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Case- control study on association of peroxisome proliferator-activated receptor -delta, and gene-gene interactions with essential hypertension in Chinese Han population

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Abstract:	Aims: To investigate the association of peroxisome proliferator-activated receptors-δ (PPAR δ), and additional gene- gene interaction with essential hypertension (EH) in Chinese Han population. Methods: A total of 1248 subjects (625 males, 623 females), with a mean age of 51.2 ± 15.1 years old, including 620 EH patients and 628 normotension subjects were included in the study, including the genotyping of polymorphisms. Logistic regression model was used to examine the association between four 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 lower in carriers of C allele of the rs2016520 polymorphism than those with TT (TC+ CC versus TT, adjusted OR (95%CI) =0.61(0.49-0.78). In addition, we also found a significant association between rs9794 and EH, EH risk was also significantly lower in carriers of G allele of the rs9794 polymorphism than those with CC (CG+ GG versus CC, adjusted OR (95%CI) =0.65(0.53-0.83). potential gene-gene interaction between rs2016520 and rs9794, subjects with TC or CC of rs2016520 and CG or GG of rs9794 genotype have lowest EH risk, compared to subjects with TT of rs2016520 and CC of rs9794 genotype, OR (95%CI) was 0.32(0.23 -0.62), after covariates adjustment. Conclusions: Our results support an important association between rs2016520 and rs9794 minor allele of PPAR-δ and decreased risk of EH, and additional interaction between rs2016520 and rs9794.

Case- control study on association of peroxisome proliferator-activated receptor -delta, and gene-gene interactions with essential hypertension in Chinese Han population

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Abstract

Aims: To investigate the association of peroxisome proliferator-activated receptors-δ (PPAR δ), and additional gene- gene interaction with essential hypertension (EH) in Chinese Han population.

Methods: A total of 1248 subjects (625 males, 623 females), with a mean age of 51.2 ± 15.1 years old, including 620 EH patients and 628 normotension subjects were included in the study, including the genotyping of polymorphisms. Logistic regression model was used to examine the association between four 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 lower in carriers of C allele of the rs2016520 polymorphism than those with TT (TC+ CC *versus* TT, adjusted OR (95%CI) =0.61(0.49-0.78). In addition, we also found a significant association between rs9794 and EH, EH risk was also significantly lower in carriers of G allele of the rs9794 polymorphism than those with CC (CG+ GG *versus* CC, adjusted OR (95%CI) =0.65(0.53-0.83). potential gene–gene interaction between rs2016520 and rs9794, subjects with TC or CC of rs2016520 and CG or GG of rs9794 genotype have lowest EH risk, compared to subjects with TT of rs2016520 and CC of rs9794 genotype, OR (95%CI) was 0.32(0.23 -0.62), after covariates adjustment.

Conclusions: Our results support an important association between rs2016520 and rs9794 minor allele of PPAR-δ and decreased risk of EH, and additional interaction between rs2016520 and rs9794.

[Keywords] Essential hypertension, PPAR-δ, Polymorphism, Interaction

Introduction

Hypertension is a multifactorial disorder in which genetic and environmental factors are involved, including genetic factor and many environmental factors (Yagil and Yagil 2005). Therefore, genetic factors affecting insulin resistance may be involved as a common genetic basis of susceptibility to hypertension. Peroxisome proliferator-activated receptors (PPARs) are nuclear transcription factors involved in the regulation of lipid and glucose metabolism. Three closely related members belong to the PPAR subgroup designated PPAR-α, PPAR-δ and PPAR-γ. In contrast to the two other PPARs, PPAR-δ is ubiquitously expressed. Existing evidence indicates that activation of PPAR-δ in the endothelium stimulates angiogenesis (Piqueras et al. 2007; Wang et al. 2006), protects endothelial cells from apoptosis (Liou et al. 2006), and suppresses atherosclerosis (Barish et al. 2008). Although some studies (Zhu et al. 2014; Gu et al. 2013; Usuda and Kanda 2014) have focused on the association between PPAR-γ and hypertension, however, the association between PPAR-δ and hypertension was not extensively studied previously. 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 (Fisher 1919) exists among PPARs genotypes and other EH-related genes. So it is necessary to investigate the interaction among many SNP, which has not been studied in previous studies. So the aim of this study was to investigate the association between PPAR-δ and additional gene-gene interactions on hypertension risk.

