Original Article

Study on Association of Peroxisome Proliferator-Activated Receptor α with C-Reactive Protein, and Additional Gene–Gene Interaction in Chinese Han

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Abstract

Background: The aim was to examine the association between 6 single nucleotide polymorphisms (SNPs) of peroxisome proliferator-activated receptors α (PPAR α) poly morphisms and C-reactive protein (CRP) level, as well as additional gene-gene interaction among the 6 SNPs.

Methods: A total of 1260 subjects (583 men, 677 women), with a mean age of 41.3 ± 14.6 years old, were selected. Six SNPs of PPAR α were selected for genotyping in the study including: rs135539, rs135551, rs135549, rs1800206, rs1800243 and rs4253623. Linear regression analysis was performed to verify the polymorphism association between SNP with CRP levels. Generalized MDR (GMDR) was employed to analysis the interaction among six SNPs.

Results: Linear regression results indicated a significant negative correlation between mutation of rs1800206 and CRP level. The carriers of the V allele (LV + VV) of rs1800206 were associated with a significant decreased level of CRP (regression coefficients was -0.533, standard error was 0.148 ($P < 0.001$)). However, the other 5 SNPs in PPAR α were not significantly associated with CRP level before or after covariate adjustment. GMDR model indicated that there was a significant two-locus model ($P = 0.0107$) involving rs1800206 and rs135539, indicating a potential gene–gene interaction between rs1800206 and rs135539. Overall, the two- locus models had a cross-validation consistency of 10 of 10, respectively, and had the testing accuracy of 55.9%, respectively.

Conclusions: Our results support an important association between rs1800206 minor allele (V) of PPAR α and lower CRP level. The interaction analysis showed a combined effect between rs1800206 and rs135539 on the lower CRP level.

Keywords: CRP, interaction, polymorphisms, PPAR α, SNP

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Introduction

Inflammation plays important roles in the pathogenesis of a variety of diseases, including insulin resistance, type 2 diabetes mellitus (DM2), fatty liver and cardiovascular disease (CVD).[1] In the several inflammation mechanisms, the secretion of several molecular mediators capable of activating, or suppressing, and numerous transcription factors have been reported in recent years, such as peroxisome proliferator-activated receptors (PPARs).[2,3]

PPARs that were orphan nuclear receptors belong to the steroid, retinoid, and thyroid hormone receptor super-family of ligand-activated transcription factors.[4,5] Three subtypes have been characterized, including PPAR α, PPAR β/δ, and PPAR γ.[6] PPAR α was the first PPAR isotype to be cloned.[7,8] The human PPAR α gene is located on chromosome 22q12–q13.1, and it spanned about 93kb, including 8 exons which could encode a protein of 468 amino acids. The coding region of the PPAR α protein begins in exon 3, thus the first two exons and part of exon 3 constitute the 5'-untranslated region of the gene. PPAR α is highly expressed in many organs, such as heart, liver, skeletal muscle and kidney. The function of PPAR α in the liver is to induce hepatic peroxisomal fatty acid oxidation. Expression of PPAR α is reported not only in macrophage foam cells but also in vascular endothelium. The activation of anti-inflammatory and anti-atherogenic effects were considered as the main role in these cells.

C-reactive protein (CRP), a plasma protein synthesized by the liver, is a sensitive and dynamic systemic marker of inflammation,[9] current evidence shows that CRP could be influenced by both clinical and genetic factors.[10] The family study has suggested that genetic factors account for 27% – 40 % of the variance in CRP level,[11] suggesting a role for genetic variation in determining serum levels. PPARs have attracted enormous attention on inflammation. However, the associations between variants of the PPAR α, corresponding gene–gene interactions with CRP level were rarely studied. So in this study, we sought to examine the association between 6 PPAR α polymorphisms with CRP level, and the additional interaction among the 6 SNPs.

Materials and Methods

Subjects

This was a cross-sectional study. Chinese participants were consecutively recruited between January 2012 and December 2013. A total of 1320 subjects were included in investigated population. Subjects with abnormal increasing of CRP level (≥ 10 mg/L) be-

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cause of any reasons was excluded, including subjects with infection (n = 22), trauma (n = 19) and any other factor (n = 9), and CRP were missing (n = 10). At last, a total of 1260 subjects (583 men, 677 women) were included in the study, including the genotyping of polymorphisms, The mean age of all participants was 41.3 ± 14.6 years old. There was no significant difference between the selected subjects and those who were not selected in terms of age, sex, smoking status, and alcohol consumption. Informed consent was obtained from all participants.

