Relationship of PPARG, PPARGC1A, and PPARGC1B polymorphisms with susceptibility to hepatocellular carcinoma in an eastern Chinese Han population

Sheng Zhang,1,3,* Jiakai Jiang,1,3 Zhan Chen,2 Yafeng Wang,1 Weifeng Tang,4 Yu Chen,5–7 Longgen Liu6

1 Department of General Surgery, Changzhou Third People’s Hospital, Changzhou, Jiangsu Province, China; 2Department of Thoracic Surgery, Fujian Medical University Union Hospital, Fuzhou, Fujian Province, China; 3Department of Cardiology, People’s Hospital of Xishuangbanna Dai Autonomous Prefecture, Jinghong, Yunnan Province, China; 4Department of Cardiothoracic Surgery, Affiliated People’s Hospital of Jiangsu University, Zhenjiang, Jiangsu Province, China; 5Cancer Bio-immunotherapy Center, Changzhou Cancer Hospital and Fujian Medical University Cancer Hospital, Fuzhou, Fujian Province, China; 6Department of Medical Oncology, Fujian Cancer Hospital and Fujian Medical University Cancer Hospital, Fuzhou, Fujian Province, China; 7Department of Liver Disease, Changzhou Third People’s Hospital, Changzhou, Jiangsu Province, China

*These authors contributed equally

Background: PPARG, PPARGC1A, and PPARGC1B polymorphisms may be implicated in the development of cancer.

Participants and methods: In this study, we selected PPARG rs1801282 C>G and rs3856806 C>T, PPARGC1A rs2970847 C>T, and PPARGC1B rs7732671 G>C and rs17572019 G>A single-nucleotide polymorphisms to explore the relationship between these polymorphisms and hepatocellular carcinoma (HCC) risk. A total of 584 HCC patients and 923 controls were enrolled.

Results: We found that PPARG rs1801282 C>G polymorphism was correlated with a decreased susceptibility of HCC (CG vs CC, adjusted OR 0.47, 95% CI 0.27–0.82, P=0.007; CG/GG vs CC, adjusted OR 0.52, 95% CI 0.31–0.88, P=0.015). However, PPARG rs3856806 C>T polymorphism was a risk factor for HCC (TT vs CC, adjusted OR 2.33, 95% CI 1.25–4.36, P=0.008; TT vs CT/CC, adjusted OR 2.26, 95% CI 1.22–4.17, P=0.010). In a subgroup analysis by chronic hepatitis B virus (HBV)-infection status, age, sex, alcohol use, and smoking status, a significant association between PPARG rs1801282 C>G polymorphism and a decreased risk of HCC in male, ≥ 53 years, never-smoking, never-drinking, and nonchronic HBV-infection-status subgroups was found. However, we found PPARG rs3856806 C>T polymorphism increased the risk of HCC in never-smoking, never-drinking, and nonchronic HBV-infection-status subgroups. Haplotype-comparison analysis indicated that C $\text{rs1801282}$ T, $\text{rs3856806}$ C $\text{rs2970847}$ G, and $\text{rs7732671}$ G $\text{rs17572019}$ A haplotypes increased the risk of HCC. PPARG $\text{rs1801282}$ T, $\text{rs3856806}$ C $\text{rs2970847}$ G, and $\text{rs7732671}$ A $\text{rs17572019}$ T haplotypes also influenced the risk of HCC.

Conclusion: In conclusion, our findings suggest PPARG polymorphisms may influence the susceptibility of HCC. The PPARG, PPARGC1A, and PPARGC1B haplotypes might be associated with HCC risk.

Keywords: PPARG, PPARGC1A, PPARGC1B, polymorphism, risk, hepatitis B virus, hepatocellular carcinoma

Introduction

In 2012, an estimated 782,500 new liver cancer (LC) patients and 745,500 related deaths occurred worldwide.1 China accounts for almost half the total number of LC cases and deaths annually. Hepatocellular carcinoma (HCC) is the most common subtype of LC. A large number of HCC cases are diagnosed annually, with a high mortality rate, which encourages people to explore the potential risk factors for HCC. Due to the chronic infection of hepatitis B virus (HBV), the incidence of HCC in parts of sub-Saharan Africa and Asia is much higher than other regions.2–4 However, other
risk factors might also contribute to the etiology of HCC. Recently, many hereditary factors have been found to confer susceptibility to HCC.

 Peroxisome proliferator-activated receptors (PPARs), a cluster of important nuclear transcription factors, may be involved in the process of cellular differentiation and regulate carbohydrate/lipid metabolism and energy balance. There are three predominant subtypes in PPARs: PPARα, PPARβ, and PPARγ. PPARγ, also known as PPARγ, is located on chromosome 3p25. PPARγ interacts with RXR and forms a dimer to regulate its target genes, which are involved in adipocyte differentiation and insulin sensitization. It has been reported that PPARγ possessed anti-inflammatory roles and can restrain the production of many inflammatory mediators, such as IL6, IL8, and TNFα. Several studies have found that obesity, metabolic syndrome, insulin resistance/insufficiency, type 2 diabetes mellitus (T2DM), and inflammation have a common molecular basis, in which PPARγ can influence the process of these diseases and might alter the risk of cancer. Two coactivators of PPARγ, PPARGC1A and PPARGC1B, are vital regulators of energy metabolism. In addition, Li et al reported that PPARGC1A might be a potential biomarker for lung cancer prognosis. Eichner et al found that miR378 was embedded within PPARGC1B, which encodes PPARGC1B, and miR378 expression correlated with progression of breast cancer in humans. Recently, a meta-analysis found that PPARG rs1801282 C>G single-nucleotide polymorphism (SNP) was associated with cancer risk in Asians; however, the studies included were limited. PPARG rs3856806 C>T polymorphism is believed to be related to inflammatory response and is associated with the development of ovarian carcinoma, follicular lymphoma, and colorectal cancer. Studies have reported that PPARGC1A rs2970847 C>T SNP increased the risk of T2DM. However, the association between this SNP and cancer risk is unknown. Martinez-Nava et al studied the association of PPARGC1B rs7732671 G>C and rs17572019 G>A SNPs with risk of breast cancer and found that the PPARGC1B rs7732671 C allele was a protective factor for breast cancer. In view of these previous studies, the potential role of PPARG, PPARGC1A, and PPARGC1B SNPs in determining HCC risk was unclear. Understanding the possible relationship might be beneficial for HCC prevention. Therefore, in this case–control study, we selected PPARG rs1801282 C>G and rs3856806 C>T, PPARGC1A rs2970847 C>T, and PPARGC1B rs7732671 G>C and rs17572019 G>A SNPs to explore the relationship between these polymorphisms and HCC risk in an eastern Chinese Han population.