Materials and methods

Subjects

This was a case-control study. Participants were consecutively recruited between January 2010 and November 2012 from the First Hospital of Jilin University. We excluded participants with diabetes, CVD, missing data and participants with BMI< 18.5 kg/m^2, A total of 1248 subjects (625 males, 623 females), with a mean age of 51.2 ± 15.1 years old, including 620 EH patients and 628 normotension 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-δ gene, which have been reported associations with metabolic abnormalities and minor allele frequency (MAF) greater than 1%. Four SNPs of PPAR-δ were selected for genotyping in the study: rs2016520, rs9794, rs1053046 and rs1053049. 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 25μl reaction mixture including 1.25ul SNP Genotyping Assays (20×), 12.5μl Genotying Master Mix (2×), 20ng 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 ≥140mmHg and/or DBP ≥90mmHg and/or use of antihypertensive medication (World Health Organization 1999).

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) (Lou et al. 2007) 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.

Results

1. A total of 1248 subjects (625 males, 623 females), with a mean age of 51.2 ± 15.1 years old, were selected, including 620EH patients and 628 normotension subjects. Participants characteristics stratified by cases and controls are shown in Table 2. The distribution of smoking, alcohol consumption, sedentary behavior and family history of EH were significantly different between cases and controls. The mean of WC, BMI, FPG, HDL, TG and TC were significantly different between cases and controls.

2. All genotypes were distributed according to Hardy–Weinberg equilibrium in controls (P>0.05). The frequencies for C allele of rs2016520 was significantly lower in EH cases (22.3% *vs*28.5%), and G allele of rs9794 was also significantly lower in EH cases (21.4% *vs*28.9%). Logistic regression analysis showed a significant association between genotypes of variants in rs2016520 and rs9794 with decreased EH risk, after adjustment for gender, age, smoking and alcohol status, TC, TG, HDL and family history of EH, EH risk was significantly lower in carriers of C allele of the rs2016520 polymorphism than those with TT (TC+CC *versus* TT, adjusted OR (95%CI) =0.61 (0.49-0.78). In addition, we also found EH risk was also significantly lower in carriers of G allele of the rs9794 polymorphism than those with CC (CG+ GG *versus* CC, adjusted OR (95%CI) =0.65(0.53-0.83). However, we did not find any significant association between rs1053046 and rs1053049 with EH after covariates adjustment. (Table 3)

3. We employed the GMDR analysis to investigate the impact of the interaction among four SNP in PPAR -δ, 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

rs2016520 and rs9794, indicating a potential gene–gene interaction between rs2016520 and rs9794. Overall, the two- locus models had a cross-validation consistency of 10 of 10, and had the testing accuracy of 62.17%.

4. In order to obtain the odds ratios and 95%CI for the joint effects of rs2016520 and rs9794 on EH, we conducted interaction analysis between two SNP by using logistic regression. We found that subjects with TC or CC of rs2016520 and CG or GG of rs9794 genotype have lowest EH risk, compared to subjects with TT of rs2016520 and CC of rs9794 genotype, OR (95%CI) was 0.32(0.23 -0.62), after covariates adjustment. (Table 5)

