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Case-control study on peroxisome proliferator-activated receptor gamma polymorphism and interaction with HDL on essential hypertension in Chinese Han

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ABSTRACT

Objective(s): To investigate the association of single nucleotide polymorphisms (SNPs) in the peroxisome proliferator-activated receptors gamma (PPARG) with essential hypertension (EH) and additional role of gene– high-density lipoprotein cholesterol (HDL) interaction.

Materials and Methods: A total of 1640 patients with EH (806 males, 834 females), with a mean age of 52.5±12.6 years, were selected, including 816 EH patients and 824 controls, who were enrolled from the community. Three SNPs were selected for genotyping in the case–control study: rs10865710, rs709158, rs1805192. Logistic regression model was used to examine the interaction between SNP and HDL on EH, odds ratio (OR) and 95% confidence interval (95% CI) were also calculated.

Results: All genotypes were distributed according to Hardy–Weinberg equilibrium in controls. Logistic regression analysis showed an association between genotypes of variants in rs1805192 and decreased EH risk, EH risk was significantly lower 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) =0.65 (0.53–0.83), after covariate adjustment. In addition, the Ala allele of the rs1805192 polymorphism was also associated with diastolic blood pressure (DBP), but not systolic blood pressure (SBP), we also found, by interaction analysis, combined effect of rs1805192 and HDL on EH risk after covariate adjustment.

Conclusion: Our results support an important association between rs1805192 minor allele (Ala allele) of PPARG and lower EH risk, the interaction analysis showed a combined effect of Ala- HDL on lower EH risk.

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Introduction

Essential hypertension (EH) is a multifactorial disorder in which genetic and environmental factors are involved (1). Elevated high-density lipoprotein cholesterol (HDL-C) has been suggested to be strongly related to the reduction of EH events in large population-based studies (2-5). One study also indicated that each 1 mg/dl increase in HDL-C was associated with a decrease of 2–3% in the risk of coronary heart disease events (5). Genetic determinant may be prominent in the development of EH in view of the heritability of blood pressure (6), peroxisome proliferator-activated receptors gamma (PPARG) gene, for instance, which was a candidate gene for EH, seems to attract particular interest in relation to hypertension (7). PPARG is a ligand-activated transcription factor that plays a critical role in metabolism. Although thiazolidinediones, a high-affinity PPARG ligands, was used clinically to treat hypertension and provide other cardiovascular benefits, however, PPARG gene in the pathogenesis of EH or the changes in arterial blood pressure remains inconsistent (8-10). In addition, so far, no study evaluating the combined effects of PPARG and HDL on the risk of developing hypertension has been conducted, in particular in the Chinese population. Hence, in this study, we aimed to investigate the PPARG polymorphism and EH, and additional interaction between PPARG and HDL.

Materials and Methods

Subjects

The current study was a population-based case-control study. EH patients were consecutively recruited between March 2009 and December 2014 from the affiliated hospital of Qingdao university, Qingdao, Shandong province, China. Subjects with cardiovascular disease (CVD), type 2 diabetes and

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Table 1. Probe sequence for three single-nucleotide polymorphisms (SNPs) used for TaqMan fluorescence probe detection

SNP ID	SNP	Chromosome	Position	Nucleotide substitution	Probe sequence
rs709158	Intron A>G	3	12403176	A>G	5'-AGATACGGGGGAGGAAATTCACTGG[A/G] TTTTACAATATATTTTTCAAGGCAA-3'
rs10865710	C681G	3	12293198	C>G	5'-TTGGCATTAGATGCTGTTTTGTCTT[C/G] ATGGAAAATACAGCTATTCTAGGAT-3'
rs1805192	Pro12Ala	3	12361238	C>G	5'-ACCTCAGACAGATTGTCACGGAACA[C/G] GTGCAGCTACTGCAGGTGATCAAGA-3'

cancer were excluded. A total of 1640 Chinese Han subjects (806 males and 834 females), with a mean age of 52.5±12.6 years, were selected, including 816 EH patients and 824 healthy controls, who were enrolled from the community. Informed consent was obtained from all patients and healthy donors.