Participants and methods

Subjects

As part of an ongoing study carried out in an eastern Chinese Han population, the first 584 incident HCC patients and 923 hospital-based controls were recruited in this study. Our case–control study was approved by Fujian Medical University Ethics Committee (Fuzhou, China). HCC cases were recruited from the Department of Hepatobiliary Surgery at Fuzong Clinical Medical College and Union Clinical Medical College of Fujian Medical University. All HCC patients were diagnosed by pathology. Major selection criteria of HCC patients were sporadic HCC cases, HCC patients without chemoradiotherapy, Chinese Han population, and living in eastern China. Corresponding exclusion criteria were HCC patients with autoimmune disease history, had received prior chemoradiotherapy, had other malignancy history, and without a pathological diagnosis. Meanwhile, a total of 923 participants who attended a physical examination in the hospitals mentioned were enrolled as controls. Additionally, criteria for control selection were healthy subjects without a history of malignancy, without autoimmune disease, without chronic liver disease, and eastern Chinese Han. HCC patients and controls matched well by age and sex. All subjects were recruited between January 2002 and December 2016 consecutively. Demographic variables and risk factors (eg, smoking, drinking, and chronic HBV-infection status) were collected by our colleagues. Written informed consent was signed by all subjects. Information is listed in Table 1.

DNA extraction and genotyping

Extraction of genomic DNA from EDTA anticoagulant blood samples was performed using a DNA-purification kit (Promega, Madison, WI, USA). Purity and concentration of the DNA samples obtained was assessed by spectrophotometry with the NanoDrop ND-1000 and 1.5% agarose gel electrophoresis. Genomic DNA was stored at –80°C. Genotyping of PPARG rs1801282 C>G and rs3856806 C>T, PPARGC1A rs2970847 C>T, and PPARGC1B rs7732671 G>C and rs17572019 G>A SNPs was carried out with a genotyping assay (SNPscan; Genesky Biotechnologies, Shanghai, China) on a 3730XL (Thermo Fisher Scientific, Waltham, MA, USA). Data were observed using GeneMapper 4.1 software (Thermo Fisher Scientific). Sixty (4%) randomly selected samples were tested again by a different technologist. The results were not altered.

Statistical analysis

All statistical analyses were done with SAS 9.4 software (SAS Institute, Cary, NC, USA) using Student’s t-test,
Table 1: Distribution of selected demographic variables and risk factors in HCC cases and controls

| Variable                      | Cases (n=584) | Controls (n=923) | P-value * |
|-------------------------------|--------------|-----------------|-----------|
|                               | n            | %               | n         | %         |          |
| Mean age (years)              | 53.17 (±11.76) | 53.72 (±9.97)   | 0.327     |           |          |
| Age (years)                   |              |                 |           |           |          |
| <53                           | 264          | 45.21           | 395       | 42.80     |          |
| ≥53                           | 320          | 54.79           | 528       | 57.20     |          |
| Sex                           |              |                 |           |           |          |
| Male                          | 525          | 89.90           | 835       | 90.47     |          |
| Female                        | 59           | 10.10           | 88        | 9.53      |          |
| Smoking status                |              |                 |           |           |          |
| Never                         | 374          | 64.04           | 596       | 64.57     | 0.834    |
| Ever                          | 210          | 35.96           | 327       | 35.43     |          |
| Alcohol use                   |              |                 |           | <0.001    |          |
| Never                         | 414          | 70.89           | 775       | 83.97     |          |
| Ever                          | 170          | 29.11           | 148       | 16.03     |          |
| Chronic HBV infection         |              |                 |           | <0.001    |          |
| Yes                           | 412          | 70.55           | 85        | 9.21      |          |
| No                            | 172          | 29.45           | 838       | 90.79     |          |
| BCLC classification           |              |                 |           |           |          |
| A                             | 392          | 67.12           |           |           |          |
| B                             | 175          | 29.97           |           |           |          |
| C                             | 17           | 2.91            |           |           |          |

Note: *Two-sided χ²-test and Student’s t-test.

Abbreviations: BCLC, Barcelona Clinic Liver Cancer; HBV, hepatitis B virus; HCC, hepatocellular carcinoma.

Fisher’s exact test, and χ²-test. Age was expressed as the mean ± SD. We used Student’s t-test to determine the differences in age distribution between HCC cases and controls, and χ²-test or Fisher’s exact test used to assess potential differences in age, sex, smoking status, alcohol use, chronic HBV-infection status, and genotypes. Deviation from Hardy–Weinberg equilibrium (HWE) was determined using an Internet-based calculator (http://ihg.gsdf.de/cgi-bin/hw/hwa1.pl)26,27 to compare the obtained genotype frequencies in controls with the expected frequencies. Using different models of inheritance (allele, additive, homozygote, dominant, and recessive), associations between PPARG rs1801282 C>G and rs3856806 C>T, PPARGC1A rs2970847 C>T, and PPARGC1B rs7732671 G>C and rs17572019 G>A SNPs and risk of HCC were determined by crude/adjusted ORs and CIs. SHEsis software (Bio-X, Shanghai, China), an online calculator, was used for construction of PPARG, PPARGC1A, and PPARGC1B haplotypes.28 P<0.05 (two-tailed) was used as the threshold for significance. In this study, Bonferroni correction was performed for multiple testing.29,30 Power and Sample Size Calculation software (http://biostat.mc.vanderbilt.edu/twiki/bin/view/Main/PowerSampleSize) was used to assess the statistical power of this study (α=0.05).31

Results
Baseline characteristics
HCC patients and cancer-free controls comprised 584 and 923 subjects, respectively. Mean ages were 53.17±11.76 (range 20–83) years in the HCC group and 53.72±9.97 (range 21–83) years in controls. Baseline characteristics of HCC patients and controls are given in Table 1. In addition, Table 1 shows that our study was well matched by sex and age. Corresponding SNP information for PPARG rs1801282 C>G and rs3856806 C>T, PPARGC1A rs2970847 C>T, and PPARGC1B rs7732671 G>C and rs17572019 G>A polymorphisms is summarized in Table 2. The success rate of genotyping was >99% (Table 2). The minor-allele frequency in controls is listed in Table 2, and results were similar to the data for Chinese Han population. In controls, except for PPARG rs3856806 C>T, the distribution of PPARG rs1801282 C>G, PPARGC1A rs2970847 C>T, and PPARGC1B rs7732671 G>C and rs17572019 G>A genotype frequencies accorded with HWE.

