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BDNF Val66Met polymorphism is associated with unstable angina

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ABSTRACT

Background: Brain-derived neurotrophic factor (BDNF) is involved in the pathophysiology of coronary artery disease (CAD). The human BDNF Val66Met polymorphism has been shown to be associated with altered susceptibility to neuropsychiatric disorders. However it is unknown whether this polymorphism plays a role in cardiovascular disease.
Methods: Genotyping of BDNF Val66Met polymorphism was carried out in 513 controls, 628 unstable angina pectoris (UAP) and 276 stable angina pectoris (SAP) patients. The plasma concentrations of BDNF and high-sensitivity C-reactive protein (hsCRP) were measured by ELISA. The general clinical data in patients and controls were obtained.
Results: There was a significant association between genotype and allele frequency of the BDNF Val66Met polymorphism and UAP (all $P<0.05$). Multivariate logistic regression analysis revealed that the $BDNF_{Met/Met}$ genotype had a protective effect on the occurrence of UAP after controlling for known risk factors of CAD (OR 0.53, $P=0.005$). Subjects with $BDNF_{Met/Met}$ genotype also had decreased plasma hsCRP levels compared with the Val carriers ($P<0.01$).
Conclusion: The $BDNF_{Met/Met}$ genotype has a protective effect on the occurrence of UAP, which might in part be due to the decreased plasma hsCRP level in $BDNF_{Met/Met}$ carriers. To our knowledge, this is the first study that demonstrates the link between BDNF Val66Met polymorphism and CAD.

1. Introduction

Coronary artery disease (CAD) is a complex disorder that is influenced by genetic and environmental factors. There are various hypotheses regarding the involvement of genetic factors in the development of CAD. However, these hypotheses cannot explain the overall genetic susceptibility and additional genes may be involved in the development of CAD.

Brain-derived neurotrophic Factor (BDNF) is a member of the neurotrophin family of growth factors, which promotes survival, differentiation, and maintenance of neurons in peripheral and central nervous system [1]. It has been shown that BDNF and its receptors are expressed in both nonneuronal tissues and various cell types such as developing heart [2], atherosclerotic vessels [3–5], macrophages [6,7], lymphocytes [8,9], endothelial cells [2] and vascular smooth muscle cells [3], suggesting that BDNF may also play a role in cardiovascular system. Previous reports demonstrated that BDNF plays a critical role in regulating both vascular development and response to injury [10]. BDNF deficiency results in a reduction in endothelial cell–cell contacts and endothelial cell apoptosis, leading to intraventricular wall hemorrhage, depressed cardiac contractility and early postnatal death [2]. BDNF is expressed in atherosclerotic coronary arteries, preferentially localized in the atheromatous intima and around the vasa vasorum in the adventitia. In contrast, BDNF is barely detected in nonatherosclerotic coronary arteries [4]. The localization of BDNF in the cardiovascular system and its potential biological activities suggest a possible role of BDNF in the pathogenesis of CAD.

A single nucleotide polymorphism (SNP) in the BDNF gene leading to G (Val) to A (Met) substitution at position 196 (codon 66) in the prodomain has been found to be associated with neuropsychiatric disorders including Alzheimer's disease, Parkinson's disease, depression, and bipolar disorder [11–14]. Humans carrying the Met allele have smaller hippocampal volumes and perform poorly on hippocampal-dependent memory tasks [15,16]. It has previously been shown that Met variant alters the intracellular trafficking and activity-dependent secretion of BDNF in neurosecretory cells and neurons [15,17]. Moreover, we have found that these trafficking abnormalities are likely to reflect

Abbreviations: BDNF, brain-derived neurotrophic factor; CAD, coronary artery disease; hsCRP, high-sensitivity C-reactive protein; SAP, stable angina pectoris; UAP, unstable angina pectoris.

