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Original Article

Correlation between single nucleotide polymorphism of thrombospondin-1 and acute myocardial infarction

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Abstract: Background: Thrombospondin (TSP) gene is a candidate gene for susceptibility to acute myocardial infarction (AMI). No consensus has been reached yet as to the correlation between the single nucleotide polymorphism (SNP) of TSP-1 and susceptibility to AMI. This study focused on the correlation between the N700S SNP in TSP-1 and AMI in the Chinese population. Material and methods: SNP phenotyping of TSP-1 N700S was performed for 436 AMI cases and 450 controls. Unconditional logistic regression was applied to analyze the correlation between the N700S SNP in TSP-1 and susceptibility to AMI. Results: Of all cases studied, we found no obvious correlation between the N700S SNP in TSP-1 and susceptibility to AMI (χ^2=2.767, *P*=0.251). After stratification by influence factors, the frequency of AG+GG genotypes was 1.776 times of that of AA genotype among cases aged above 55 years old (OR=1.776, 95% CI=1.034-3.050, *P*=0.037); the difference was of statistical significance between different age groups. Conclusion: For the Chinese population, the N700S SNP in TSP-1 was not obviously correlated with susceptibility to AMI. But for the older age group, the risk of AMI among cases carrying AG+GG genotypes was higher compared to cases carrying AA genotype. The G allele may be a risk factor of susceptibility to AMI.

Keywords: Thrombospondin gene, acute myocardial infarction, single nucleotide polymorphism

Introduction

Acute myocardial infarction (AMI) is a severe consequence of the progression of coronary atherosclerotic heart disease (CAD) and probably the most critical form of CAD [1]. China has witnessed a doubling of CAD-related deaths in the past 20 years [2]. In China, there were 290 million people suffering from cardiovascular diseases and about 2.5 million people suffering from AMI in 2012 [3]. CAD brings great burden, psychologically and economically, to both patients and their families.

Thrombospondins (TSPs) are a family of multifunctional glycoproteins [4], consisting of 5 members, which are TSP-1, TSP-2, TSP-3, TSP-4 and TSP-5 [5]. As the most important member of the TSP family, TSP-1 can be synthesized and secreted into the extracellular matrix by endothelial cells, fibroblasts and vascular smooth muscle cells, thus fulfilling various functions by binding to the receptors. TSP can promote platelet activation and aggregation [6]. Topol et al. showed that SNP of the TSP-1, 2 and -4 genes was associated with AMI by inducing vascular endothelial hyperplasia and prothrombotic state [7]. The N700S SNP in TSP-1 is the substitution of asparagine by serine at codon 700 in intron 13 (A8831G). Mutant homozygote is reported to correlate significantly with AMI. Boekholdt et al. [8] did not confirm this, while Zwicker et al. [9] observed a significant correlation between N700S SNP in TSP-1 and AMI. The existing studies on the correlation between N700S SNP in TSP-1 and AMI for the Chinese populations generally gave a negative answer [10, 11].

Based on the existing studies, we performed an analysis on the correlation between N700S SNP in TSP-1 and AMI among the Chinese population, so as to provide reference for the screening and treatment of CAD and AMI.

Materials and methods

Subjects

A total of 436 AMI cases and 450 controls were included. AMI cases were treated at XX hospital

Table 1. Comparison between groups in general physiological and biochemical indexes

Characteristics	AMI Cases (n=436)	Controls (n=450)	χ^2/t	*P*-Value
Age (years)	58.450±10.590	58.930±10.089	0.680	0.497
Sex			0.938	0.333
Male	296 (67.9)	319 (70.9)		
Female	140 (32.1)	131 (29.1)		
Smoke			1.786	0.181
Ever	273 (62.6)	261 (58.0)		
Never	163 (37.4)	188 (42.0)		
BMI (kg/m^2)	26.769±3.572	24.596±3.432	9.236	<0.001
TG (mmol/L)	1.785±0.873	1.581±0.838	3.545	<0.001
TC (mmol/L)	4.668±0.860	4.375±0.759	5.390	<0.001
LDL-C (mmol/L)	3.031±0.809	2.677±0.724	6.864	<0.001
HDL-C (mmol/L)	1.156±0.260	1.270±0.258	6.552	<0.001
SBP (mmHg)	132.014±18.578	126.410±16.931	4.696	<0.001
DBP (mmHg)	77.448±10.795	79.662±10.621	3.078	0.002
Hypertension			12.311	<0.001
Yes	160 (36.7)	116 (25.8)		
No	276 (63.3)	334 (74.2)		
History of CAD			92.785	<0.001
Yes	98 (22.5)	7 (1.6)		
No	338 (77.5)	443 (98.4)		