Body measurements

Demographic data and lifestyle risk factors for all participants were obtained by using a standard questionnaire administered by trained staffs. The first and fifth Korotkoff sounds were recorded as systolic (SBP) and diastolic (DBP) pressures, respectively. The mean of the three BP measurements was used in the analysis. Body weight and height were measured, and body mass index (BMI) was calculated as weight in kilograms divided by the square of the height in meters. Waist circumference (WC) was measured by the same physician at the umbilical level with the participants standing and breathing normally during the physical examination. Blood samples were collected in the morning after at least 8 hr of fasting. All plasma and serum samples were frozen at –80 ˚C until laboratory testing. Plasma glucose was measured using an oxidase enzymatic method. All analysis was performed by the same lab.

Genomic DNA extraction and genotyping

Three single nucleotide polymorphisms (SNPs) were selected for genotyping in the case–control study, including rs10865710, rs709158 and rs1805192. Participant Genomic DNA was extracted from EDTA-treated whole blood, using the DNA Blood Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. Three SNPs were detected by TaqMan fluorescence probe. ABI Prism7000 software and allelic discrimination procedure were used for genotyping of the fore-mentioned three SNPs. The 25 μl reaction mixture included 1.25 ul SNP Genotyping Assays (20×), 12.5 μl Genotyping Master Mix (2×) and 20 ng DNA, and the conditions were as follows: initial denaturation for 10 min at 95 ˚C, denaturation for 15 sec at 92 ˚C, annealing and extension for 90 sec at 60 ˚C, 50 cycles. Probe sequences of the three SNPs are shown in Table 1.

Diagnostic criteria

Hypertension was defined as SBP ≥140 mmHg and/or DBP≥90 mmHg and/or use of antihypertensive medication (11).

High HDL level was defined as ≥40 mg/dl in men and ≥50 mg/dl in women (12).

Statistical analysis

The mean and standard deviation (SD) for normally distributed continuous variables, and percentages for the categorical variable, were calculated and compared between EH patients and control participants. The genotype and allele frequencies were obtained by direct count. Genotype distributions in EH patients and controls were evaluated by the χ^2 test using SPSS (version 19.0; SPSS Inc., Chicago, IL, USA). Hardy-Weinberg equilibrium (HWE) test was used to detect genotype typing errors for quality control by the Fisher's exact χ^2 test using HWE software (13, 14). Logistic regression model was used to examine the association between SNP polymorphisms and HDL and EH, and additional interaction, odds ratio (OR) and 95% confidence interval (95% CI) were calculated. Odds were adjusted for the potential confounding factors, such as gender, age, smoking and alcohol status.

Results

A total of 1640 subjects (806 males and 834 females), with a mean age of 52.5 ± 12.6 years, were selected, including 816 EH patients and 824 controls, who were enrolled from the community. Participant characteristics of EH cases and controls are shown in Table 2. Mean of WC, BMI, fasting plasma glucose (FPG), total cholesterol (TC), and triglyceride (TG) were significantly higher in EH subjects than in controls (All *P*-values<0.05). Mean of HDL was significantly higher in controls (*P*=0.003). The distributions of high-fat diet, low fiber diet, alcohol consumption, income per month/person, sedentary behavior, and family history of EH were also significantly different between EH cases and controls (*P*<0.05 for all comparisons).

All genotypes were distributed according to Hardy–Weinberg equilibrium in controls. There were significant differences in rs1805192 alleles and genotypes distributions between cases and controls (Table 3). The frequencies for Ala allele of rs1805192 were lower in EH cases (19.4% *vs* 29.0%). Logistic regression analysis showed an association between genotypes of variants in rs1805192 and decreased EH risk, after adjustment for gender, age, smoking, and alcohol status, EH risk was significantly lower in carriers of Ala allele of the rs1805192 polymorphism

Table 2. General characteristics of 1640 study participants in case and control groups

