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Interaction between peroxisome proliferator-activated receptor gamma and smoking on cardiovascular disease

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HIGHLIGHTS

- The first study to examine the combined effects of PPAR-γ and smoking on cardiovascular disease.
- Independent effects of PPAR-γ single nucleotide polymorphism and cardiovascular disease in a Chinese population

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ABSTRACT

The aim of this study was to investigate the association between peroxisome proliferator-activator receptor-γ (PPAR-γ) genotype and additional gene–smoking interaction on cardiovascular disease (CVD) based on a Chinese population. A total of 1248 subjects (613 men, 635 women), with a mean age of 55.5 ± 11.8 years old, were selected, including 620 CVD patients and 628 normal controls. Logistic regression was performed to investigate association between single nucleotide polymorphism (SNP) and CVD. Generalized MDR (GMDR) was used to analysis the gene–environment interaction, cross-validation consistency, the testing balanced accuracy, and the sign test, to assess each selected interaction were calculated. The carriers of homozygous mutant of two SNP revealed increased CVD risk than those with wild-type homozygotes, OR (95% CI) were 1.31 (1.16–1.95) and 1.68 (1.29–2.06), respectively. GMDR analysis for one- to three-locus models indicated that there was a significant two-locus model ($p = 0.0107$) involving rs1805192 and smoking, indicating a potential gene–gene interaction between rs1805192 and smoking. Overall, the two- locus models had a cross-validation consistency of 10 of 10, and had the testing accuracy of 62.17%. We found that smokers with Pro/Ala or Ala/Ala genotype have highest CVD risk, compared to non-smokers with Pro/Pro genotype, OR (95% CI) was 3.46 (1.31–3.42), after covariates adjustment. We found a significant association between genotypes of variants in rs10865710 and rs1805192 with increased CVD risk and a potential gene–gene interaction between rs1805192 and smoking.

1. Introduction

The peroxisome proliferator-activator receptor-γ (PPAR-γ) gene, located at 3p25-24, is a member of the nuclear hormone receptor superfamily [1]. PPAR-γ plays an important role in the differentiation of adipocytes and regulation of insulin sensitivity, and its variation may be associated with diabetes and metabolic syndrome [2]. PPAR-γ is a key regulator of fatty acid metabolism, promoting its storage in adipose tissue and reducing circulating concentrations of free fatty acids. Activation of PPAR-γ has favorable effects on measures of adipocyte function, insulin sensitivity, lipoprotein metabolism, and vascular structure and function [3,4]. Clinical trials of thiazolidinedione, which was PPAR-γ activators, have not provided conclusive evidence that they reduce cardiovascular morbidity and mortality. Many studies [5–9] have been performed to explore the association between PPAR-γ polymorphism and cardiovascular disease (CVD), but the results are inconsistent. The multiple phenotypes were result of many gene polymorphism, gene- gene interactions and gene–environment interactions, furthermore, Wu et al. [10] conducted a meta-regression analysis, and found a link of the PPAR-γ polymorphism with coronary artery disease (CAD) risk in population with lower smoking proportion, it indicated that smoking is a major risk factor of CAD [11,12] and potential interaction of the PPAR-γ genotype with smoking factor is existed. Amoruso et al. [13] conducted a study on the impact of tobacco smoke on expression of PPAR-γ gene, they found that in coronary heart disease (CHD) patients, exposure to tobacco smoke profoundly affected PPAR-γ expression, and this was related to levels of secretion of pro-inflammatory cytokines. Monocyte-derived macrophages (MDMs) from CHD smokers showed the lowest PPAR-γ expression and released more inflammatory cytokines. However, till now, no study focused on

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this the interaction between PPAR-γ genotype and smoking in Chinese population, so the aim of this study was to investigate the association between PPAR-γ genotype and additional gene–smoking interaction on CVD risk, based on a Chinese hospital based case–control study.

2. Materials and methods

2.1. Subjects

This was a hospital based case–control study. Participants were consecutively recruited between January 2012 and November 2013 from PLA 161 hospital. All cases were confirmed by clinical diagnosis. A total of 1248 subjects (613 men, 635 women), with a mean age of 55.5 ± 11.8 years old, were selected, including 620 CVD patients and 628 normal controls. Healthy controls were randomly selected from a population screening program for risk factors of CVD in the same regions and 1:1 matched to cases on the basis of age (±3 years) and sex. Participants with diabetes, hypertension, missing data and participants with BMI < 18.5 kg/m^2 were not included in the controls. Blood samples were collected from each participant. Informed consent was obtained from all participants. The protocol of this study was approved by the Ethics Committee of PLA 161 Hospital.