Note: BMI (Body Mass Index); TG (triglyceride); TC (Total Cholesterol); LDL-C (Low density Lipoprotein Cholesterol); HDL-C (High Density Lipoprotein Cholesterol); SBP (Systolic Blood Pressure); DBP (Diastolic Blood Pressure); CAD (coronary atherosclerotic heart disease).

from January 2015 to March 2016, and the diagnosis was made according to the WHO's diagnostic criteria for AMI. The age-matched control cases were those receiving physical checkup in the same period, for which CAD was excluded by ECT, coronary computed tomography angiography (CCTA) or coronary arteriography (CAG). All AMI cases had no blood relationships with the controls, and cases with congenital heart diseases, myocardiopathy, and severe liver and kidney diseases. Clinical information was collected from all cases, including age, gender, height, weight, body mass index (BMI), smoking history, alcohol consumption, blood pressures, triglyceride (TG) and cholesterol. The experiment was approved by the hospital ethics committee. Informed consent was obtained from all cases.

Genomic DNA extraction

From each case 2 ml of fasting venous blood was drawn in the morning and placed into a tube containing EDTA K2 anti-coagulant. Genomic DNA extraction was conducted using blood genomic DNA extraction kit (Promega, USA) and the extracted genomic DNA samples were preserved at -80°C.

Genotyping

SNP genotyping was performed using the SNaPshot® Multiplex Kit. Primers were designed and synthesized by Shanghai Invitrogen Corporation. The upstream primer was 5'-GCATGGTGTACCCTCAGGTG-3 and the downstream primer was 5'-TGTTTTGATAAGGTGATGGGC, using a 15 μion. The upstream primer was 5'-GCATGGTGTACCCTCAGGTG-3 and the downstr °C for 3 min, denaturation at 94°C for 15 s, annealing at 60°C for 30 s, extension at 72°C for 30 s, a total of 11 annealing cycles, with a temperature decrease by 0.5°C for each cycle; denaturation at 94°C for 15 s, annealing at 54°C for 15 s, extension at 72°C for 30 s, 24 cycles; final extension at 72°C for 4 min. Excess dNTP was removed with Exol (Fermentas) and FastAP (Fermentas), and the purified PCR products were subjected to extension using a 6 μl system, by denaturation at 96°C for 1 min, denaturation at 96°C for 15 s, annealing at 54°C for 10 s, extension at 60°C for 30 s, 30 cycles. PCR reaction was maintained at 4°C. Finally, the extension products were sequenced on the ABI 3730XL analyzer.

Quality control

The raw data were input by two staff members independently. All procedures were conducted in strict accordance with the protocol. After the first genotyping, 5% of the DNA samples were randomly selected from the two groups, respectively, for second genotyping. The results of the two genotyping operations were consistent.

Statistical analysis

IBM SPSS 19.0 was used for statistical analysis. Measurements were presented as mean ±

Table 2. Relationship between N700S -1 and acute myocardial infarction in patients with acute myocardial infarction

N700S	Cases	Controls	OR (95% CI)	*P*	OR (95% CI)[a]	*P*
Genotype						
AA	376 (86.2)	398 (88.4)				
AG	52 (11.9)	49 (10.9)	1.123 (0.742-1.701)	0.583	1.124 (0.739-1.712)	0.584
GG	8 (1.8)	3 (0.7)	2.823 (0.743-10.719)	0.127	3.130 (0.812-12.068)	0.098
AA vs. AG+GG	376/60	398/52	1.221 (0.821-1.817)	0.324	1.233 (0.824-1.848)	0.308
AA+AG vs. GG	428/8	447/3	2.785 (0.734-10.567)	0.132	3.082 (0.800-11.871)	0.102
Allele						
A	804 (92.2)	845 (93.9)				
G	68 (7.8)	55 (6.1)	0.770 (0.532-1.112)	0.162		

Note: [a]adjusted by gender, age, smoking.

Table 3. Stratified analysis of vs. AG+GG AA genotype and susceptibility to AMI disease

Stratification factors		AMI Cases	Controls	Adjusted OR (95% CI)	*P*-Value
Age (years)	≤55	192 (44.0)	185 (41.1)	0.752 (0.406-1.394)	0.366
	>55	244 (56.0)	265 (58.9)	1.776 (1.034-3.050)	0.037*
Sex	Male	296 (67.9)	319 (70.9)	1.174 (0.753-1.831)	0.478
	Female	140 (32.1)	131 (29.1)	1.517 (0.569-4.045)	0.398
Smoke	Ever	273 (62.6)	261 (58.0)	1.264 (0.791-2.020)	0.328
	Never	163 (37.4)	188 (42.0)	1.137 (0.513-2.523)	0.752
BMI (kg/m^2)	<25	133 (30.5)	242 (53.8)	1.498 (0.790-2.839)	0.216
	≥25	303 (69.5)	208 (46.2)	1.161 (0.669-2.015)	0.596
Hypertension	Yes	160 (36.7)	116 (25.8)	0.970 (0.551-1.707)	0.915
	No	276 (63.3)	334 (74.2)	1.079 (0.565-2.060)	0.817

