoma. *Oncotarget*. 2016;7(31):49743–49750.
8. Liu G, Zhu T, Cui Y, et al. Correlation between IGF2BP2 gene polymorphism and the risk of breast cancer in Chinese Han women. *Biomed Pharmacother*. 2015;69:297–300.
9. Sainz J, Rudolph A, Hoffmeister M, et al. Effect of type 2 diabetes predisposing genetic variants on colorectal cancer risk. *J Clin Endocrinol Metab*. 2012;97(5):E845–E851.
10. Chang YC, Liu PH, Yu YH, et al. Validation of type 2 diabetes risk variants identified by genome-wide association studies in Han Chinese population: a replication study and meta-analysis. *PLoS One*. 2014;9(4):e95045.
11. Nemr R, Echtay A, Dashti EA, et al. Strong association of common variants in the IGF2BP2 gene with type 2 diabetes in Lebanese Arabs. *Diabetes Res Clin Pract*. 2012;96(2):225–229.
12. Zhai Y, Zhao WH, Chen CM. [Verification on the cut-offs of waist circumference for defining central obesity in Chinese elderly and tall adults]. *Zhonghua Liu Xing Bing Xue Za Zhi*. 2010;31(6):621–625. Chinese.
13. Zhang X, Zhang S, Li Y, et al. Association of obesity and atrial fibrillation among middle-aged and elderly Chinese. *Int J Obes (Lond)*. 2009;33(11):1318–1325.
14. Yin J, Wang X, Wei J, et al. Interleukin 12B rs3212227 T>G polymorphism was associated with an increased risk of gastric cardiac adenocarcinoma in a Chinese population. *Dis Esophagus*. 2015;28(3):291–298.
15. Zhang S, Wang Y, Jiang H, et al. Association between the CD28 IVS3 +17T>C (rs3116496) polymorphism and cancer susceptibility: a meta-analysis involving 8,843 subjects. *Int J Clin Exp Med*. 2015;8(10):17353–17361.
16. Zhang S, Wang Y, Jiang H, et al. Peroxisome proliferator-activated receptor gamma rs1801282 C>G polymorphism is associated with polycystic ovary syndrome susceptibility: a meta-analysis involving 7,069 subjects. *Int J Clin Exp Med*. 2015;8(10):17418–17429.
17. Tang W, Wang Y, Jiang H, et al. Programmed death-1 (PD-1) rs2227981 C>T polymorphism is associated with cancer susceptibility: a meta-analysis. *Int J Clin Exp Med*. 2015;8(12):22278–22285.

18. Qiu H, Cheng C, Wang Y, et al. Investigation of cyclin D1 rs9344 G>A polymorphism in colorectal cancer: a meta-analysis involving 13,642 subjects. *Onco Targets Ther*. 2016;9:6641–6650.

19. Tang W, Qiu H, Ding H, et al. Association between the STK15 F31I polymorphism and cancer susceptibility: a meta-analysis involving 43,626 subjects. *PLoS One*. 2013;8(12):e82790.

20. Tang W, Qiu H, Jiang H, et al. Lack of association between cytotoxic T-lymphocyte antigen 4 (CTLA-4)-1722T/C (rs733618) polymorphism and cancer risk: a case-control study to a meta-analysis. *PLoS One*. 2014;9(4):e94039.

21. Tang W, Wang Y, Chen S, et al. Investigation of cytotoxic T-lymphocyte antigen 4 polymorphisms in gastric cardia adenocarcinoma. *Scand J Immunol*. 2016;83(3):212–218.

22. Shi YY, He L. SHEsis, a powerful software platform for analyses of linkage disequilibrium, haplotype construction, and genetic association at polymorphism loci. *Cell Res*. 2005;15(2):97–98.

23. Tang W, Chen S, Chen Y, et al. Programmed death-1 polymorphisms is [sic] associated with risk of esophagogastric junction adenocarcinoma in the Chinese Han population: a case-control study involving 2,740 subjects. *Oncotarget*. 2017;8(23):37308–37320.

24. Zhu L, Huang Q, Xie Z, et al. PPARGC1A rs3736265 G>A polymorphism is associated with decreased risk of type 2 diabetes mellitus and fasting plasma glucose level. *Oncotarget*. 2017;8(23):37308–37320.

25. Szanto A, Nagy L. The many faces of PPARG: anti-inflammatory by any means? *Immunobiology*. 2008;213(9–10):789–803.

26. Youssef J, Badr M. Peroxisome proliferator-activated receptors and cancer: challenges and opportunities. *Br J Pharmacol*. 2011;164(1):68–82.

27. Ogino S, Shima K, Baba Y, et al. Colorectal cancer expression of peroxisome proliferator-activated receptor γ (PPARG, PPARγ) is associated with good prognosis. *Gastroenterology*. 2009;136(4):1242–1250.

28. Deeb SS, Fajas L, Nemoto M, et al. A Pro12Ala substitution in PPARG2 associated with decreased receptor activity, lower body mass index and improved insulin sensitivity, *Nat Genet*. 1998;20(3):284–287.

29. Wang Y, Chen Y, Jiang H, et al. Peroxisome proliferator-activated receptor γ (PPARG) rs1801282 C>G polymorphism is associated with cancer susceptibility in Asians: an updated meta-analysis. *Int J Clin Exp Med*. 2015;8(8):12661–12673.

30. Sauna ZE, Kimchi-Sarfaty C, Ambudkar SV, Gottesman MM. The sounds of silence: synonymous mutations affect function. *Pharmacogenomics*. 2007;8(6):527–532.

31. Xu W, Li Y, Wang X, et al. PPARγ polymorphisms and cancer risk: a meta-analysis involving 32,138 subjects. *Oncol Rep*. 2010;24(2):579–585.

32. Wu J, Wu J, Zhou Y, et al. Quantitative assessment of the variation in IGF2BP2 gene and type 2 diabetes risk. *Acta Diabetol*. 2012;49 Suppl1:S87–S97.

33. Tabara Y, Osawa H, Kawamoto R, et al. Replication study of candidate genes associated with type 2 diabetes based on genome-wide screening. *Diabetes*. 2009;58(2):493–498.

34. Horikawa Y, Miyake K, Yasuda K, et al. Replication of genome-wide association studies of type 2 diabetes susceptibility in Japan. *J Clin Endocrinol Metab*. 2008;93(8):3136–3141.