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Original Article

Association of peroxisome proliferator-activated receptor γ, and gene-gene interactions with essential hypertension in Chinese Han population

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Abstract: Aims: To investigate the association between four single nucleotide polymorphisms (SNP) of peroxisome proliferator-activated receptor-γ (PPAR γ), and additional gene-gene interaction with essential hypertension (EH) in Chinese Han population. Methods: A total of 1280 subjects (625 males, 655 females), with a mean age of 51.2±15.1 years old, including 628 EH patients and 652 normal subjects were included in the study, including the genotyping of polymorphisms. Logistic regression model was used to examine the association between 4 SNP and EH, odds ratio (OR) and 95% confident interval (95% CI) were calculated. Generalized MDR (GMDR) was employed to analysis the interaction among four SNPs. Results: EH risk was significantly higher in carriers of Ala allele of the rs1805192 polymorphism than those with Pro/Pro (Pro/Ala + Ala/Ala *versus* Pro/Pro, adjusted OR (95% CI) = 1.54 (1.20-1.89). In addition, we also found a significant association between rs4684847 and EH, EH risk was significantly higher in carriers of T allele of the rs4684847 polymorphism than those with CC (CT + TT *versus* CC, adjusted OR (95% CI) = 1.32 (1.16-2.43). There was a significant two-locus model (P = 0.0107) involving rs1805192 and rs4684847, indicating a potential gene-gene interaction between rs1805192 and rs4684847. Subjects with Pro/Ala or Ala/Ala and CT or TT genotype have highest EH risk, compared to subjects with Pro/Pro-CC genotype, OR (95% CI) was 3.26 (1.90-5.80), after covariates adjustment. Conclusions: Our results support an important association between rs1805192 and rs4684847 minor allele of PPAR γ and EH, and additional interaction between rs1805192 and rs4684847.

Keywords: Essential hypertension, PPAR γ, polymorphism, interaction

Introduction

Hypertension is a multifactorial disorder in which genetic and environmental factors are involved, including genetic factor and many environmental factors, such as insulin resistance and hyperinsulinemia [1]. Therefore, genetic factors affecting insulin resistance may be involved as a common genetic basis of susceptibility to hypertension. Peroxisome proliferator-activated receptor-γ (PPAR γ) is a ligand-activated nuclear transcription factor that forms a heterodimeric complex with the retinoid X receptor-α [2]. PPAR γ is a regulator of important target genes involved in glucose, lipid metabolism, adipogenesis and insulin signaling [3]. PPAR γ is an established mediator of improved insulin signaling mechanisms induced by thiazolidinediones (TZDs). Some studies have indicated that the importance of PPAR γ on blood pressure comes from human genetic studies of patients with mutated PPAR γ proteins. The characteristics of these patients often include insulin resistance, elevated triglycerides, and hypertension [3-5]. Although recent studies [6-8] have documented the association between PPAR γ polymorphism and essential hypertension (EH), however the results were inconsistent. So the aim of this study was to investigate the association between four single nucleotide polymorphisms (SNP) of PPAR γ, and additional gene-gene interaction with EH in Chinese Han population.

Materials and methods

Subjects

This was a case-control study. Participants were consecutively recruited between January

Table 1. Description and Probe sequence for 4 SNPs used for Taqman fluorescence probe analysis

SNP ID	SNP	Chromosome	Nucleotide substitution	Probe sequence
rs709158	Intron A>G	3:12403176	A>G	5'-AGATACGGGGGAGGAAATTCACTGG [A/G] TTTTACAATATATTTTTCAAGGCAA-3'
rs3856806	C1341T	3:12434058	C>T	5'-ACCTCAGACAGATTGTCACGGAACA [C/T] GTGCAGCTACTGCAGGTGATCAAGA-3'
rs1805192	Pro12Ala	3:12361238	C>G	5'-ACCTCAGACAGATTGTCACGGAACA [C/T] GTGCAGCTACTGCAGGTGATCAAGA-3'
rs4684847	Intron C>T	3:12326337	C>G	5'ATTTATTTAAATCATCTCTAATTCT [C/T] ACAACTCCGAAAAGATAAGAAAACA-3'

Table 2. General characteristics of 1280 study participants in case and control group