Note: *P<0.05. There was a statistically significant difference in the incidence of AMI between AG+GG and AA genotype patients aged above 55.

standard deviation, and the counts were presented as frequencies (percentages). Continuous quantitative variables were compared between the groups by using a t-test. Categorical data were compared between the groups by using a chi-square test. Hardy-Weinberg equilibrium was tested using a chi-square test. Correlation between genotypes and AMI was analyzed by bivariate logistic regression, with the significance level α=0.05.

Results

General physiological and biochemical indicators in the two groups

The two groups showed no significant differences in age, gender and smoking status. However, there were significant differences in BMI, TG, TC, LDL-C, HDL-C, systolic pressure, diastolic pressure, and familial history of hypertension and CAD (P<0.05). BMI, TG, TC, LDL-C, systolic pressure, incidence of hypertension and CAD were significantly higher in the case group, while HDL-C and diastolic pressure were significantly lower in the case group, as compared with the control group (**Table 1**).

SNP analysis and testing of Hardy-Weinberg equilibrium

Genotype distribution of N700S SNP in TSP-1 obeyed Hardy-Weinberg equilibrium in the control group (χ^2=1.175, P=0.278), indicating the absence of considerable selection and migration in the sample population. The chi-square test showed no significant differences in genotype distribution of N700S SNP in TSP-1 between the two groups (χ^2=2.767, P=0.251). According to unconditional logistic regression, N700S SNP in TSP-1 was not significantly correlated with AMI (P>0.05) (**Table 2**).

Stratified analysis

Stratified analysis was conducted based on the factors of age, gender, smoking status, BMI and hypertension. Logistic regression analysis indicated that after stratification based on the above factors, AA genotype or AG+GG genotypes did not correlate with the susceptibility to AMI. However, age-stratified correlation analysis indicated that the AG+GG genotypes predicted a higher risk of AMI for those aged above

55 (P<0.05). Therefore, the G allele may be a risk factor of AMI (**Table 3**).

Discussion

AMI is caused by the combined action of multiple environmental and genetic factors [12]. TSP-1 is believed to be the susceptibility gene of AMI, but the correlation between N700S SNP in TSP-1 and AMI has been rarely investigated [13]. According to our analysis, there was no obvious correlation between the two for the Chinese population. But after stratification by age factor, it was found that the risk of AMI for AG+GG genotypes was 1.776 times of that for the AA genotype among cases aged above 55 (OR=1.776, 95% CI=1.034-3.050, P=0.037). Hence age may be an influence factor in the susceptibility to AMI and G allele is a risk factor of AMI.

Comparison of general physiological and biochemical indicators between the two groups suggested that AMI was usually accompanied by hypertension, hyperlipidemia and diabetes, which are the risk factors for AMI, as has been confirmed by a large number of studies so far [14]. Smoking is another risk factor for AMI, but we did not observe significant differences between smokers and non-smokers. This is probably due to the small sample size and the lack of further discrimination of the smoking status instead of simply dividing the cases into smokers and non-smokers. We also found significant differences in the prevalence of familial history of CAD between the two groups, indicating the importance of genetic variation in the susceptibility to AMI.

The identified susceptibility genes of AMI include genes related to the renin-angiotensin system, lipid metabolism, cytokines & adhesion molecules, growth factors & coagulation system, and fibrinolytic system [15]. TSPs are components of the coagulation system and TSP polymorphism may lead to AMI by inducing vascular endothelial hyperplasia and prothrombotic state. This study compared genotype frequencies of N700S SNP in TSP-1 between the two groups and did not find significant difference for the Chinese population. This agreed with the conclusions made by Lei et al. [10] and Zhou et al. [11]. However, correlations were confirmed for the USA and Italian populations [9, 16], which suggested the role of geographic and ethnic factors. Given the complex pathogenesis and regulatory network of CAD, disagreement across the studies may arise from the impact of sample selection and confounding factors. The significance of gene-environment interaction and gene-gene interaction is also noteworthy [17] in the discussion on susceptibility to diseases.

AMI involves the action of multiple factors and genes. Our study provides reference for the screening and treatment of CAD and AMI. Considering the multiplicity of susceptibility genes of AMI, small-sample-size single-factor experiments usually have low repeatability and intrinsic limitations, as variations in geographic and environmental factors and living habits can all lead to difference in the susceptibility to AMI. To confirm our findings, multi-center large-sample-size studies involving cases from different regions need to be conducted.

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Disclosure of conflict of interest

None.

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