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Original Article

Association of rs5368 and rs3917406 polymorphisms in E-selectin gene with premature coronary artery disease in Chinese Han population

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Abstract: Objectives: Genetics polymorphism of the E-selectin affects the pathogenesis of atherosclerosis and associated with coronary artery disease (CAD). We aimed to investigate the association between the rs5368 and rs3917406 polymorphisms in E-selectin genes and premature CAD (PCAD) in Chinese Han population. Methods: PCAD 628 patients and 732 controls were included in the study. E-selectin of rs5368 and rs3917406 polymorphisms were analyzed by polymerase chain reaction (PCR). Results: The frequencies of T allele of the rs5368 and rs3917406 polymorphisms were 27.2% and 47.8%, respectively, in the PCAD group, and 30.5% and 42.8% in the control group. The frequency of the T allele of the rs3917406 polymorphism was significantly higher in the PCAD group than in the control group (x^2 = 6.857, P = 0.009). In contrast, no statistically significant difference was found between controls and patients in the frequency of T allele of the rs5368 polymorphism. The univariate analysis showed that the E-selectin rs3917406 polymorphisms was associated with the PCAD in additive model (OR = 1.226, 95% CI = 1.05-1.43, P = 0.010) and dominant model (OR = 1.406, 95% CI = 1.11-1.78, P = 0.005). After adjusting for potential confounding variables the rs3917406 polymorphisms was independently associated with PCAD in additive model (OR = 1.347, 95% CI = 1.12-1.62, P = 0.002) and dominant model (OR = 1.669, 95% CI = 1.26-2.21, P < 0.001). The E-selectin rs5368 polymorphisms were not associated with PCAD in univariate and multivariate analyses of three models. Conclusion: Among the Chinese Han population, the rs3917406 polymorphism of the E-selectin gene was associated with PCAD in univariate and multivariate analysis, however, no significant correlation between the E-selectin rs5368 polymorphism and PCAD.

Keywords: E-selectin, premature coronary artery disease, gene polymorphism

Introduction

Coronary artery disease (CAD) is one of the most important health problems globally causing the highest rate of mortality and morbidity in recent years. The pathogenesis of CAD is not entirely clear at present. It is believed that CAD is a multifactorial disease resulting from genetic predisposition and environmental influences and their interactions [1]. Genetic factors have been defined as more important risk contributors for the pathogenesis of the premature CAD (PCAD). Previous studies have showed that genetic factors are more likely to affect young rather than old people [2].

E-selectin, belongs to the selectin superfamily of adhesion molecule, is a cell-surface membrane glycoprotein expressed on endothelial cell after activation by cytokines such as interleukin-1, lipopolysaccharide, and tumor necrosis factor a [3, 4]. E-selectin play a prominent role in the process of the adhesion of circulating leukocytes to endothelial cells [5]. It mediates monocytes and lymphocytes activation and induces a series of inflammatory, leading to atherosclerosis and a variety of vascular disease [6].

Single nucleotide polymorphisms (SNPs) of the E-selectin gene are currently considered to be a high risk factor for atherosclerosis development. It has been previously reported that was identified associations between the A561C, G98T and C1839T variations of the E-selection gene with CAD, hypertension and ischemic

cerebrovascular diseases [7-11]. Recently, results from a Taiwan population reported that the E-selectin rs5368 and rs3917406 polymorphisms was associated with MMP9 and the soluble E-selectin (sE-selectin) levels [12]. However, no data are available on association of the two polymorphisms with CAD. Accordingly, the purpose of our study was to determine the association of the E-selectin rs5368 and rs3917406 polymorphisms with the PCAD in a Chinese Han population.