Discussion

In the present study, we found that there was a significant association between PPAR -δ genotypes of variants in rs2016520 and rs9794 with decreased EH risk. There were lower EH risks in the C allele of rs2016520 and G allele of rs9794 carriers, suggesting that variants in two SNP could increase EH risk. The rs2016520 polymorphism in exon 4 of the PPAR-δ gene was described by Skogsberg et al (2003). Polymorphism has been associated with lipid metabolism, body mass index (BMI) and coronary heart disease in many studies (Skogsberg et al. 2003; Aberle et al. 2006). Yan et al (2005) reported a relationship between rs2016520 gene polymorphism and lipid profile, obesity and left ventricular hypertrophy in 300 patients with metabolic syndrome, 174 patients with essential hypertension, and 143 patients with type 2 diabetes mellitus. But the frequencies of the rs2016520 genotypes were not different among three groups. Yin et al (2012) reported that the genotypic frequencies of PPAR –δ rs2016520, were different between normotensive and hypertensive subjects, it suggested that SBP, DBP and PP were associated with PPAR –δ genotypes or alleles, subjects with CC genotype have higher BP, SBP and DBP levels than that in subjects with TT genotype. Lin et al (2012) reported a significant association between rs9794 with lower risk of hypertension and high SBP in a Chinese population. Kojonazarov et al (2014) reported that the PPAR β/δ agonist GW0742 has direct protective effects on the right heart in vivo, it suggested that these observations identify PPAR β/δ as a viable therapeutic target to treat pulmonary hypertension that may complement current and future vasodilator drugs.

As we all known that genetic susceptibility to any phenotype was related to multiple genes, most of which were minor 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 rs2016520 and rs9794, subjects with TC or CC of rs2016520 and CG or GG of rs9794 genotype have lowest EH risk, compared to subjects with TT of rs2016520 and CC of rs9794 genotype, OR (95%CI) was 0.32(0.23 -0.62), after covariates adjustment. Zarzuelo et al (2011) provide the first evidence that chronic treatment with the highly selective PPAR δ agonist GW0742 reduces systolic blood pressure, mesenteric vascular hypertrophy, vascular inflammation, systemic and vascular oxidative stress, and endothelial dysfunction in an experiments study. The function of PPAR δ was first examined in PPAR δ- deficient mice, and later studied using the recently developed synthetic PPAR δ ligand. GW501516, a high-affinity PPAR δ ligand, can reduce weight gain and reduce triglycerides, and increase high-density lipoprotein (HDL) -cholesterol in obese mice by increasing peripheral fatty acid catabolism (Wang et al. 2003; Lee et al. 2006). PPAR δ also regulates glucose homeostasis. GW501516 also lowers the plasma insulin level and improves glucose tolerance and insulin sensitivity in diet-induced obese mice (Tanaka et al. 2003). Collectively, these findings suggest that PPAR δ may be regulated the blood pressure levels by regulating risk factors of EH, including obesity, HDL-C, triglycerides and fatty acid catabolism, it could be a potential therapeutic target for combating obesity and insulin resistance.

Several limitations of this study should be considered. Firstly, limited number of SNP in PPAR δ was chosen in this study. More SNPs, not only in PPAR δ, but also in PPAR α and PPAR γ, 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 δ polymorphisms and EH in a Chinese Han population. We found that there was a significant association between PPAR δ genotypes of variants in four SNP and decreased EH risk. There were lower EH risks in the C allele of rs2016520, or G allele of rs9794 carriers, suggesting that variants in two SNP could decrease EH risk. In addition, we also found a potential gene–gene interaction between rs2016520 and rs9794. The results of this study may help to clarify the role of the PPAR δ gene in blood pressure regulation and the evaluation of its polymorphisms and multiple gene- gene interaction as being characterized as genetic risk factors for EH.

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Conflict of interest

There is no conflict of interest.