Table 2: Primary information for PPARG rs1801282 C>G, rs3856806 C>T, PPARGC1A rs2970847 C>T, and PPARGC1B rs7732671 G>C, rs17572019 G>A polymorphisms

| Genotyped SNPs                  | PPARG rs1801282 C>G | PPARG rs3856806 C>T | PPARGC1A rs2970847 C>T | PPARGC1B rs7732671 G>C | PPARGC1B rs17572019 G>A |
|---------------------------------|---------------------|---------------------|------------------------|------------------------|------------------------|
| Chromosome                      | 3                   | 3                   | 4                      | 5                      | 5                      |
| Function                        | Missense            | Coding-synonymous   | Coding-synonymous       | Missense               | Missense               |
| “Chr Pos” (NCBI build 37)       | 12393125            | 12475557            | 23815924               | 149212243              | 149212471              |
| MAF for Chinese in database     | 0.07                | 0.25                | 0.28                   | 0.09                   | 0.07                   |
| MAF in our controls (n=923)     | 0.05                | 0.22                | 0.22                   | 0.06                   | 0.06                   |
| P-value for HWE test in controls| 0.883               | 0.009               | 0.498                  | 0.241                  | 0.543                  |
| Genotyping method               | SNP scan            | SNP scan            | SNP scan               | SNP scan               | SNP scan               |
| Percentage genotyping value     | 99.27%              | 99.27%              | 99.27%                 | 99.27%                 | 99.27%                 |

Abbreviations: MAF, minor-allele frequency; HWE, Hardy–Weinberg equilibrium; SNP, single-nucleotide polymorphism.
Association of PPARG rs1801282 C>G and rs3856806 C>T, PPARGC1A rs2970847 C>T, and PPARGC1B rs7732671 G>C and rs17572019 G>A SNPs with HCC

The frequencies of PPARG rs1801282 genotypes in HCC patients and controls are summarized in Table 3. We found that the PPARG rs1801282 G allele was associated with a decreased risk of HCC (CG vs CC, crude OR 0.47, 95% CI 0.31–0.72, P=0.001; CG/GG vs CC, crude OR 0.51, 95% CI 0.34–0.77, P=0.001; G vs C, crude OR 0.56, 95% CI 0.38–0.82, P=0.003). After adjustments for age, sex, smoking, drinking, and chronic HBV-infection status, the results were not essentially changed (CG vs CC, adjusted OR 0.47, 95% CI 0.27–0.82, P=0.007; CG/GG vs CC, adjusted OR 0.52, 95% CI 0.31–0.88, P=0.015; Table 3).

Table 3 lists the frequencies of PPARG rs3856806 genotypes in HCC patients and controls. We found that the PPARG rs3856806 T allele conferred risk to HCC (TT vs CC, crude OR 2.12, 95% CI 1.31–3.44, P=0.002; TT vs CC/CT, crude OR 2.13, 95% CI 1.33–3.43, P=0.002; TT vs CC, crude OR 1.21, 95% CI 1.02–1.44, P=0.029). After adjustments for age, sex, smoking, drinking, and chronic HBV-infection status, the results were not materially altered (TT vs CC, adjusted OR 2.33, 95% CI 1.25–4.36, P=0.008; TT vs CC/CT, adjusted OR 2.26, 95% CI 1.22–4.17, P=0.010; Table 3). However, PPARGC1A rs2970847 C>T and PPARGC1B rs7732671 G>C and rs17572019 G>A polymorphisms were not associated with HCC risk in all genetic models (Table 3).

We performed a Bonferroni correction for multiple testing. The genotype distribution of PPARG polymorphisms was still significantly different between HCC cases and controls (P=0.007 for rs1801282 C>G, P=0.008 and P=0.010 for rs3856806 C>T, respectively). We also calculated the statistical power of this study (α=0.05) using Power and Sample Size Calculation. For PPARG rs1801282 C>G, the power value was 0.955 in CG vs CC, 0.906 in GG/GC vs CC, and 0.859 in G vs C. For PPARG rs3856806 C>T, the power value was 0.932 in TT vs CC, 0.921 in TT vs CC/CT, and 0.584 in T vs C.

Association of PPARG rs1801282 C>G and rs3856806 C>T, PPARGC1A rs2970847 C>T, and PPARGC1B rs7732671 G>C and rs17572019 G>A SNPs with HCC in different subgroups

Table 4 shows the relationship of PPARG rs1801282 C>G polymorphism with risk of HCC in the stratified analyses.

After adjustment by logistic regression analysis, we found that the PPARG rs1801282 G allele decreased the risk of HCC (male subgroup, CG vs CC, adjusted OR 0.52, 95% CI 0.29–0.92, P=0.025 and CG/GG vs CC, adjusted OR 0.52, 95% CI 0.29–0.91, P=0.022; ≥53 years subgroup, CG vs CC, adjusted OR 0.36, 95% CI 0.17–0.74, P=0.006 and CG/GG vs CC, adjusted OR 0.38, 95% CI 0.19–0.77, P=0.007; never-smoking subgroup, CG vs CC, adjusted OR 0.32, 95% CI 0.15–0.66, P=0.002 and CG/GG vs CC, adjusted OR 0.39, 95% CI 0.20–0.77, P=0.007; never-smoking subgroup, CG vs CC, adjusted OR 0.40, 95% CI 0.21–0.76, P=0.005 and CG/GG vs CC, adjusted OR 0.47, 95% CI 0.26–0.86, P=0.015; nonchronic HBV-infection subgroup, CG vs CC, adjusted OR 0.42, 95% CI 0.20–0.89, P=0.024 and CG/GG vs CC, adjusted OR 0.46, 95% CI 0.22–0.93, P=0.030).

As listed in Table 5, we found that the PPARG rs3856806 T allele was associated with a risk of HCC in some subgroups (never-smoking subgroup, TT vs CC, adjusted OR 2.24, 95% CI 1.29–4.00, P=0.015; nonchronic HBV-infection subgroup, TT vs CC, adjusted OR 2.21, 95% CI 1.09–4.39, P=0.027; never-drinking subgroup, TT vs CC, adjusted OR 2.10, 95% CI 1.05–4.19, P=0.036 and TT vs CT/CC, adjusted OR 2.08, 95% CI 1.05–4.11, P=0.035; nonchronic HBV-infection subgroup, TT vs CC, adjusted OR 2.44, 95% CI 1.22–4.88, P=0.012 and TT vs CT/CC, adjusted OR 2.34, 95% CI 1.19–4.60, P=0.014). However, PPARGC1A rs2970847 C>T and PPARGC1B rs7732671 G>C and rs17572019 G>A polymorphisms were not associated with HCC risk in any subgroup (data not shown).