Materials and methods

Study population

Two groups of unrelated Chinese individuals were recruited for the present study from the Cardiology Department of Beijing Anzhen Hospital, between May 2011 and April 2014. The PCAD group comprised 628 candidates of Chinese Han population that males age were 45 years or less and females age were 55 years or less with coronary arteriography documented CAD. The inclusion criterion for CAD was the presence of coronary arteriography determined narrowing of coronary vessels by more 50% at least one of the three main coronary arteries, or the history of myocardial infarction. Exclusion criteria were evidence of significant concomitant diseases, such as myocardiopathy, thyroid disease, chronic inflammatory disease, and cancer. The control group comprised 732 candidates of Chinese Han population that males age were 45 years or less and females age were 55 years or less with no significant coronary stenosis by angiography. Exclusion criteria for this group included among others diseases such as myocardiopathy, thyroid disease, chronic inflammatory disease, and cancer. All candidates were characterized on the basis of medical interview in respect of concomitant risk factors of atherosclerosis such as hypertension, diabetes mellitus, hyperlipidemia, cigarette smoking, and over-weight. Hypertension was diagnosed on basis of previous medical records, or more two times clinical examination showed systolic blood pressure exceeding 140 mmHg and/or diastolic blood pressure greater than 90 mmHg. Diabetes mellitus were diagnosed as such according to the American Diabetes Association criteria [13], or the patients had a known history of diabetes mellitus. Hyperlipidemia was defined as triglyceride levels > 1.8 mmol/L and total cholesterol levels > 5.2 mmol/L by laboratory examination. Individuals who smoked at least 2 cigarettes daily for more than 1 year were classified as smokers. Body mass index (BMI) was calculated by dividing weight in kilogram by square of height in meters and it was defined as increased when one was higher than 25 kg/m^2.

This study was approved in accordance with the regulations laid down by the Beijing Anzhen Hospital Ethics Committee and informed written consent was obtained from all participants entering the study.

Biochemical investigation

Venous blood sample was drawn after an overnight fast. Serum total cholesterol (CH), LDL-cholesterol, HDL-cholesterol, triglyceride (TG), and blood glucose were measured by automated enzymatic methods at Olympus-1000 automated analyzer (Tokyo, Japan). All procedures were performed according to the manufacturer's instructions.

Genotype determination

Venous blood from all individuals in the study was collected into vials containing EDTA. The samples were stored frozen at -80°C until the extraction of DNA. Genomic DNA was extracted from peripheral blood leukocytes with QIAamp DNA Mini Kit (Quiagen, Germany). The polymorphism of E-selectin gene was analyzed by polymerase chain reaction-restriction fragment length polymorphism technique. Primers used include 5'-ACGTTGGATGTCCATTGTCCCTGAGATGTG-3' (forward) and 5'-ACGTTGGATGAAGTCCTCTTGTGCCTTCAG-3' (reverse) for rs5368, 5'-ACGTTGGATGTGGCCCAGACTTTTCTCATC-3' (forward) and 5'-ACGTTGGATGGGGTGGCAGTCACA-AAAGAG-3' (reverse) for rs3917406. The sequences of the primers and probes for each SNP are available on request. Genomic DNA (10 ng) and 0.5 uM primer mix was used for each reaction, and amplification was set to perform 15 min of initial denaturation at 94°C, followed by 11 cycles 94°C for 20 sec, 56°C for 30 sec, and 72°C for 1 min; and then 72°C for 3 min, and a final extension at 4°C for 5 min. Genotyping was done by matrix- assisted laser desorption/ionization time of fight mass spectrometry (MALDI-TOF MS) using the MassARRAY system (Sequenom, San Diego, CA, USA), and analyzed using MassARRAY Tyer software (versopm4.0; Sequenom). For quality control, repeated analyses were undertaken on 10% of

Table 1. Clinical characteristics of the study subjects

	PCAD patients (n = 628)	Controls (n = 732)	P
Age (years)	44.87 ± 6.18	46.23 ± 6.30	0.001
Gender (M/F)	406/222	300/432	< 0.001
BMI, kg/m^2	26.95 ± 3.45	26.55 ± 3.57	0.085
Smoking, n (%)	325 (51.75)	200 (27.32)	< 0.001
Hypertension, n (%)	345 (54.94)	374 (51.09)	0.157
Diabetes, n (%)	160 (25.48)	108 (14.75)	< 0.001
Total cholesterol (mmoL/l)	4.54 ± 1.03	4.43 ± 1.20	0.135
Triglycerides (mmoL/l)	2.15 ± 1.72	1.94 ± 1.74	0.066
HDL cholesterol (mmoL/l)	1.01 ± 0.27	1.09 ± 0.29	< 0.001
LDL cholesterol (mmoL/l)	2.73 ± 0.85	2.70 ± 0.93	0.515

Table 2. The distribution of the E-selectin genotypic and allelic in the PCAD group and the control group