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Table 1
Patient characteristics

Group variables	UAP (n=628)	P vs. SAP	SAP (n=276)	P vs. control	Control (n=513)	P vs. UAP
Age, y	62.5±11.4	NS	64.8±12.3	NS	62.1±9.5	NS
Gender M/F (%)	68.6/31.4	NS	66.7/33.3	NS	65.5/34.5	NS
BMI (kg/m^2)	25.8±3.8	NS	24.7±3.8	NS	24.2±3.6	NS
Systolic BP (mm Hg)	130±19	NS	130±16	<0.01	112±11	<0.01
Diastolic BP (mm Hg)	75±11	NS	76±13	<0.01	70±12	<0.01
Total cholesterol (mmol/l)	4.9±1.1	NS	4.9±1.2	<0.01	4.4±0.9	<0.01
LDL cholesterol (mmol/l)	3.1±0.9	NS	3.0±1.0	<0.01	2.1±0.7	<0.01
Triglycerides (mmol/l)	1.7±0.9	NS	1.6±1.3	<0.01	1.1±0.4	<0.01
Glycemia (mmol/l)	6.0±2.1	NS	5.9±2.0	<0.01	5.0±0.8	<0.01
Left ventricular ejection fraction (%)	60±13	NS	62±12	–	Not available	–
Diabetes mellitus (%)	24.0	NS	20.7	<0.01	0	<0.01
Hypertensives (%)	64.7	NS	61.9	<0.01	0	<0.01
Lipid abnormality (%)	57.3	NS	52.9	<0.01	0	<0.01
Smoking (%)	42.5	NS	42.2	<0.01	0	<0.01
Coronary Gesini score	55.7±42.5	NS	54.9±47.6	–	Not available	–
Numbers of diseased vessels	2.3±0.9	NS	2.0±1.0	–	Not available	–
Cortisol (ng/ml)	446.2±14.5	<0.05	379.3±11.6	NS	373.5±10.8	<0.05

impaired binding of BDNF to a sorting protein, sortilin, which interacts with BDNF in the prodomain region that encompasses the 196 substitution position [18]. Although the studies on associations between BDNF Val66Met polymorphism and neuropsychiatric disorders are vast, there is no report on the role of this polymorphism in CAD patients. In this study, we examined whether the BDNF Val66Met polymorphism is associated with CAD.

2. Materials and methods

2.1. Patients and controls

Nine hundred four patients with angina and 513 controls were recruited from the Qilu hospital, Shandong University. All patients had >50% stenotic lesions in at least one major coronary vessel determined by coronary angiography. The unstable angina pectoris (UAP) group consisted of 628 patients who had anginal episodes at rest within 48 h of the study without a significant increase in cardiac troponin levels (according to the American College of Cardiology/American Heart Association 2007 guidelines for the management of patients with unstable angina [19]). The stable angina pectoris (SAP) group consisted of 276 patients with typical symptoms of angina during exertion or positive treadmill exercise testing but no episodes of angina at rest. The control group consisted of 513 health subjects screened during routine physical check-up in the same period of time as the study patients. In the control subjects, it was unethical to perform coronary angiography to rule out the presence of asymptomatic CAD. Therefore, the following inclusion criteria were used: no abnormal Q wave or ST-T changes on electrocardiography; negative family history of CAD and stroke; nonsmoking status; absence of hypercholesterolemia, hypertriglyceridemia, diabetes mellitus, and hypertension. Based on available data from epidemiologic and family studies, a cohort fulfilling these criteria is expected to have a very low prevalence of asymptomatic CAD [20]. All individuals were Han Chinese in ethnic origin. No individual had acute infection or acute inflammation (individuals with plasma hs-CRP concentration> 10 mg/l were excluded from the study) and no individual had taken steroids, or nonsteroidal antiinflammatory drugs except for aspirin. In our study, we did not enroll patients with mental disorders, or patients taking antidepressant drugs or tranquilizers. Smoker was defined as consecutive smoking≤10 y and 10 pieces of everyday. Hypertension was defined as systolic pressure>140 mm Hg, and/or diastolic pressure>90 mm Hg, or use of any antihypertensive agents. Lipid abnormality was defined as total cholesterol>6 mmol/l, and/or triglycerides>1.7 mmol/l, and/or LDL cholesterol>3.1 mmol/l. Diabetes mellitus was defined as a previous diagnosis of the disease, history of antidiabetic medications, or plasma fasting levels of glucose>7.0 mmol/l on at least two occasions. The patients with diabetes in our study were all Type 2 Diabetes mellitus patients. The diabetic duration, the number of patients with diabetes and the number of patients with satisfied glucose control were 4.5±2.3 y, 150(24%), 75(50%) and 4.3±2.1 y, 57(20.7%), 29(50.9%) in UAP and SAP patients, respectively. Written informed consent was obtained from all individuals before enrollment in the study, and the study was approved by the Medical Research Ethics Committee of Shandong University, and was carried out in accordance with the Declaration of Helsinki (1996) of the World Health Organization.