SNP haplotypes

Using the SHESIS online calculator, we constructed several haplotypes of PPARG, PPARGC1A, and PPARGC1B genes (Table 6). Haplotype comparison analysis indicated that C rs1801282 T rs3856806 C rs2970847 G rs7732671 G rs17572019 G rs1801282 T rs3856806 T rs2970847 G rs7732671 G rs17572019 C rs3856806 C rs2970847 C rs7732671 A rs17572019 C rs1801282 C rs3856806 C rs2970847 C rs7732671 A rs17572019 were associated with risk of HCC (OR 1.29, 95% CI 1.05–1.59, P=0.017; OR 1.56, 95% CI 1.08–2.25, P=0.017; and OR 1.63, 95% CI 1.11–2.39, P=0.011, respectively). In addition, PPARG C rs1801282 T rs3856806 and G rs1801282 C rs3856806 haplotypes also influenced the risk of HCC (OR 1.31, 95% CI 1.09–1.57, P=0.004 and OR 0.46, 95% CI 0.21–1.00, P=0.046, respectively).

Discussion

PPARG, PPARGC1A, and PPARGC1B genes may have an impact on inflammatory response, insulin sensitization, cell differentiation, and cellular apoptosis and alter the risk of cancer. In this study, we examined the relationship between PPARG rs1801282 C>G and rs3856806 C>T,
Table 3 Logistic regression analyses of associations between PPARG rs1801282 C>G, rs3856806 C>T, PPARGC1A rs2970847 C>T, and PPARGC1B rs7732671 G>C, and rs17572019 G>A polymorphisms and risk of HCC

| Genotype           | HCC cases (n=584) | Controls (n=923) | Crude OR (95% CI) P-value | Adjusted OR (95% CI) P-value |
|--------------------|------------------|-----------------|---------------------------|-----------------------------|
| Total              |                  |                 |                           |                             |
| PPARC rs1801282 C>G |                  |                 |                           |                             |
| CC                 | 542              | 823             | 94.26                     | 89.36                       | 1.00 (1.00)                 | 0.007                           |
| CG                 | 30               | 95              | 5.22                      | 10.31                       | 0.47 (0.31–0.72)            | 0.001                           |
| GG                 | 3                | 3               | 0.52                      | 0.33                        | 0.001                       | 1.00 (0.02–48.26)              | 0.007                           |
| GC + GG            | 33               | 98              | 5.74                      | 10.64                       | 0.51 (0.34–0.77)            | 0.001                           |
| GC + GC            | 572              | 918             | 99.48                     | 99.67                       | 1.00 (0.00)                 | 0.001                           |
| GG                 | 3                | 3               | 0.52                      | 0.33                        | 1.61 (0.32–7.98)            | 0.563                           |
| C allele           | 1,114            | 1,741           | 96.87                     | 94.52                       | 1.00 (0.00)                 | 0.001                           |
| G allele           | 36               | 101             | 3.13                      | 5.48                        | 0.56 (0.38–0.82)            | 0.003                           |
| PPARC rs3856806 C>T |                  |                 |                           |                             |
| CC                 | 320              | 543             | 55.65                     | 58.96                       | 1.00 (1.00)                 | 0.007                           |
| CT                 | 214              | 346             | 37.22                     | 37.57                       | 1.03 (0.82–1.28)            | 0.828                           |
| TT                 | 41               | 32              | 7.13                      | 3.47                        | 2.12 (1.31–3.44)            | 0.002                           |
| CT + TT            | 255              | 378             | 44.35                     | 41.04                       | 1.15 (0.93–1.41)            | 0.208                           |
| CC + CT            | 534              | 889             | 92.87                     | 96.53                       | 1.00 (1.00)                 | 0.001                           |
| TT                 | 41               | 32              | 7.13                      | 3.47                        | 2.13 (1.33–3.43)            | 0.002                           |
| C allele           | 854              | 1,432           | 74.26                     | 77.74                       | 1.00 (1.00)                 | 0.001                           |
| T allele           | 296              | 410             | 25.74                     | 22.26                       | 1.21 (1.02–1.44)            | 0.029                           |
| PPARGC1A rs2970847 C>T |                  |                 |                           |                             |
| CC                 | 356              | 557             | 61.91                     | 60.48                       | 1.00 (1.00)                 | 0.007                           |
| CT                 | 194              | 323             | 33.74                     | 35.07                       | 0.92 (0.74–1.15)            | 0.460                           |
| TT                 | 25               | 41              | 4.35                      | 4.45                        | 0.93 (0.56–1.56)            | 0.794                           |
| CT + TT            | 219              | 364             | 38.09                     | 39.52                       | 0.94 (0.76–1.17)            | 0.580                           |
| CC + CT            | 550              | 880             | 95.65                     | 95.55                       | 1.00 (1.00)                 | 0.001                           |
| TT                 | 25               | 41              | 4.35                      | 4.45                        | 0.98 (0.59–1.62)            | 0.924                           |
| C allele           | 906              | 1,437           | 78.78                     | 78.01                       | 1.00 (1.00)                 | 0.001                           |
| T allele           | 244              | 405             | 21.22                     | 21.99                       | 0.96 (0.80–1.14)            | 0.619                           |
| PPARGC1B rs7732671 G>C |                  |                 |                           |                             |
| GG                 | 497              | 819             | 86.43                     | 88.93                       | 1.00 (1.00)                 | 0.007                           |
| GC                 | 77               | 101             | 13.39                     | 10.97                       | 1.24 (0.90–1.70)            | 0.188                           |
| CC                 | 1                | 1               | 0.17                      | 0.11                        | 1.62 (1.00–2.60)            | 0.732                           |
| GC + CC            | 78               | 102             | 13.57                     | 11.07                       | 1.26 (0.92–1.73)            | 0.150                           |
| GG + GC            | 574              | 920             | 99.83                     | 99.89                       | 1.00 (1.00)                 | 0.001                           |
| CC                 | 1                | 1               | 0.17                      | 0.11                        | 1.60 (1.05–25.68)           | 0.739                           |
| G allele           | 1,071            | 1,739           | 93.13                     | 94.41                       | 1.00 (1.00)                 | 0.001                           |
| C allele           | 79               | 103             | 6.87                      | 5.59                        | 1.25 (0.92–1.69)            | 0.155                           |
| PPARGC1B rs17572019 G>A |                  |                 |                           |                             |
| GG                 | 496              | 818             | 86.26                     | 88.82                       | 1.00 (1.00)                 | 0.007                           |
| GA                 | 78               | 101             | 13.57                     | 10.97                       | 1.25 (0.92–1.72)            | 0.160                           |
| AA                 | 1                | 2               | 0.17                      | 0.22                        | 0.81 (0.07–8.98)            | 0.865                           |
| GA + AA            | 79               | 103             | 13.74                     | 11.18                       | 1.27 (0.92–1.73)            | 0.142                           |
| GG + GA            | 574              | 919             | 99.83                     | 99.78                       | 1.00 (1.00)                 | 0.001                           |
| AA                 | 1                | 2               | 0.17                      | 0.22                        | 0.80 (0.07–8.85)            | 0.856                           |
| G allele           | 1,070            | 1,737           | 93.04                     | 94.30                       | 1.00 (1.00)                 | 0.001                           |
| A allele           | 80               | 105             | 6.96                      | 5.70                        | 1.24 (0.92–1.67)            | 0.165                           |