Body Measurements

Data on demographic information, lifestyle risk factors for all participants were obtained using a standard questionnaire administered by trained staffs. Body weight and height were measured according to standardized procedures.[12] Body mass index (BMI) was calculated as weight in kilograms divided by the square of the height in meters. Cigarette smokers were those who self-reported smoking cigarettes at least once a day for 1 year or more. Alcohol consumption was expressed as the sum of milliliters of alcohol per week from wine, beer, and spirits. Blood samples were collected in the morning after at least 8 hours of fasting. All plasma and serum samples were frozen at –80°C until laboratory testing. Plasma glucose was measured using an oxidase enzymatic method. Particle enhanced immune latex agglutination high sensitive assay was used to detect CRP level, and the Human C-Reactive Protein- ELISA Kit Reagent box (Shanghai EXcell biology, Inc., Shanghai, China) was used. All analysis was performed by the same lab.

Genomic DNA extraction and genotyping

We selected SNPs within the PPAR α gene using the following methods: 1) previously reported associations with metabolic abnormalities; 2) known heterozygosity and a minor allele frequency (MAF) greater than 2%. Six SNPs of PPAR α were selected for genotyping in the study: rs135539, rs135551, rs135549, rs1800206, rs1800243, and rs4253623. 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 were used for genotyping of fore-mentioned six SNPs. A 25 μL reaction mixture including 1.25 uL 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 seconds and 92°C, annealing and extension for 90 seconds and 60°C, 50 cycles. Probe sequences of all SNPs were shown in Table 1.

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 or the Fisher exact test if necessary. 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). Linear regression analysis was performed to verify the polymorphism association between SNP with CRP levels. Logistic regression was used to test the interaction among different SNPs, using gender, age, smoking and alcohol status, as well as physical activity as covariates in the model. And those less than 0.05 were considered as statistical significance.

Generalized MDR (GMDR)[13] was employed to analysis the interaction among six SNPs, some parameters were calculated, including the testing balanced accuracy, cross-validation consistency, and the sign test. 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. And those less than 0.05 were considered as statistical significance.

Results

A total of 1260 subjects (583 men, 677 women), with a mean age of 41.3 ± 14.6 years old, were selected. Minor allele frequencies of rs135539, rs135551, rs135549, rs1800206, rs1800243, and rs4253623 were 25.2%, 22.5%, 23.2%, 23.3%, 23.9%, and

Table 1. Description and Probe sequence for 6 SNPs used for Taqman fluorescence probe analysis

SNP ID	Chromosome	Exon/Intron	Nucleotide substitution	Probe sequence
rs135539	22:46163368	Intron	A>C	5'-AGCAGAATTTAAATCCTAGGTGATT[A/C] TTAACTCTAATCATACATCTAATGA-3'
rs135551	22:46168728	Intron	G>A	5'-TCTGCCCTCAAGGAGTTAGACTCAG[C/T] GAGGACAGTCAGACTAACAGAAACA-3'
rs135549	22:46157413	Intron	T>C	5'-CTCTCTCTCAGTCTAGGTGTGGGGG[A/G] AGCTGCAGAGGTCTGGGACAATTTC-3'
rs1800206	22:46218377	Exon	L>V	5'-CCAGTATTGTCGATTTCACAAGTGC[C/G] TTTCTGTCGGGATGTCACACAACGG-3'
rs1800243	22:46235199	Exon	G>C	5'CAGGAGGGTATTGTACATGTGCTCA[C/G] ACTCCACCTGCAGAGCAACCACCCG-3'
rs4253623	22:46154203	Intron	A>G	5'-CAGAATATAAAAAAGAAACTTAAAG[A/G] TAATCCTCATCATGGTAAAAGATGA-3'