2.2. Body measurements

Data on demographic information, diet, smoking and drinking information for all participants were obtained using a standard questionnaire administered by trained staffs. We defined currently alcohol consumption as more than 1 drink of any type per month or not currently drinking as less than 1 drink of any type per month [14]; Current smokers were defined as those who have smoked for at least 100 cigarettes and still smoked at the time of blood collection, the duration of smoking was more than 1 year, individuals with no history of cigarette smoking were considered as never smokers [15,16]. Body weight, height, and waist circumference (WC) were also measured according to standardized procedures [17]. Body mass index (BMI) was calculated as weight in kilograms divided by the square of the height in meters. Blood samples were collected in the morning after at least 8 h of fasting. All plasma and serum samples were frozen at −80 °C until laboratory testing.

2.3. Genomic DNA extraction and genotyping

We selected SNPs within the PPAR γ gene, which have been reported associations with metabolic abnormalities and minor allele frequency (MAF) greater than 2%. Three SNP of PPAR **γ** were selected for genotyping in the study: rs3856806, rs709158 and rs1805192. Genomic DNA from participants was extracted from EDTA-treated whole blood, using the DNA Blood Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. All SNPs were detected by TaqMan fluorescence probe. ABI Prism7000 software and allelic discrimination procedure was used for genotyping of fore-mentioned 3 SNP. A 25 μl reaction mixture including 1.25 μl SNP Genotyping Assays (20×), 12.5 μl Genotying Master Mix (2×), 20 ng DNA, and the conditions were as follows: initial denaturation for 10 min and 95 °C, denaturation for 15 s and 92 °C, annealing and extension for 90 s and 60 °C, 50 cycles. Probe sequences of all SNPs were shown in Table 1.

2.4. Diagnostic criteria

For this study, we defined CVD as any of the following events: interventional therapy of coronary artery (cardiac catheterization, percutaneous coronary intervention, or coronary artery bypass grafting), stable angina pectoris, unstable angina pectoris, the first occurrence of acute myocardial infarction, congestive heart failure caused by myocardial ischemia after baseline investigation, ischemic stroke, hemorrhagic stroke, and peripheral vascular disease. The diagnosis of CVD and the determination of its pathological type were based on the standard questionnaires and medical records.

2.5. Statistical analysis

The mean and standard deviation (SD) for normally distributed continuous variables, and percentages for categorical variable, were calculated and compared. The genotype and allele frequencies were obtained by direct count. The categorical data were analyzed using χ^2 test. Further, continuous variables were analyzed using Student's t test or one-way analysis of variance, followed by the least significant difference multiple-range tests for comparison between groups. Hardy–Weinberg equilibrium (HWE) was performed by using SNPStats (available online at http://bioinfo.iconcologia.net/SNPstats). Logistic regression was performed to investigate association between SNP and CVD using gender, age, high fat diet, low fiber diet, smoking and alcohol status, BMI and WC as covariates in the model.

Generalized MDR (GMDR) [18] was used to analysis the gene–environment interaction, cross-validation consistency, the testing balanced accuracy, and the sign test, to assess each selected interaction were calculated. The cross-validation consistency score is a measure of the degree of consistency with which the selected interaction is identified as the best model among all possibilities considered. The testing balanced accuracy is a measure of the degree to which the interaction accurately predicts case–control status with scores between 0.50 (indicating that the model predicts no better than chance) and 1.00 (indicating perfect prediction). Finally, a sign test or a permutation test (providing empirical *p*-values) for prediction accuracy can be used to measure the significance of an identified model.

3. Results

A total of 1248 subjects (613 men, 635 women), with a mean age of 55.5 ± 11.8 years old, were selected, including 620 CVD patients and 628 normal controls. Participants characteristics stratified by cases and controls are shown in Table 2. The distribution of smoking and alcohol consumption were significantly different between cases and controls.

Table 1
Description and Probe sequence used for TaqMan fluorescence probe analysis for 3 SNPs.

SNP ID	SNP	Chromosome	Exon/intron	Nucleotide substitution	Probe sequence
rs709158	Intron A>G	3	Intron_2	A>G	5′-AGATACGGGGAGGAAATTCACTGG[A/G] TTTTACAATATATTTTTCAAGGCAA-3′
rs10865710	C681G	3	Exon_A2	C>G	5′-TTGGCATTAGATGCTGTTTTGTCTT[C/G] ATGGAAAATACAGCTATTCTAGGAT-3′
rs1805192	Pro12Ala	3	Exon_B	C>G	5′-ACCTCAGACAGATTGTCACGGAACA[C/T] GTGCAGCTACTGCAGGTGATCAAGA-3′