Notes: *Adjusted for age, sex, smoking status, alcohol use, and chronic HBV infection status. Bold represents statistically significant values (P<0.05).

Abbreviations: HCC, hepatocellular carcinoma; HBV, hepatitis B virus.

PPARGC1A rs2970847 C>T, and PPARGC1B rs7732671 G>C and rs17572019 G>A SNPs and HCC risk. We found that PPARG rs1801282 C>G polymorphism decreased the risk of HCC in male, ≥53 years, never-smoking, never-drinking, and nonchronic HBV-infection-status subgroups. However, PPARG rs3856806 C>T polymorphism increased the risk of HCC in never-smoking, never-drinking, and nonchronic
Table 4 Stratified analyses between PPARG rs1801282 C>G polymorphism and HCC risk by chronic HBV infection, sex, age, smoking status, and alcohol consumption

| Case/control | Adjusted OR (95% CI), P-value |
|--------------|------------------------------|
|              | CC  | GC  | GG  | GC/GG | GG vs (GC/CC) |
| Sex          |     |     |     |       |               |
| Male         | 486/741 | 30/89 | 1/3 | 31/92 | 1.00 | 0.52 (0.29–0.92), P=0.025 |
| Female       | 56/82 | 0/6  | 2/0 | 2/6  | 1.00 | 0.67 (0.12–3.82), P=0.651 |
| Age (years)  |     |     |     |       |     |               |
| <53          | 244/360 | 14/31 | 2/2 | 16/33 | 1.00 | 0.69 (0.29–1.62), P=0.389 |
| ≥53          | 298/463 | 16/64 | 1/1 | 17/65 | 1.00 | 0.36 (0.17–0.74), P=0.006 |
| Smoking status |     |     |     |       |     |               |
| Never        | 350/532 | 15/59 | 3/3 | 18/62 | 1.00 | 0.32 (0.15–0.66), P=0.002 |
| Ever         | 192/291 | 15/36 | 0/0 | 15/36 | 1.00 | 0.87 (0.37–2.04), P=0.743 |
| Alcohol use  |     |     |     |       |     |               |
| Never        | 386/692 | 19/79 | 3/2 | 22/81 | 1.00 | 0.40 (0.21–0.76), P=0.005 |
| Ever         | 156/131 | 11/16 | 0/1 | 11/17 | 1.00 | 0.79 (0.26–2.35), P=0.667 |
| Chronic HBV infection |     |     |     |       |     |               |
| Yes          | 380/78 | 22/7  | 2/0 | 24/7  | 1.00 | 0.53 (0.21–1.35), P=0.180 |
| No           | 162/745 | 8/88  | 1/3 | 9/91  | 1.00 | 0.42 (0.20–0.89), P=0.024 |

Notes: *Genotyping successful in 584 (98.46%) HCC cases and 923 (99.78%) controls for PPARG rs1801282 C>G; †adjusted for age, sex, smoking status, chronic HBV infection, and alcohol use (besides stratified factors accordingly) in a logistic regression model. Bold represents statistically significant values (P<0.05).

Abbreviations: HCC, hepatocellular carcinoma; HBV, hepatitis B virus.

Table 5 Stratified analyses between PPARG rs3856806 C>T polymorphism and HCC risk by chronic HBV infection, sex, age, smoking status, and alcohol consumption

| Case/control | Adjusted OR (95% CI), P-value |
|--------------|------------------------------|
|              | CC  | CT  | TT  | CT/TT | TT vs (CT/CC) |
| Sex          |     |     |     |       |               |
| Male         | 285/491 | 196/315 | 36/27 | 232/342 | 1.00 | 1.08 (0.79–1.48), P=0.635 |
| Female       | 35/52 | 18/31 | 5/5  | 23/36  | 1.00 | 1.06 (0.45–2.50), P=0.888 |
| Age (years)  |     |     |     |       |     |               |
| <53          | 143/230 | 99/150 | 18/13 | 117/163 | 1.00 | 1.25 (0.81–1.92), P=0.320 |
| ≥53          | 177/313 | 115/196 | 23/19 | 138/215 | 1.00 | 1.00 (0.68–1.47), P=0.982 |
| Smoking status |     |     |     |       |     |               |
| Never        | 204/347 | 137/222 | 27/25 | 164/247 | 1.00 | 1.06 (0.75–1.50), P=0.757 |
| Ever         | 116/196 | 77/124 | 14/7  | 91/131  | 1.00 | 1.27 (0.75–2.16), P=0.133 |
| Alcohol use  |     |     |     |       |     |               |
| Never        | 230/457 | 149/288 | 29/28 | 178/316 | 1.00 | 1.05 (0.76–1.44), P=0.779 |
| Ever         | 90/86 | 65/58 | 12/4  | 77/62  | 1.00 | 1.39 (0.72–2.67), P=0.328 |
| Chronic HBV infection |     |     |     |       |     |               |
| Yes          | 230/52 | 147/29 | 27/4  | 174/33  | 1.00 | 1.01 (0.60–1.71), P=0.971 |
| No           | 90/491 | 67/317 | 14/28 | 81/345  | 1.00 | 1.12 (0.79–1.59), P=0.523 |

Notes: *Genotyping successful in 584 (98.46%) HCC cases and 923 (99.78%) controls for PPARG rs3856806 C>T; †adjusted for age, sex, smoking status, chronic HBV infection, and alcohol use (besides stratified factors accordingly) in a logistic regression model. Bold represents statistically significant values (P<0.05).