Table 2. The genotype and allelic frequencies distribution in the all subjects

SNPs	Genotypes and Alleles	Frequencies N (MAF, %)	HWE test
rs135539	AA	718 (57.0)	0.107
	AC	452 (35.9)	
	CC	90 (7.0)	
	A	1888 (74.9)	
	C	632 (25.2)	
rs135551	GG	746 (59.2)	0.106
	GA	460 (36.5)	
	AA	54 (4.3)	
	G	1952 (77.5)	
	A	568 (22.5)	
rs135549	TT	738 (58.6)	0.370
	TC	460 (36.5)	
	CC	62 (4.9)	
	T	1936 (76.8)	
	C	584 (23.2)	
rs1800206	LL	741 (58.8)	0.764
	LV	453 (36.0)	
	VV	66 (5.2)	
	L	1935 (76.8)	
	V	585 (23.2)	
rs1800243	GG	726 (57.6)	0.545
	GC	466 (37.0)	
	CC	68 (5.4)	
	G	1918 (76.1)	
	C	602 (23.9)	
rs4253623	AA	749 (59.4)	0.704
	AG	448 (35.6)	
	GG	63 (5.0)	
	A	1946 (77.2)	
	G	574 (22.8)	

Table 3. Linear regression analysis on association between 6 SNPs and CRP level

SNP ID	Genotypes	CRP (mg/L)	Regression coefficients	Standard error	*P*-values [a]
rs135539			-0.165	0.134	0.13
	AA	1.12 ± 2.58			
	AC+CC	0.81 ± 1.53			
rs135551			-0.013	0.162	0.88
	GG	0.98 ± 2.12			
	GA+AA	0.95 ± 2.26			
rs135549			-0.172	0.140	0.09
	TT	1.08 ± 1.59			
	TC+CC	0.84 ± 2.79			
rs1800206			-0.533	0.148	< 0.001
	LL	1.47 ± 1.45			
	LV+VV	0.74 ± 2.86			
rs1800243			-0.130	0.136	0.103
	GG	1.02 ± 2.34			
	GC+CC	0.86 ± 1.98			
rs4253623			-0.089	0.137	0.23
	AA	1.07 ± 2.07			
	AG+GG	0.90 ± 2.23			

[a] Adjusted for gender, age, smoke and alcohol status, physical activity

Table 4. Best gene–gene interaction models, as identified by GMDR

Locus no.	Best combination	Cross-validation consistency	Testing accuracy	*P*-values[a]
2	rs1800206 rs135539	10/10	0.5590	0.0107
3	rs135539 rs1800206 rs135551	7/10	0.5669	0.1719
4	rs135539 rs1800206 rs135551 rs1800243	9/10	0.5577	0.1719
5	rs135539 rs135549 rs1800206 rs135551 rs1800243	5/10	0.5236	0.3770
6	rs135539 rs135549 rs1800206 rs135551 rs1800243 rs4253623	9/10	0.6217	0.0547

[a] Adjusted for gender, age, smoke and alcohol status, physical activity

22.8%, respectively. All genotypes were distributed according to Hardy–Weinberg equilibrium (all *P*-values more than 0.05) (Table 2).

Linear regression analysis was performed to determine the association between 6 SNPs and CRP level. Six SNPs were included in the linear regression analysis, we found that the CRP level was lower in the subjects with minor alleles, compared to subjects with wild genotype. We also found a significant negative correlation between mutation of rs1800206 and CRP. The carriers of the V allele (LV + VV) of rs1800206 were associated with a significant decreased level of CRP, regression coefficients was -0.533, standard error was 0.148 ($P < 0.001$). However, the other 5 SNPs in PPAR α were not significantly associated with CRP level before or after covariate adjustment (Table 3).

We also employed the GMDR analysis to assess the impact of the interaction among six SNPs, after adjustment for covariates including: gender, age, smoke and alcohol status, as well as physical activity. Table 4 summarizes the results obtained from GMDR analysis for two- to six-locus models with covariates adjustment. There was a significant two-locus model ($P = 0.0107$) involving rs1800206 and rs135539, indicating a potential gene–gene interaction between rs1800206 and rs135539. Overall, the two- locus models had a cross-validation consistency of 10 of 10, and had the testing accuracy of 55.9%. To obtain ORS and 95% CIs for the joint effects of candidate SNPs (rs1800206 and rs135539) on CRP, we conducted interaction analysis among SNPs in the 2-locus models. In the two-locus model, subjects with rs1800206- LV or VV and rs135539- CG or GG genotypes have the lowest CRP level. The difference (95% CI) = -0.49 (-0.67 to -0.26) ($P < 0.001$), after covariates adjustment for gender, age, smoke and alcohol status, physical activity (Table 5).