Genotype	PCAD (n = 628)	Controls (n = 732)	*P* value of HWE testing
rs5368			0.086
CC	325 (51.8)	343 (46.9)	
CT	264 (42.0)	332 (45.3)	
TT	39 (6.2)	57 (7.8)	
C allele	914 (72.8)	1018 (69.5)	
T allele	342 (27.2)[a]	446 (30.5)	
rs3917406			0.381
CC	162 (25.8)	240 (32.8)	
CT	332 (52.9)	358 (48.9)	
TT	134 (21.3)	134 (18.3)	
C allele	656 (52.2)	838 (57.2)	
T allele	600 (47.8)[b]	626 (42.8)	

[a]x^2 = 3.438, P = 0.064, OR = 0.854, 95% CI = 0.732-1.009. [b]x^2 = 6.857, P = 0.009, OR = 1.224, 95% CI = 1.052-1.425.

randomly selected samples with high DNA quality.

Statistical analysis

The analysis of clinical characteristics was performed using SPSS 19.0 (SPSS, Chicago, USA) statistical analysis software. The continuous variables were expressed as means ± SDs and were tested using a two-sided t-test. The categorical variables were tested using the Pearson chi-square test. Further statistical analysis was conducted in Plink 1.07 software (http://pngu.mgh.harvard.edu/purcell/plink/). Hardy-Weinberg equilibrium (H-WE), genotype and allele frequencies was examined using the Pearson Chi-square test. An association analysis based on unconditional univariate logistic regression and multivariate logistic regression were carried out to determine the odds ratio (OR) and 95% confidence interval (95% CI) for two SNPs in three genetic models (dominant, recessive and additive). A two-tailed P < 0.05 was considered statistically significant.

Results

A total of 1360 subjects were included in this case-control study. The clinical characteristics of all the subjects are shown in **Table 1**. The PCAD group had a higher prevalence of male gender, smoking, and diabetes compared with the control group (P < 0.001). But, age was notably lower in the PCAD group (P = 0.001). Lipid profiles revealed that the PCAD patient group had a significantly lower mean level of HDL-cholesterol (P < 0.001). BMI, Hypertension, plasma total cholesterol, triglycerides, and LDL cholesterol levels were not significantly different between the control and PCAD groups.

The genotypic and allelic distributions of the polymorphisms in the studied genes for patients and controls are show in **Table 2**. SNP genotypes were tested for departures from HWE for controls and patients, and all polymorphisms were in H-WE. The same there genotypes of the E-selectin rs5368 and rs3917406 polymorphisms were observed and the alleles were C and T. The frequencies of T allele of the rs5368 and rs3917406 polymorphisms were 27.2% and 47.8%, respectively, in the PCAD group, and 30.5% and 42.8% in the control group. The frequency of the T allele of the rs3917406 polymorphism was significantly higher in the PCAD group than in the control group (x^2 = 6.857, P = 0.009). In contrast, no statistically significant difference was found between controls and patients in the frequency of T allele of the rs5368 polymorphism.

The association between the E-selectin rs5368 and rs3917406 polymorphisms and PCAD was analyzed the results using a logistic regression analysis by alternative models such as additive

Table 3. Association of E-selectin rs5368 and rs3917406 polymorphisms with PCAD in univariate and multivariate analyses

		Univariate			Multivariate		
		OR	95% CI	P	OR	95% CI	P
rs5368	Additive	0.818	0.63-1.05	0.121	0.802	0.63-1.01	0. 065
	Dominant	0.829	0.64-1.07	0.155	0.756	0.56-1.02	0.063
	Recessive	0.718	0.44-1.18	0.187	0.689	0.40-1.19	0.184
rs3917406	Additive	1.226	1.05-1.43	0.010	1.347	1.12-1.62	0.002
	Dominant	1.406	1.11-1.78	0.005	1.669	1.26-2.21	< 0.001
	Recessive	1.231	0.94-1.61	0.128	1.332	0.98-1.82	0.071

Multivariate: adjustment for potential confounding variables (age, gender, smoking, diabetes and HDL cholesterol).

model (CC vs. CT vs. TT), dominant model [(CC + CT) vs. TT)] and recessive model [CC vs. (CT + TT)] in **Table 3**. The univariate analysis showed that the E-selectin rs3917406 polymorphisms was found to be associated with the PCAD in additive model (OR = 1.226, 95% CI = 1.05-1.43, P = 0.0108) and dominant model (OR = 1.406, 95% CI = 1.11-1.78, P = 0.005). After adjusting for potential confounding variables (age, gender, smoking, diabetes and HDL cholesterol) the E-selectin rs3917406 polymorphisms was independently associated with PCAD in additive model (OR = 1.347, 95% CI = 1.12-1.62, P = 0.002) and dominant model (OR = 1.669, 95% CI = 1.26-2.21, P < 0.001). The E-selectin rs5368 polymorphisms were not found to be associated with PCAD in univariate and multivariate analyses of three models.