Abbreviations: HCC, hepatocellular carcinoma; HBV, hepatitis B virus.
Table 6 PPARG, PPARGC1A, and PPARGC1B haplotype frequency (%) in cases and controls and HCC risk

|         | Cases n (%) | Controls n (%) | Crude OR (95% CI) | P-value |
|---------|-------------|----------------|-------------------|---------|
| PPARG   |             |                |                   |         |
| C<sub>rs1801282</sub>C<sub>rs3856086</sub>C<sub>rs2970847</sub>C<sub>rs17572019</sub> | 847 (73.59) | 1,403 (76.17) | 1.00 |         |
| C<sub>rs1801282</sub>T<sub>rs3856086</sub>C<sub>rs2970847</sub>C<sub>rs17572019</sub> | 267 (23.20) | 338 (18.35) | 1.31 (1.09–1.57) | 0.004 |
| G<sub>rs1801282</sub>T<sub>rs3856086</sub>T<sub>rs2970847</sub>C<sub>rs17572019</sub> | 29 (2.52) | 72 (3.91) | 0.67 (0.43–1.04) | 0.069 |
| G<sub>rs1801282</sub>T<sub>rs3856086</sub>C<sub>rs2970847</sub>C<sub>rs17572019</sub> | 8 (0.70) | 29 (1.57) | 0.46 (0.21–1.00) | 0.046 |
| PPARGC1B |             |                |                   |         |
| G<sub>rs7732671</sub>T<sub>rs17572019</sub>A<sub>rs17572019</sub> | 1,070 (93.04) | 1,737 (94.30) | 1.00 |         |
| C<sub>rs7732671</sub>T<sub>rs17572019</sub>A<sub>rs17572019</sub> | 79 (6.87) | 103 (5.59) | 1.25 (0.92–1.69) | 0.155 |
| PPARGC1A |             |                |                   |         |
| C<sub>rs2970847</sub>T<sub>rs17572019</sub> | 906 (78.78) | 1,437 (78.01) | 1.00 |         |
| T<sub>rs2970847</sub>T<sub>rs17572019</sub> | 244 (21.22) | 405 (21.99) | 0.96 (0.80–1.14) | 0.619 |
| PPARG, PPARGC1A, and PPARGC1B |             |                |                   |         |
| C<sub>rs1801282</sub>C<sub>rs3856086</sub>C<sub>rs2970847</sub>C<sub>rs17572019</sub> | 618 (53.79) | 1,044 (56.71) | 1.00 |         |
| C<sub>rs1801282</sub>T<sub>rs3856086</sub>C<sub>rs2970847</sub>C<sub>rs17572019</sub> | 198 (17.23) | 259 (14.07) | 1.29 (1.05–1.59) | 0.017 |
| C<sub>rs1801282</sub>C<sub>rs3856086</sub>T<sub>rs2970847</sub>C<sub>rs17572019</sub> | 159 (13.84) | 277 (15.05) | 0.97 (0.78–1.21) | 0.783 |
| G<sub>rs1801282</sub>C<sub>rs3856086</sub>T<sub>rs2970847</sub>C<sub>rs17572019</sub> | 59 (5.13) | 64 (3.48) | 1.56 (1.08–2.25) | 0.017 |
| C<sub>rs1801282</sub>T<sub>rs3856086</sub>C<sub>rs2970847</sub>C<sub>rs17572019</sub> | 56 (4.87) | 58 (3.15) | 1.63 (1.11–2.39) | 0.011 |
| G<sub>rs1801282</sub>T<sub>rs3856086</sub>T<sub>rs2970847</sub>C<sub>rs17572019</sub> | 21 (1.83) | 42 (2.28) | 0.84 (0.50–1.44) | 0.534 |
| G<sub>rs1801282</sub>T<sub>rs3856086</sub>T<sub>rs2970847</sub>T<sub>rs17572019</sub> | 12 (1.04) | 23 (1.25) | 0.88 (0.44–1.78) | 0.725 |
| G<sub>rs1801282</sub>T<sub>rs3856086</sub>C<sub>rs2970847</sub>T<sub>rs17572019</sub> | 7 (0.61) | 26 (1.41) | 0.45 (0.20–0.95) | 0.060 |
| Others  | 19 (1.65) | 48 (2.61) | 0.67 (0.39–1.15) | 0.142 |

Note: Bold represents statistically significant values (P<0.05). Abbreviation: HCC, hepatocellular carcinoma.

PPARG has been considered a HCC suppressor that contributes to the suppression of HCC-cell growth, angiogenesis, and migration. These primary results indicate that PPARG plays an important role in tumor suppression and may be a therapeutic target in HCC. An SNP can lead to abnormal expression or to the generation of a defective form of the protein. Therefore, SNPs may be associated with the development of disease. PPARG rs1801282 C>G polymorphism is a missense SNP, which encodes a proline-to-alanine substitution. Compared to the PPARG rs1801282 C allele, the PPARG rs1801282 G allele might influence the binding affinity to DNA elements and alter expression of PPARG-target genes and could then decrease transcriptional activation of the PPARG gene in vitro. It has been suggested that PPARG rs1801282 C>G substitution could improve insulin sensitivity and decrease body mass index (BMI) and susceptibility of T2DM. As such, it is believed that PPARG rs1801282 C>G polymorphism may decrease cancer susceptibility through insulin-related mechanisms. In the present case–control study, we found that rs1801282 C>G polymorphism decreased the risk of HCC. Several meta-analyses found that this SNP decreased the susceptibility of colorectal cancer in Caucasians. Our findings were similar to those results. In future, more case–control studies are needed to confirm our findings and assess the interaction of genetic predisposition with environmental factors.

Rs3856806 C>T polymorphism, located in PPARG exon 6, is correlated with higher BMI. A C→T substitution is a synonymous SNP that encodes a histidine amino-acid residue in PPARG protein with either the rs3856806 C or T allele. PPARG rs3856806 C>T variants could influence energy metabolism and then presumably confer risk of T2DM. The association between this SNP and the risk of cancer is unknown. Recently, some case–control studies found positive signals of PPARG rs3856806 C>T variants with the development of malignancy. The results of a meta-analysis suggested that PPARG rs3856806 C>T variants did not alter the susceptibility of cancer; however, only four case–control studies with small samples were included in this pooled analysis. In this study, we found that PPARG rs3856806 C>T polymorphism was associated with an increased risk of HCC. It was proposed that synonymous SNPs may affect mRNA stability/structure, splicing accuracy, and codon usage. In combination with our findings, these results showed that PPARG rs3856806 C>T variants might be a risk factor for HCC. The interaction between genetic predisposition and environmental factors might confer risk of HCC.
factor for HCC, probably through altering mRNA processing or translation and influencing the expression of PPARG. Therefore, in future, the function of PPARG rs3856806 C>T polymorphism needs to be explored further.