Discussion

PPARs are ligand-activated transcription factors belonging to the nuclear hormone receptor superfamily1, which includes three PPAR isoforms (PPAR α, PPAR γ and PPAR β/δ). Some studies have focused on the association between PPAR γ gene polymorphism and C-reactive protein level, however few studies involved in PPAR α, which was the first PPAR isotype to be cloned. In this study, we found that rs1800206 in PPAR α was significantly associated with CRP level. The CRP level was lower in subjects with minor alleles (LV or VV) than subjects with LL genotype. The carriers of the V allele (LV + VV) of rs1800206 were associated with a significant decreased level of CRP. PPAR α regulate lipid metabolism, and anti-inflammatory activities. Clinical trials[14] have shown that PPAR α agonist could decrease inflammation. In animal trial study, PPAR α deficiency induces a prolonged inflammatory response in a mouse ear-swelling model. Some studies[15,16] have indicated that fibrates, which was PPAR α agonist, could ameliorate systemic inflammation and improve vascular reactivity. Belfort, et al.[17] also indicated that PPAR α agonist could significantly reduce plasma hsCRP (about 50%) levels. Kleemann, et al.[18] reported that activators of PPAR α could directly suppress huCRP expression, and reduce huCRP gene expression by direct anti-inflammatory activities unrelated to cholesterol. Gu, et al.[19] conducted a similar study in a Chinese population, found that rs1805192 was associated with lower CRP level. However, this relation just existed in normal weight subjects.

Several mechanisms of association between activation of PPAR α and decreased inflammation have been suggested. PPAR ligands could inhibit the expression of cyclooxygenase-2 and production of IL-6, by PPAR α- induced inhibition of signaling of the pro-inflammatory mediator NF-$_k$B and induction of apoptosis.[20] The anti-inflammatory action of fibrates on huCRP expression is based on up-regulation of NF-$_k$B, I$_k$Bα and reduction of NF-$_k$B activity. PPAR α activators could increase the inhibition of CRP promoter activity,[18] and decrease expression levels of p50–NF-$_k$B and C/EBP-β in human hepatocytes. PPAR α also was a negative regulator of the inflammatory response through direct combination with p65–NF-$_k$B, to inactive the NF-$_k$B transcription factor pathways.[21,22] PPAR α activators also prevent p50 and p65 translocation into the nucleus by influencing hepatic expression of I$_k$B.[18,23]

It has been suggested that the genetic susceptibility of the pheno-

Table 5. Interaction analysis for two- locus models by using logistic regression

rs1800206	rs135539	Difference (95% CI)[a]	*P*-values
LL	CC	0.00	- - -
LV or VV	CC	0.29 (-0.18 to 0.76)	0.23
LL	CG or GG	-0.23 (-0.61to - 0.17)	0.01
LV or VV	CG or GG	-0.49 (-0.67 to -0.26)	< 0.001

[a] Adjusted for gender, age, smoke and alcohol status, physical activity

type was related to multiple-gene, and most of which were minor genes. For this reason, interaction analysis, among six SNPs was needed. We employed the GMDR analysis to assess the impact of the interaction among the six SNPs on CRP level with covariates adjustment. The results indicated that there was a significant one-locus model ($P = 0.0107$) involving rs1800206 and rs135539, indicating a potential gene–gene interaction between rs1800206 and rs135539. In order to obtain ORs and 95% CIs for the joint effects of candidate SNPs (rs1800206 and rs135539) on CRP, we conducted an interaction analysis among SNPs in the 2-locus models. The results indicated that subjects with rs1800206- LV or VV, rs135539- CG or GG genotypes have the lowest CRP level, compared to subjects with rs1800206- LL and rs135539-CC genotypes. In this study, although rs135539 was not associated with CRP level in the linear regression model, in the interaction analysis we found a significant interaction between rs1800206 and rs135539. It is well known that the CRP was influenced by many genes, and the impact of one or two genes or SNPs was very small However, minor gene could provide strong effect for CRP, due to the existing of gene-gene interaction.

Limitations of this study should be considered. Firstly, only six SNPs of PPAR α were chosen. The selected SNPs were not sufficient to capture most genetic information of the PPAR α. Further studies should include more SNPs, even the other PPAR isoforms, such as PPAR δ or PPAR γ. Secondly, there was a relatively small sample size of the study, though the number of study participants met the requirement for analysis, future studies should be conducted in different races.

In conclusion, our results support an important association between PPAR α rs1800206 and decreased CRP level. In addition, the results also shown a combined effect of gene-gene interaction between rs1800206 and rs135539 on decreased CRP level.

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