Variables	EH group (n = 628)	Normotension group (n = 652)	*p*-values
Age (year)	50.8±14.2	51.4±16.1	0.480
Males N (%)	321 (51.1)	304 (46.6)	0.120
Smoke N (%)	221 (35.2)	225 (34.5)	0.838
Alcohol consumption N (%)	232 (36.9)	175 (26.8)	0.0001
WC (cm)	90.4±16.8	82.2±16.9	<0.001
BMI (kg/m^2)	26.6±9.1	23.6±9.3	<0.001
FPG (mmol/L)	5.9±1.3	5.2±1.2	<0.001
TG (mmol/L)	1.3±0.6	1.2± 0.5	0.001
TC (mmol/L)	4.7±0.9	4.4±0.8	<0.001
HDL (mmol/L)	1.25±0.32	1.32±0.30	<0.001
Sedentary behavior N (%)	194 (30.9)	142 (21.8)	0.0003
Family history of EH N (%)	237 (37.7)	192 (29.4)	0.002

Note: Means ± standard deviation for age, WC, BMI, FPG, TC, TG, HDL-C; TC, total cholesterol; HDL, high density lipoprotein; FPG, fast plasma glucose; TG, triglyceride.

2011 and November 2013 from Cangzhou central Hospital. We excluded participants with diabetes, CVD, missing data and participants with BMI <18.5 kg/m^2, a total of 1280 subjects (625 males, 655 females), with a mean age of 51.2±15.1 years old, including 628 EH patients and 652 normal subjects were included in the study, including the genotyping of polymorphisms. 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, height, waist circumference were measured, and BMI was calculated as weight in kilograms divided by the square of the height in meters. WC was measured two times at 1 cm above the umbilicus at minimal respiration by trained observers; the mean of the two WC measurements was utilized in the analysis. 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. The concentrations of HDL cholesterol and triglycerides were assessed enzymatically using an automatic biochemistry analyzer (Hitachi Inc., Tokyo, Japan) and commercial reagents. All analysis was performed by the same lab.

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%. Four SNP of PPAR γ were selected for genotyping in the study: rs3856806, rs709158, rs1805192, rs4684847. 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 four SNP. A 25 μl reaction mixture including 1.25 μl SNP Genotyping Assays (20×), 12.5 μl Genotyping

Table 3. Genotype and allele frequencies of 4 SNPs between case and control group

SNPs	Genotypes and Alleles	Frequencies N (%)		OR (95% CI)*			H-W test for controls
		Control (n = 652)	Case (n = 628)	SBP ≥140 mmHg	DBP ≥90 mmHg	HBP	
rs3856806							
	CC	395 (60.6)	330 (52.5)	1.00	1.00	1.00	0.087
	CT	215 (33.0)	242 (38.5)	1.12 (0.95-1.39)	1.06 (0.72-1.34)	1.08 (0.75-1.36)	
	TT	42 (6.4)	56 (9.0)	1.24 (0.92-1.64)	1.10 (0.88-1.52)	1.14 (0.86-1.58)	
	TT + CT	257 (39.4)	298 (47.4)	1.20 (0.93-1.41)	1.07 (0.80-1.48)	1.18 (0.87-1.39)	
	C	902 (77.1)	354 (71.8)				
	T	299 (22.9)	354 (28.2)				
rs709158							
	AA	380 (58.3)	335 (53.3)	1.00	1.00	1.00	0.168
	AG	227 (34.8)	247 (39.3)	1.02 (0.83-1.37)	1.01 (0.68-1.45)	0.98 (0.65-1.49)	
	GG	45 (6.9)	46 (7.3)	1.06 (0.79-1.44)	1.05 (0.83-1.39)	1.02 (0.80-1.63)	
	GG + AG	272 (41.7)	293 (46.6)	1.10 (0.81-1.65)	1.07 (0.83-1.47)	1.04 (0.74-1.56)	
	A	987 (75.7)	917 (73.0)				
	G	317 (24.3)	339 (27.0)				
rs1805192							
	Pro/Pro	428 (65.6)	325 (51.8)	1.00	1.00	1.00	0.264
	Pro/Ala	195 (29.9)	242 (38.5)	1.42 (1.12-1.82)[1]	1.30 (1.04-1.69)[1]	1.52 (1.22-1.85)[1]	
	Ala/Ala	29 (4.5)	61 (9.7)	1.32 (0.88-2.27)	2.50 (1.45-4.35)[1]	2.08 (1.43-2.94)[1]	
	Ala/Ala + Pro/Ala	224 (34.4)	303 (48.2)	1.28 (0.94-1.67)	1.50 (1.11-1.96)[1]	1.54 (1.20-1.89)[1]	
	Pro	1051 (80.6)	892 (71.1)				
	Ala	253 (19.4)	364 (28.9)				
rs4684847							
	CC	426 (65.3)	321 (51.1)	1.00	1.00	1.00	0.073
	CT	193 (29.6)	240 (38.2)	1.40 (1.19-1.76)[1]	1.28 (1.07-1.66)[1]	1.42 (1.18-1.70)[1]	
	TT	33 (5.1)	67 (10.7)	1.36 (1.10-1.82)[1]	1.17 (0.96-3.35)	1.28 (1.05-2.87)[1]	
	TT + CT	226 (34.7)	307 (48.9)	1.39 (1.17-1.69)[1]	1.22 (0.98-2.69)	1.32 (1.16-2.43)[1]	
	C	1045 (80.1)	882 (70.2)				
	T	259 (19.9)	374 (29.8)				