Discussion

In the present study, we found that the significant association between the E-selectin rs3917406 polymorphism and PCAD risk in Chinese Han population. We found that the T allele of rs3917406 accounted for 47.8% in PCAD group, which was significantly higher than in control group (42.8%, P < 0.05). Further analysis indicated the genotype of rs3917406 was significantly different between PCAD and control group by different models of the logistic regression analysis (additive model P = 0.010, dominant model P = 0.005). The odds ratio for the risk of PCAD associated with the polymorphism of rs3917406 was 1.226 (95% CI = 1.05-1.44) and 1.406 (95% CI = 1.11-1.78) in additive and dominant model. Although the frequencies of several risk factors for CAD, including male, smoking, and diabetes, were higher compared with controls, and age and HDL-cholesterol were notably lower in the PCAD group), the association still remained statistically significant after multiple comparisons adjusting (additive model P = 0.002, dominant model P < 0.001). It indicates that T allele of rs3917406 may be a risk factor for PCAD in Chinese Han population. In addition to classical risk factors genetic predisposition may thus play an important role in the pathogenesis of PCAD in Chinese Han population.

In 1994, Wenzel et al. [14] first reported an adenine to cytosine substitution at nucleotide position 561 resulting in an amino acid exchange from serine to arginine (position 128), and the Ser128Arg E-selectin gene polymorphism may be associated with a higher risk for early severe atherosclerosis. Li et al. [7] found significantly higher frequency of R allele of the Ser128Arg E-selectin gene polymorphism in patients (6.7%) with CAD versus controls (3.1%) in Central China (OR = 2.21; 95% CI = 1.20-4.07). It was reported the association between the C allele of A561C in E-selectin gene polymorphism and CAD risk in Asian and Caucasian populations [15-17]. Several polymorphisms in the E-selectin gene, such as G98T [10, 17], L554F [18], G2692A and C1091T [19], were successively reported to be associated with atherosclerosis and CAD.

Atherosclerosis is considered to be a chronic inflammation process [20]. One early phase of atherosclerosis involves the recruitment of inflammatory cells from the circulation and their trans-endothelial migration. E-selectin mediated this process, which are expressed on the vascular endothelium cell in response to several inflammatory stimuli [21]. It is believed that circulating levels of the sE-selectin do reflect endothelial expression of this cell adhesion molecules, and that increases of sE-selectin are indicative of the presence of a systemic inflammatory condition [22]. Elevated levels of sE-selectin have been found in patients of atherosclerosis and CAD [23], and some authors found such an association between the poly-

morphism of E-selectin gene and the plasma level of E-selectin in CAD patients [16]. Recently Wu S [12] showed that the E-selectin rs3917406 polymorphism was associated with sE-selectin levels (P = 0.031) after adjusting for age, gender, smoking status, BMI, MMP9 and sICAM1 levels, but rs5368 polymorphism was not associated with sE-selectin levels (P = 0.336) in the Taiwan population. At the same time, this study showed that the E-selectin rs5368 polymorphism was independently associated with MMP9 levels (P < 0.001) by multivariate analysis. The MMP9 play a critical role in migration of monocytes into intima during inflammatory reaction and plaque disruption in the late stage of atherosclerosis [24]. The plasma levels of MMP9 have been associated with CAD patients [25]. In our study, we found that there was no significant correlation between the E-selectin rs5368 polymorphism and PCAD in Chinese Han population.

In conclusion, in this study, we showed that there is an association between the rs3917406 polymorphism of the E-selectin gene and PCAD risk in Chinese Han population. By adjusting a few risk factors for mismatched PCAD with controls, including male, smoking, diabetes, age and HDL-cholesterol, the association was more significant between the rs3917406 polymorphism of the E-selectin gene and PCAD risk. But, there was no significant correlation between the E-selectin rs5368 polymorphism and PCAD in Chinese Han population. Further studies are required to confirm these findings.

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Disclosure of conflict of interest

None.

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