Variables	EH cases group (n=816)	Control group (n=824)	*P*-values
Age (year)	52.8±16.2	51.4±17.6	0.281
Males N (%)	387 (47.4)	419 (50.8)	0.184
High fat diet N (%)	296 (36.3)	240 (29.1)	0.002
Low fiber diet N (%)	152 (18.6)	116 (14.1)	0.016
Smoke N (%)	320 (39.2)	289 (35.1)	0.095
Alcohol consumption N (%)	268 (32.8)	217 (26.3)	0.005
WC (cm)	92.4±17.8	83.2±16.9	<0.001
BMI (kg/m^2)	26.9±9.7	24.6±10.8	<0.001
FPG (mmol/l)	5.8±1.2	5.3±1.1	<0.001
TG (mmol/l)	1.3±0.6	1.2± 0.5	<0.001
TC (mmol/l)	4.8±0.9	4.5±1.0	<0.001
HDL (mmol/l)	1.24±0.33	1.31±0.27	0.003
Income per month/person N (%)			
<2000	538 (65.9)	578 (70.2)	
2000-	215 (26.3)	212 (25.7)	
>=5000	63 (7.8)	34 (4.1)	0.006
Sedentary behavior N (%)	180 (22.0)	142 (17.3)	0.019
Family history of EH N (%)	237 (29.1)	194 (23.5)	0.011

Note: Mean± standard deviation for age, WC, BMI, FPG, TC, TG, HDL-C; TC, total cholesterol; HDL, high-density lipoprotein; LDL, low-density lipoprotein; FPG, fasting plasma glucose; TG, triglyceride; WC, waist circumference; BMI, body mass index

Table 3. Genotype and allele frequencies of three single-nucleotide polymorphisms between case and control groups

SNPs	Genotypes and Alleles	Frequencies N (%)		OR (95%CI)*			*H-W* test for controls
		Cases (n=816)	Controls (n=824)	SBP≥140mmHg	DBP≥90mmHg	HBP	
rs10865710							
	CC	428 (52.4)	432 (52.4)	1.00	1.00	1.00	0.728
	CG	316 (38.7)	332 (40.3)	0.86 (0.79-1.38)	0.89 (0.72-1.34)	0.87 (0.75-1.36)	
	GG	72 (8.8)	60(7.3)	1.05 (0.92-1.41)	1.08 (0.88-1.37)	1.10 (0.84-1.43)	
	GG+CG	386 (47.5)	392 (47.6)	1.01 (0.74-1.69)	1.03 (0.80-1.54)	1.04 (0.70-1.58)	
	C	1172 (71.8)	1203 (73.0)				
	G	460 (28.2)	445 (27.0)				
rs709158							
	AA	415 (50.8)	439 (53.3)	1.00	1.00	1.00	0.988
	AG	330 (40.4)	325 (39.4)	1.02 (0.83-1.37)	1.01 (0.68-1.45)	0.98 (0.65-1.49)	
	GG	71(8.8)	60 (7.3)	1.06 (0.79-1.44)	1.05 (0.83-1.39)	1.02 (0.80-1.63)	
	GG+AG	391 (49.2)	385 (46.7)	1.10 (0.81-1.65)	1.07 (0.83-1.47)	1.04 (0.74-1.56)	
	A	1160 (71.1)	1203 (73.0)				
	G	472 (28.9)	445 (27.0)				
rs1805192							
	Pro/Pro	536 (65.7)	426 (51.7)	1.00	1.00	1.00	0.071
	Pro/Ala	244 (29.9)	318 (38.6)	0.70 (0.55-0.89)	0.77 (0.59-0.98)	0.66 (0.54-0.82)[1]	
	Ala/Ala	36 (4.4)	80 (9.7)	0.76 (0.51-1.14)	0.40 (0.23-0.69)[1]	0.48 (0.34-0.70)[1]	
	Ala/Ala+ Pro/Ala	280 (34.3)	398 (48.3)	0.78 (0.60-1.01)	0.67 (0.51-0.90)[1]	0.65 (0.53-0.83)[1]	
	Pro	1316 (80.6)	1170 (71.0)				
	Ala	316 (19.4)	478 (29.0)				

*Adjusted for gender, age, smoking, and alcohol status. [1] P<0.05; The nucleotide substitution of rs1805192 was C>G

than those with Pro/Pro (Pro/Ala+ Ala/Ala *versus* Pro/Pro, adjusted OR (95% CI)=0.65 (0.53–0.83). In addition, the Ala allele of the rs1805192 polymorphism was also associated with DBP, but not SBP, however, we did not find any significant association between rs10865710 and rs709158 with EH, SBP and DBP both before and after covariates adjustment.