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

SNP ID	MAF	Chromosome	Functional Consequence	Nucleotide substitution	Probe sequence
rs2016520	0.2290	6:35411001	Intron variant, utr variant 5 prime	T>C	5’-CGGCCACATGCCGCGTCCCTGCCCC[C/G]ACCCGGGTCTGGTGCTGAGGATACA-3’
rs9794	0.1416	6:35428018	Utr variant 3 prime	C>G	5’-CCTCTGCCCAGGCTGATGGGAACCA[C/T]CCTGTAGAGGTCCATCTGCGTTCAG-3’
rs1053046	0.1681	6:35427801	Utr variant 3 prime	A > G	5’-GTCCTCCCTCCCAAGGAGCCATTCT[A/G]TGTGTGACTCTGGGTGGAAGTGCCC-3’
rs1053049	0.3359	6:35427841	Utr variant 3 prime	T > C	5’-TGGAAGTGCCCAGCCCCTGCCCCTA[C/T]GGGCGCTGCAGCCTCCCTTCCATGC-3’

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

Variables	EH group (n=620)	Normotension group (n=628)	*p*-values
Age (year)	51.8±14.2	52.4±16.1	0.485
Males N (%)	321(51.8)	304(48.4)	0.252
Smoke N (%)	221 (35.6)	181(28.8)	0.012
Alcohol consumption N (%)	232 (37.4)	175 (27.9)	<0.001
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(31.3)	142(22.6)	<0.001
Family history of EH N (%)	252(40.6)	190(30.2)	<0.001

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.

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

SNPs	Genotypes and Alleles	Frequencies N (%)		OR(95%CI)[a]	H-W test for controls
		EH group (n=620)	Normotension group (n=628)		
rs2016520					
	TT	379(61.1)	328(52.2)	1.00	0.172
	TC	205(33.1)	242(38.5)	0.70(0.57-0.82)[1]	
	CC	36(5.8)	58(9.3)	0.48(0.34-0.71)[1]	
	CC+TC	241 (38.9)	300 (47.8)	0.61(0.49-0.78)[1]	
	T	963(77.7)	898(71.5)		
	C	277(22.3)	358(28.5)		
rs1053046					
	AA	361(58.2)	332(52.9)	1.00	0.746
	AG	216(34.8)	247(39.3)	0.98(0.65-1.49)	
	GG	43(6.9)	49(7.8)	0.94(0.73-1.53)	
	GG+AG	259(41.8)	296(47.1)	0.97(0.68-1.50)	
	A	938(75.6)	911(72.5)		
	G	302(24.4)	345(27.5)		
rs9794					
	CC	392(63.2)	325(51.8)	1.00	0.109
	CG	191(30.8)	242(38.5)	0.66(0.54-0.82)[1]	
	GG	37(6.0)	61(9.7)	0.48(0.34-0.70)[1]	
	GG+CG	228(36.8)	303(48.2)	0.65(0.53-0.83)[1]	
	C	975(78.6)	892(71.1)		
	G	265(21.4)	364(28.9)		
rs1053049					
	TT	370(59.7)	365(58.1)	1.00	0.119
	TC	216(34.8)	218(34.7)	0.99(0.82-1.31)	
	CC	34(5.5)	45(7.2)	0.91(0.73-1.26)	
	CC+TC	250(40.3)	263(41.9)	0.97(0.80-1.29)	
	T	956(77.1)	948(75.5)		
	C	284(22.9)	308(24.5)		

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

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

Locus no.	Best combination				Cross-validation consistency	Testing accuracy	*p-values*[a]
2	rs2016520	rs9794			10/10	0.6217	0.0107
3	rs2016520	rs9794	rs1053046		9/10	0.5577	0.1719
4	rs2016520	rs9794	rs1053046	rs1053049	9/10	0.5590	0.0547

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

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

rs2016520	rs9794	OR (95% CI)[a]	*P-values*
TT	CC	1.00	-
TC or CC	CC	0.78 (0.64 -0.88)	0.002
TT	CG or GG	0.70 (0.68-0.91)	0.001
TC or CC	CG or GG	0.32(0.23 -0.62)	<0.001

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