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Original article

PPARD rs2016520 polymorphism and circulating lipid levels connect with brain diseases in Han Chinese and suggest sex-dependent effects

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ABSTRACT

The *PPARD* polymorphisms were shown to be associated with circulating lipoprotein metabolism in various diseases. We aimed to check the contribution of *PPARD* rs2016520 and lipid concentration to the risk of intracerebral hemorrhages (ICH) and brain tumors (BT) in Han Chinese. A total of 864 participants were included in the case-control study. The melting temperature shift (Tm-shift) method was used for rs2016520 genotyping. Under the recessive model, *PPARD* rs2016520 was shown to be associated with the risk of ICH ($P = 0.029$, odds ratio (OR) = 2.72), specifically in males ($P = 0.045$, OR = 3.98). Additionally, we also found that the levels of TC and LDL-C were significantly higher in participants with brain diseases than in the controls (TC: $P < 0.0001$; LDL-C: $P < 0.0001$). Significantly higher HDL-C and lower ApoA-I levels were observed in the male patients with brain diseases (HDL-C: $P < 0.0001$; ApoA-I: $P = 0.008$), in contrast of a higher TG level in female ICH ($P = 0.023$). Subsequent interaction analysis between *PPARD* rs2016520 and lipoprotein metabolism showed that the LDL-C level was positively correlated with ICH in the rs2016520-AA carriers ($P < 0.0001$), but not in the other genotype carriers (AG or GG, $P = 0.300$). Our results showed that *PPARD* rs2016520 displayed a strong relationship with ICH risk in the male Han Chinese. The TC and LDL-C levels were positively higher in the patients with brain diseases than in the controls. The levels of TG, HDL-C and ApoA-I were shown to affect brain disease in a gender-dependent model. The genotype rs2016520-AA showed significant interaction with the circulating LDL-C levels in ICH.

1. Introduction

The brain is the center of the nervous system in humans. Lesions in the brain have a great impact on the human body. The most common brain diseases are intracerebral hemorrhage (ICH) and brain tumors (BT). ICH is the result from the rupture of blood vessels in the brain [1]. BT is the intracranial solid neoplasm which results from abnormal growth of cells in the brain [2]. These brain diseases (ICH and BT) have become the major causes of morbidity and mortality in the developing countries such as China [3]. Recent studies have identified that inherited and environmental factors may play an important role in the pathogenesis of brain diseases, including ICH and BT [4]. However, the mechanisms of brain diseases are not fully understood.

As a member of the peroxisome proliferator-activated receptor (PPAR) family, peroxisome proliferator-activated receptor delta (PPARD) is a nuclear hormone receptor governing a variety of biological processes, such as epidermal cell proliferation, cell migration [5], glucose [6] and lipid metabolism [7]. PPARD is highly expressed in brain and heart [8]. The protein can promote inflammation and tumor growth [8] as well as increase the concentration of plasma high-density lipoprotein cholesterol (HDL-C) in cardiovascular disease [9]. Previous studies showed that the *PPARD* polymorphisms were associated with modifications of serum lipid concentrations and the cardiovascular disease risk in the general population [10–12].

SNP rs2016520 is located in the 5′-untranslated region of the *PPARD* gene [13]. This polymorphism has been studied in different diseases such as coronary heart disease (CHD) [9], obesity [14],

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metabolic disease [15], essential hypertension (EH) [16] and Alzheimer's disease [17]. However, there is no published study that focuses on the association between rs2016520 and ICH or BT in Han Chinese. In the present study, we aimed to investigate the role of *PPARD* rs2016520 to the risk of brain diseases in Han Chinese, and to identify the effects of the *PPARD* genotypes on circulating lipids in ICH and BT patients.

2. Materials and methods

2.1. Study population

A total of 256 unrelated patients were gathered between March 2013 and May 2014 from the Department of Neurosurgery of the Ningbo First Hospital in Ningbo city of Zhejiang province, China. Among them, 114 BT patients (59 males and 55 females) were diagnosed by way of brain CT or MRI and 142 ICH patients (88 males and 54 females) were diagnosed by digital subtraction angiography. The 609 controls (330 males and 279 females) were randomly selected from participants in the health examination by Ningbo First Hospital. Control participants had no symptoms or history of diseases, including stroke, autoimmune diseases, hematological diseases, and severe liver or kidney disease. All the subjects were Han Chinese residents in Ningbo city of the Eastern China. The results of the diagnoses were independently judged by at least two separate neurosurgeons. The blood samples of all individuals were collected at the same time and treated by the same investigators. The study protocol was approved by the Ethical Committee of Ningbo First Hospital and the informed written consent was obtained from all subjects.

2.2. Biochemical analysis

About 5 ml blood samples were collected into 3.2% citrate sodium-treated tubes within 12 h after fasting, short stored in 4 °C, and long stored in −80 °C. The levels of TG, TC, HDL-C, and LDL-C were determined by standard enzymatic methods [18]. ApoA-I and ApoB concentrations were estimated through the transmission turbidimetric method [18].