2.2. Genotype analysis

Genomic DNA was isolated from whole blood according to standard procedures. All genotypings were performed by the fluorescence resonance energy transfer method (FRET) using the Light Cycler 2.0 (Roche Diagnostics, Mannheim, Germany). Forward primer: 5′-AAC ATC CGA GGA CAA GGT GG-3′; reverse primer: 5′-GGA CAT GTT TGC AGC ATC TAG GTA A-3′; donor hybridization probe: 5′-GCT CTT CTA TCA CGT GTT CGA AAG TG-FL-3′; acceptor hybridization probe: 5′-LC640-CAG CCA ATG ATG TCA AGC CTC TTG AAC CTG-PH-3′ (TIB MOLBIOL, Germany). After amplification a melting curve was generated by holding the reaction at 50 °C for 30 s and then heating slowly to 70 °C with a ramp rate of 0.1 °C/s. Peaks were obtained at 58 °C for the Met-allele and at 65 °C for the Val-allele.

2.3. Measurement of plasma BDNF, cortisol and hsCRP levels

Blood samples were obtained between 7:30 am and 8:00 am, within 24 h of hospitalization. The blood samples were drawn into tubes containing EDTA (pH 7.5) and immediately centrifuged at 3000 rpm for 10 min at 4 °C, and then the plasma samples were collected and stored at −80 °C. The plasma BDNF and hsCRP concentrations were measured by their respective ELISA kits (BDNF Emax® ImmunoAssay System, Promega, USA; Human hs-CRP ELISA kit, USCNLIFE, USA). For plasma cortisol concentration measurement, a commercial radioimmunoassay kit was employed (Cortisol-RIA, Beijing North Biotechnology Research Institute, China). The intra- and interassay CVs were <10%.

2.4. Statistical analysis

All the continuous variables are presented as mean±S.D. Hardy–Weinberg equilibrium expectations were tested using a χ^2 goodness-of-fit test. ANOVA test was used to compare quantitative variables among genotypes or different group subjects. Chi-squared analysis was

Table 2
Distribution of BDNF Val66Met polymorphism in UAP patients, SAP patients and controls

Subjects	BDNF Val66Met genotypes (%)			Allele frequencies	
	Met/Met	Val/Met	Val/Val	Met	Val
Control (n=513)	128 (25.0)	246 (48.0)	139 (27.1)	0.49	0.51
SAP (n=276)	68 (24.6)	135 (48.9)	73 (26.4)	0.49	0.51
UAP (n=628)	104 (16.6)*	343 (54.6)	181 (28.8)	0.44**	0.56

* χ^2=15.037, df=4, P=0.005 for the comparisons genotypes frequencies between UAP, SAP and controls; χ^2=12.506, df=2, P=0.002 for the comparisons genotypes frequencies between UAP patients and controls; χ^2=8.916, df=2, P=0.01 for the comparisons genotypes frequencies between UAP patients and SAP patients.
** χ^2=7.359, df=2, P=0.02 for the comparisons allele frequencies between UAP, SAP and controls; χ^2=5.814, df=1, P=0.016 for the comparisons allele frequencies between UAP patients and controls; χ^2=4.222, df=1, P=0.03 for the comparisons allele frequencies between UAP patients and SAP patients.