Table 4. Interaction analysis for rs1805192 and HDL on essential hypertension by using logistic regression

rs1805192	High HDL[1]	OR (95% CI)*	*P*-values
Pro/Pro	No	1.00	-
Pro/Ala or Ala/Ala	No	0.85 (0.62-0.94)	0.013
Pro/Pro	Yes	0.62 (0.37-0.85)	<0.001
Pro/Ala or Ala/Ala	Yes	0.46 (0.28-0.59)	<0.001

*Adjusted for gender, age, smoking, and alcohol status
[1] HDL cholesterol (HDL-C more than1.0 mmol/l in men and more than 1.3 mmol/l in women)

In order to obtain the ORs and 95% CI for the joint effects of rs1805192 genotype and HDL on EH, we conducted interaction analysis between rs1805192 and HDL on EH risk. The results indicated that subjects with high HDL and Pro/Ala or Ala/Ala genotype have the lowest EH risk, compared to subjects with low HDL level and Pro/Pro genotype (OR=0.46, 95% CI=0.28–0.59), after adjustment for gender, age, smoking, and alcohol status (Table 4).

Discussion

The result of this study indicate that rs1805192 minor allele (Ala allele) of PPARG is significantly associated with lower EH risk, However, we did not find any association between the other SNPs (rs709158 and rs10865710) and EH. Pro12Ala was the first identified in PPARG2 polymorphism, which may have important consequences related to obesity, insulin resistance and type 2 diabetes (15). Subsequently, more and more studies (8- 10, 16) focused on PPARG and EH, however, the results were inconsistent. Study (8) indicated that Pro12Ala polymorphism was not associated with hypertension in type 2 diabetes mellitus patients. Another study (16) indicated that the Pro12Ala polymorphism of the PPARG2 gene is associated with hypertension and triglycerides levels in Chinese nonagenarians/ centenarians, Ala of PPAR-γ2 gene and decreased prevalence of hypertension. Results of study by Gu *et al* (17) suggested 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. In a meta-analysis, Wang and Liu (18) found that there was a significant association of the Pro12Ala polymorphism with hypertension susceptibility among East Asians. These findings, in part, corroborated our findings.

The association between elevated HDL-C and reduction of EH events have been studied in large population-based studies (2-5). Although Oda *et al* (19) suggested that HDL cholesterol was independently and positively associated with hypertension in apparently healthy Japanese men and women. However, most studies indicated that higher HDL was a protective factor for EH. In this study, the mean HDL levels were higher in EH cases than those in controls. Hence, we conducted an interaction analysis for HDL and Ala allele of rs1805192. We found a significant interaction between HDL and the Ala allele, subjects with high HDL and Pro/Ala or Ala/Ala genotypes have lowest EH risk, compared to subjects with low HDL level and the Pro/Pro genotype. Some mechanisms of association between PPARG and EH were suggested. PPARG demonstrates pleiotropic beneficial effects on vasculature (20). The effect may possibly be due to PPARG-mediated inhibition of Ang-Ⅱ type 1 receptor (AT1R) expression as well as Ang-Ⅱ-mediated signaling pathways, which may result in suppression of the renin- angiotensin system (RAS) and lead to a lower blood pressure (20). Moreover, PPARG ligands have been shown to suppress Ang-Ⅱ-induced phosphatidylinositol 3-kinase and MAP kinase and to ameliorate Ang Ⅱ-mediated inflammatory responses by interfering with the Toll-like receptor 4-dependent signaling pathway (21-23). Therefore, PPARG not only downregulates AT1R expression but also inhibits Ang Ⅱ- mediated signaling pathways, which may result in RAS suppression.

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

Conclusion

Our results support an important association between rs1805192 minor allele (Ala allele) of PPARG and lower EH risk, and the interaction analysis showed a combined effect of Ala- HDL interaction between rs1805192 and low HDL on reduction of EH risk.

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Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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