In this study, after Bonferroni correction, genotype distributions of PPARG rs1801282 C>G and rs3856806 C>T polymorphisms were still significantly different between HCC cases and controls, which indicated that our results were reliable. In addition, the results of power analysis also confirm the stability of our findings. We constructed seven haplotypes of PPARG rs1801282 C>G, PPARG rs3856806 C>T, PPARGC1A rs2970847 C>T, PPARGC1B rs7732671 G>C, and PPARGC1B rs17572019 G>A polymorphisms to evaluate the potential inherited patterns of haplotype. We found that CTCGG, CTTGG, and CCCCA haplotypes might increase the susceptibility to HCC. In addition, PPARG C<rs1801282 T<rs3856806 and G<rs1801282 C<rs3856806 haplotypes also influenced the risk of HCC. To the best of our knowledge, this study is the first investigation to explore the association of haplotypes in PPARG rs1801282 C>G, PPARG rs3856806 C>T, PPARGC1A rs2970847 C>T, PPARGC1B rs7732671 G>C, and PPARGC1B rs17572019 G>A polymorphisms with HCC susceptibility.

Just as with all epidemiological case–control studies, some limitations should be acknowledged. Firstly, this case–control study was hospital-based. All HCC cases and cancer-free controls were included from eastern China hospitals. Although the minor-allele frequency in controls was very close to data of Chinese populations (Table 2), the selection bias could not have been avoided. Secondly, we selected only five functional polymorphisms based on the publications, which could not represent an extensive view of these genetic predisposition in PPARG, PPARGC1A, and PPARGC1B genes. In future, a fine-mapping case–control study is needed to explore the potential relationships of PPARG, PPARGC1A, and PPARGC1B SNPs with HCC risk further. Thirdly, samples were moderate or small in some subgroups, and the power of the study might be limited in stratification analyses. Fourthly, for lack of sufficient samples, a replication study was not performed. Fifthly, as the distribution of PPARG rs3856806 C>T genotype frequencies did not accord with HWE in controls, our findings should be interpreted with much caution. Finally, due to lack of data for BMI, family history of HCC, other environmental factors, and lifestyle, these potential risk factors were not considered in our study. In future, large-sample studies with detailed individual data are needed to confirm our results.

In summary, to the best of our knowledge, this study is the first to explore the relationship of PPARG, PPARGC1A, and PPARGC1B polymorphisms with HCC risk. Our findings suggest that PPARG polymorphisms may influence susceptibility to HCC. In addition, PPARG, PPARGC1A, and PPARGC1B haplotypes were associated with HCC risk.

Acknowledgments
We appreciate all subjects who participated in this study. We wish to thank Dr Yan Liu (Genesky Biotechnologies Inc, Shanghai, China) for technical support. This study was supported in part by the Clinical Medicine Science and Technology Development Fund of Jiangsu University (JLY20140012), the Fujian Provincial Health and Family Planning Research Talent Training Program (Grant No. 2015-CX-7, 2018-ZQN-13, 2016-1-11, 2018-1-1), the Joint Funds for the Innovation of Science and Technology, Fujian province (Grant No. 2017Y9077), and the National Clinical Key Specialty Construction Program.

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. El-Serag HB. Epidemiology of viral hepatitis and hepatocellular carcinoma. Gastroenterology. 2012;142(6):1264–1273.
3. Raffetti E, Fattovich G, Donato F. Incidence of hepatocellular carcinoma in untreated subjects with chronic hepatitis B: a systematic review and meta-analysis. Liver Int. 2016;36(9):1239–1251.
4. Cho MC, Lee K, Paik SG, Yoon DY. Peroxisome proliferators-activated receptor (PPAR) modulators and metabolic disorders. PPAR Res. 2008;2008:679137.
5. Memisoglu A, Hankinson SE, Manson JE, Colditz GA, Hunter DJ. Lack of association of the codon 12 polymorphism of the peroxisome proliferator-activated receptor gamma gene with breast cancer and body mass. Pharmacogenetics. 2002;12(8):597–603.
6. He W. PPARγ2 polymorphism and human health. PPAR Res. 2009;2009:849538.
7. Robbins GT, Nie D. PPAR gamma, bioactive lipids, and cancer progression. Front Biosci (Landmark Ed) 2012;17:1816–1834.
8. Deeb SS, Fajas L, Nemoto M, et al. A Pro12Ala substitution in PPARγ2 associated with decreased receptor activity, lower body mass index and improved insulin sensitivity. Nat Genet. 1998;20(3):284–287.
9. Hutter S, Knabl J, Andergassen U, Jeschke U. The role of PPARs in placental immunology: a systematic review of the literature. PPAR Res. 2013;2013:970276.
10. Mantovani A, Allavena P, Sica A, Balkwill F. Cancer-related inflammation. Nature. 2008;454(7203):436–444.
11. Khandekar MJ, Cohen P, Spiegelman BM. Molecular mechanisms of cancer development in obesity. Nat Rev Cancer. 2011;11(12):886–895.
12. Calle EE, Kaaks R. Overweight, obesity and cancer: epidemiological evidence and proposed mechanisms. Nat Rev Cancer. 2004;4(8):579–591.
13. Puisgruvier P, Wu Z, Park CW, Graves R, Wright M, Spiegelman BM. A cold-inducible coactivator of nuclear receptors linked to adaptive thermogenesis. Cell. 1998;92(6):829–839.
14. Li JD, Feng QC, Qi Y, Cui G, Zhao S. PPARGC1A is upregulated and facilitates lung cancer metastasis. Exp Cell Res. 2017;359(2):356–360.
15. Eichner LJ, Perry MC, Dufour CR, et al. miR-378* mediates metastatic shift in breast cancer cells via the PGC-1α/ERRγ transcriptional pathway. *Cell Metab.* 2010;12(4):352–361.

16. Wang Y, Chen Y, Jiang H, et al. Peroxisome proliferator-activated receptor gamma (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.

17. Doney A, Fischer B, Frew D, et al. Haplotypic analysis of the PPARY Pro12Ala and C143T variants reveals opposing associations with body weight. *BMC Genet.* 2002;3:21.