2.3. Single-nucleotide polymorphism genotyping

Genomic DNA was extracted from peripheral blood samples using the nucleic acid extraction analyzer (Lab-Aid 820, Xiamen City, China). The concentration of DNA was determined by the NanoDrop 1000 spectrophotometer (Thermal Scientific, Wilmington, USA). Polymerase chain reaction (PCR) amplification was performed on the ABI Geneamp® PCR System Veriti 96-Well Sample Block Module (Applied Biosystems, Foster City, CA). The PCR conditions included an initial denaturation at 95 °C for 30 s, followed by 40 cycles of 95 °C for 30 s, 59 °C for 30 s, 72 °C for 30 s, and a final extension for 30 s at 72 °C. The sequences of the primers of *PPARD* rs2016520 are as follows: first primer 5′-GCGGGCCGCAGATGGACCTCTACAGGA-3′; second primer 5′-GCGGGCAGGGCGGCCGCAGATGGACCTCTACAGGG-3′; and one common primer 5′-CTGTCTTCTCCTCTGCCCAGGC-3′. The primer extension for genotyping was performed using the melting temperature shift on the Roche Light Cycler® 480 (Roche, Mannheim, Germany) according to the manufacturer's instructions [19].

2.4. Statistical analysis

Continuous variables were compared by Student's *t*-test and described by mean and standard deviation (SD). Nonparametric indicators were compared by the Kruskal–Wallis test. All the *P* values were adjusted for the age and gender in the comparison of lipid levels. The Hardy–Weinberg equilibrium (HWE) test for the genotype frequencies was analyzed by the Arlequin program (version 3.5, Bern, Switzerland) [20]. The allele frequencies and genotype distribution between case and control groups were performed by CLUMP22 [21]. The odds ratio (OR) and 95% confidence interval (CI) were calculated by the SPSS software (version 18). Power analysis was made by Power and Sample Size Calculation software (version 3.0.43; Nashville, TN). A *P* value <0.05 was considered to be significant.

3. Results

3.1. Characteristics of study participants

As shown in Table 1, the age of the control group was significantly higher than the case group (all $P < 0.0001$). Statistically significant differences were seen in the concentrations of TC and LDL-C among the controls, all patients, and BT and ICH (all $P < 0.0001$) cases. The HDL-C levels in the ICH cases were much higher than in the controls ($P = 0.004$) and BT cases ($P = 0.029$). A strong difference of ApoA-I concentration was revealed between BT cases and controls ($P = 0.023$).

3.2. Distribution of rs2016520 in neurosurgical patients and controls

The genotype and allele frequencies of rs2016520 in neurosurgical patients and controls are shown in Table 2. The distributions of genotypes in the control groups were all within HWE ($P > 0.05$). The rs2016520-G allele frequencies were 0.260 in all patients, 0.254 in BT cases, 0.264 in ICH cases, and 0.296 in healthy controls. These were similar to the reported Asian populations (0.317 in HapMap-CHB and 0.227 in HapMap-JPT). No significant differences were observed in the genotype and allele distribution between controls and each of the three cases ($P > 0.05$, Table 2). A further breakdown analysis by gender showed no significant association of *PPARD* rs2016520 with the risk of neurosurgical patients ($P > 0.05$, Table 2).

Table 1
Characteristics of subjects from cases and controls.[a]

Characteristics	Control (609)	BT (114)	ICH (142)	AP (256)	*P* (C/BT)	*P* (C/ICH)	*P* (BT/ICH)	*P* (C/AP)
Age	58.95 ± 10.19	49.82 ± 15.36	53.93 ± 16.70	52.11 ± 16.21	<**0.0001**	<**0.0001**	**0.037**	<**0.0001**
Man (*n*)	330	59	88	148	0.791	0.377	0.389	0.603
TG (mmol/L)	1.59 ± 1.01	1.66 ± 1.12	1.57 ± 1.32	1.61 ± 1.23	0.467	0.679	0.382	0.846
TC (mmol/L)	4.39 ± 1.01	4.98 ± 0.92	4.91 ± 1.21	4.94 ± 1.09	<**0.0001**	<**0.0001**	0.598	<**0.0001**
HDL-C (mmol/L)	1.16 ± 0.29	1.17 ± 0.30	1.23 ± 0.29	1.20 ± 0.29	0.566	**0.004**	**0.029**	0.06
LDL-C (mmol/L)	2.55 ± 0.86	2.95 ± 0.76	2.85 ± 0.82	2.89 ± 0.79	<**0.0001**	<**0.0001**	0.306	<**0.0001**
ApoA-I (mg/dL)	1.04 ± 0.23	0.99 ± 0.18	1.03 ± 0.22	1.01 ± 0.20	**0.023**	0.980	0.057	0.216
ApoB (mg/dL)	0.78 ± 0.26	0.79 ± 0.22	0.77 ± 0.23	0.78 ± 0.22	0.270	0.875	0.216	0.511

[a] *P* values were adjusted for the age and sex, and *P* values less than 0.05 are in bold. AP: all patients; BT: brain tumor; ICH: intracerebral hemorrhage.