*Adjusted for gender, age, smoking and alcohol status, TC, TG, HDL. [1]p<0.05.

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**.

Diagnostic criteria

Hypertension was defined as SBP ≥140 mmHg and/or DBP ≥90 mmHg and/or use of antihypertensive medication [9].

Statistical analysis

The mean and 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 EH using gender, age, smoking and alcohol status, TC, TG, HDL and family history of EH as covariates in the model.

Generalized MDR (GMDR) [10] was used to analysis the interaction among four SNP, cross-validation consistency, the testing balanced accuracy, and the sign test, to assess each selected interaction were calculated. The

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

Locus no.	Best combination	Cross-validation consistency	Testing accuracy	*p-values**
2	rs1805192 rs4684847	10/10	0.6217	0.0107
3	rs709158 rs1805192 rs4684847	9/10	0.5577	0.1719
4	rs3856806 rs709158 rs1805192 rs4684847	9/10	0.5590	0.0547

*Adjusted for gender, age, smoking and alcohol status, TC, TG, HDL.

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

rs1805192	rs4684847	OR (95% CI)*	*P-values*
Pro/Pro	CC	1.00	-
Pro/Ala or Ala/Ala	CC	1.29 (1.14-1.56)	0.002
Pro/Pro	CT or TT	1.42 (1.10-1.48)	0.001
Pro/Ala or Ala/Ala	CT or TT	3.26 (1.90-5.80)	<0.001

*Adjusted for gender, age, smoking and alcohol status, TC, TG, HDL.

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.

Results

A total of 1280 subjects (625 males, 655 females), with a mean age of 51.2±15.1 years old, were selected, including 628 EH patients and 652 normol subjects. Participants characteristics stratified by cases and controls are shown in **Table 2**. The distribution of alcohol consumption, sedentary behavior and family history of EH were significantly different between cases and controls. The mean of duration of diabetes, WC, BMI, FPG, HDL, TG and TC were significantly different between cases and controls.

All genotypes were distributed according to Hardy-Weinberg equilibrium in controls (P> 0.05). There were significant differences in rs1805192 alleles and genotypes distributions between EH patients and controls (**Table 3**). The frequencies for Ala allele of rs1805192 was higher in EH cases (28.9% *vs*. 19.4%). Logistic regression analysis showed a significant association between genotypes of variants in rs1805192 and increased EH risk, after adjustment for gender, age, smoking and alcohol status, TC, TG, HDL and family history of EH, EH risk was significantly higher in carriers of Ala allele of the rs1805192 polymorphism than those with Pro/Pro (Pro/Ala + Ala/Ala *versus* Pro/Pro, adjusted OR (95% CI) = 1.54 (1.20-1.89). The Ala allele of the rs1805192 polymorphism was also associated with DBP, but not SBP. In addition, we also found a significant association between rs4684847 and EH, EH risk was significantly higher in carriers of T allele of the rs4684847 polymorphism than those with CC (CT + TT *versus* CC, adjusted OR (95% CI) = 1.32 (1.16-2.43). The T allele of the rs4684847 polymorphism was also associated with SBP, but not DBP. However, we did not find any significant association between rs3856806 and rs709158 with EH, SBP and DBP after covariates adjustment.