18. Smith WM, Zhou XP, Kurose K, et al. Opposite association of two PPARG variants with cancer: overrepresentation of H449D in endometrial carcinoma cases and underrepresentation of P12A in renal cell carcinoma cases. *Hum Genet.* 2001;109(2):146–151.

19. Wang SS, Davis S, Cerhan JR, et al. Polymorphisms in oxidative stress genes and risk for non-Hodgkin lymphoma. *Carcinogenesis.* 2006;27(9):1828–1834.

20. Küry S, Buecher B, Robiou-du-Pont S, et al. Low-penetrance alleles predisposing to sporadic colorectal cancers: a French case-controlled genetic association study. *BMC Cancer.* 2008;8:326.

21. Jiang J, Gajalakshmi V, Wang J, et al. Influence of the C161T but not Pro12Ala polymorphism in the peroxisome proliferator-activated receptor-gamma on colorectal cancer in an Indian population. *Cancer Sci.* 2005;96(8):507–512.

22. Vogel U, Christensen J, Dybdahl M, et al. Prospective study of interaction between alcohol, NSAID use and polymorphisms in genes involved in the inflammatory response in relation to risk of colorectal cancer. *Mutat Res.* 2007;624(1–2):88–100.

23. Yang Y, Mo X, Chen S, Lu X, Gu D. Association of peroxisome proliferator-activated receptor gamma coactivator 1 alpha (PPARGC1A) gene polymorphisms and type 2 diabetes mellitus: a meta-analysis. *Diabetes Metab Res Rev.* 2011;27(2):177–184.

24. Barroso I, Luan J, Sandhu MS, et al. Meta-analysis of the Gly482Ser variant in PPARGC1A in type 2 diabetes and related phenotypes. *Diabetologia.* 2006;49(3):501–505.

25. Martinez-Nava GA, Burguete-Garcia AI, Lopez-Carrillo L, Hernández-Ramírez RU, Madrid-Marina V, Cébrían ME. PPARY and PPARGC1B polymorphisms modify the association between plhalat metabolites and breast cancer risk. *Biomarkers.* 2013;18(6):493–501.

26. 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.

27. Tang W, Chen Y, Chen S, Sun B, Gu H, Kang M. Programmed death-1 (PD-1) polymorphism is associated with gastric carcinoma. *Int J Clin Exp Med.* 2015;8(5):8086–8093.

28. 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.

29. Bland JM, Altman DG. Multiple significance tests: the Bonferroni and related corrections for multiple comparisons. *J Pathol Inform.* 2011;2:52.

30. 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.

31. Elrod HA, Sun SY. PPARY and apoptosis in cancer. *PPAR Res.* 2008;2008:704165.

32. Gimun GD, Smith WM, Drori S, et al. APC-dependent suppression of colon carcinogenesis by PPARY. *Proc Natl Acad Sci U S A.* 2002;99(21):13771–13776.

33. Sarraf P, Mueller E, Jones D, et al. Differentiation and reversal of malignant changes in colon cancer through PPARY. *Nat Med.* 1998;4(9):1046–1052.

34. Tontonoz P, Spiegelman BM. Fat and beyond: the diverse biology of PPARγ. *Annu Rev Biochem.* 2008;77:289–312.

35. Hsu HT, Sung MT, Lee CC, et al. Peroxisome proliferator-activated receptor γ expression is inversely associated with macroscopic vascular invasion in human hepatocellular carcinoma. *Int J Mol Sci.* 2016;17(8):1226.

36. Nojima H, Kuboki S, Shinoda K, et al. Activation of peroxisome proliferator-activated receptor-gamma inhibits tumor growth by negatively regulating nuclear factor-κB activation in patients with hepatocellular carcinoma. *J Hepatobiliary Pancreas Sci.* 2016;23(9):574–584.

37. Pang X, Wei Y, Zhang Y, Zhang M, Lu Y, Shen P. Peroxisome proliferator-activated receptor-γ activation inhibits hepatocellular carcinoma cell invasion by upregulating plasminogen activator inhibitor-1. *Cancer Sci.* 2013;104(6):672–680.

38. Wu CW, Farrell GC, Yu J. Functional role of peroxisome proliferator-activated receptor γ in hepatocellular carcinoma. *J Gastroenterol Hepatol.* 2012;27(11):1665–1669.

39. Yen CJ, Beamer BA, Negri C, et al. Molecular scanning of the human peroxisome proliferator activated receptor gamma (hPPAR gamma) gene in diabetic Caucasians: identification of a Pro12Ala PPAR gamma 2 missense mutation. *Biochem Biophys Res Commun.* 1997;241(2):270–274.

40. Masugi J, Tamori Y, Mori H, Koike T, Kasuga M. Inhibitory effect of a proline-to-alanine substitution at codon 12 of peroxisome proliferator-activated receptor-γ 2 on thiazolidinedione-induced adipogenesis. *Biochem Biophys Res Commun.* 2000;268(1):178–182.

41. Douglas JA, Erdos MR, Watanabe RM, et al. The peroxisome proliferator-activated receptor-γ2 Pro12Ala variant: association with type 2 diabetes and trait differences. *Diabetes.* 2001;50(4):886–890.

42. Mori H, Ikegami H, Kawaguchi Y, et al. The Pro12—>Ala substitution in PPAR-γ is associated with resistance to development of diabetes in the general population: possible involvement in impairment of insulin secretion in individuals with type 2 diabetes. *Diabetes.* 2001;50(4):891–894.

43. Wang W, Shao Y, Tang S, Cheng X, Lian H, Qin C. Peroxisome proliferator-activated receptor-γ (PPARY) Pro12Ala polymorphism and colorectal cancer (CRC) risk. *Int J Clin Exp Med.* 2015;8(3):4066–4072.

44. Wei Z, Han G, Bai X. Effect of proliferator-activated receptor-γ Pro12Ala polymorphism on colorectal cancer risk: a meta-analysis. *Med Sci Monit.* 2015;21:1611–1616.

45. Wang C, Li X, Huang Z, Qian J. Quantitative assessment of the influence of PPARG P12A polymorphism on gestational diabetes mellitus risk. *Mol Biol Rep.* 2013;40(2):811–817.

46. 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.

47. Zhou XP, Smith WM, Gimmi O, et al. Over-representation of PPARY sequence variants in sporadic cases of globulostoma multiforme: preliminary evidence for common low penetrance modifiers for brain tumour risk in the general population. *J Med Genet.* 2000;37(6):410–414.

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

49. Chun S, Yun JW, Park G, Cho D. The synonymous nucleotide substitu

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