We employed the GMDR analysis to investigate the impact of the interaction among four SNP in PPAR γ, after adjustment for covariates including gender, age, smoking and alcohol status, TC, TG, HDL and family history of EH. **Table 4** summarizes the results obtained from GMDR analysis for two to four locus models. There was a significant two-locus model (P = 0.0107) involving rs1805192 and rs4684847, indicating a potential gene-gene interaction between rs1805192 and rs4684847. Overall, the two-locus models had a cross-validation consistency of 10 of 10, and had the testing accuracy of 62.17%. In order to obtain the odds ratios and 95% CI for the joint effects of rs1805192 and rs4684847 on EH, we conducted interaction analysis between two SNP by using logistic regression. We found that subjects with Pro/Ala or Ala/Ala and CT or TT genotype have highest EH risk, compared to subjects with Pro/Pro-CC

genotype, OR (95% CI) was 3.26 (1.90-5.80), after covariates adjustment (**Table 5**).

Discussion

In the present study, we found that there was a significant association between PPAR γ genotypes of variants in four SNP and increased EH risk. There were higher EH risks in the Ala allele of rs1805192, T allele of rs4684847 carriers, suggesting that variants in two SNP could increase EH risk. The rs1805192 (Pro12Ala) polymorphism of the exon B in the PPAR γ is the most frequently found genetic variant of the PPAR γ gene [11]. However, the results of the association between rs1805192 and EH were controversial. Douglas et al [12] indicated that there was a significant association between rs1805192 and blood pressure. However, Swarbriek et al [13] found that no significant association between them was obtained. Gu et al [7] indicated that the Pro12Ala polymorphism appeared to be associated with the decrease in the risk for EH. Subjects carrying the 12Ala allele were associated with a 0.70-fold decreased risk of EH. However, C681G polymorphism appeared to be associated with the increase in the risk for EH. Subjects carrying the G allele were associated with a 1.54-fold increased risk of EH. Zhu et al [6] indicated that PPAR γ polymorphism including rs4684847 and rs10865710, were positively associated with EH. Yliharsila et al [14] found that the Prol2Ala polymorphism was associated with hypertension. In a meta-analysis, Wang et al [15] found that there was a significant association of the Pro12Ala polymorphism with hypertension susceptibility among East Asians.

As we all known that genetic susceptibility to any phenotype was related to multiple genes, most of which were minor genes. Because of the distance among genes, epistasis [16] exists among PPARs genotypes and other EH-related genes. For this reason, an interaction analysis of 4 SNP was necessary. We used GMDR analysis to assess interaction among the 4 SNP on obesity risk after covariate adjustment. The results showed potential gene-gene interaction between rs1805192 and rs4684847, and subjects with Pro/Ala or Ala/Ala and CT or TT genotype have highest EH risk, compared to subjects with Pro/Pro-CC genotype, OR (95% CI) was 3.26 (1.90 -5.80). It has been reported recently that independent of its blood glucose-lowering effects, PPAR γ demonstrates pleiotropic beneficial effects on vasculature [17]. The effect may possibly be due to PPAR γ- mediated inhibition of Ang-II type 1 receptor (AT1R) expression. PPAR γ agonists are known to lower blood pressure in humans, possibly through the suppression of the RAS, by mechanisms including the inhibition of AT1R expression, Ang-II-mediated signaling pathways, and Ang-II-induced adrenal aldosterone synthesis/secretion [18, 19].

Several limitations of this study should be considered. Firstly, only four SNP of PPAR γ were chosen. The limited SNPs were not sufficient to capture most genetic information of PPAR γ, even for PPAR family gene. More SNPs, not only in PPAR γ, but also in PPAR α and PPAR δ, should be included in the further studies. Secondly, more environmental factors should be included in the PPAR- environment studies, including lifestyle, diet and activity factors.

In conclusion, we tested the association between PPAR γ polymorphisms and EH in a Chinese Han population based on single-locus and interaction analyses. We found that there was a significant association between PPAR γ genotypes of variants in four SNP and increased EH risk. There were higher EH risks in the Ala allele of rs1805192, T allele of rs4684847 carriers, suggesting that variants in two SNP could increase EH risk. In addition, we also found a potential gene-gene interaction between rs1805192 and rs4684847.

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Disclosure of conflict of interest

None.

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