

A lack of association between the *CRP* rs2794520 polymorphism and coronary artery disease

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Abstract. Coronary artery disease (CAD) is mainly caused by atherosclerosis, which is closely associated with the C-reactive protein (*CRP*), a systemic inflammatory mediator. The aim of the present study was to examine whether the *CRP* rs2794520 polymorphism played a role in the risk of CAD. A total of 459 CAD patients and 432 non-CAD controls were recruited in the case-control study. Genotyping was performed on the SEQUENOM® Mass-ARRAY iPLEX® platform according to the manufacturer's instructions. The results showed that *CRP* rs2794520 was not associated with CAD. A further breakdown analysis by age or gender also indicated a lack of association between rs2794520 and CAD. In addition, the *CRP* rs2794520 polymorphism was not associated with the severity of CAD, which was represented by the number of coronary arteries with stenosis. In conclusion, there was no contribution of the *CRP* rs2794520 polymorphism to the risk of CAD.

Introduction

Coronary artery disease (CAD) is characterized by the narrowed or blocked coronary arteries that can cause a lack of myocardial oxygen supply. CAD is the main cause of human fatalities worldwide (1). CAD is a complex disorder contributed by environmental and genetic factors (2). C-reactive protein (*CRP*) is a widely used inflammatory factor (3) in the detection

of systemic inflammation diseases, such as atherosclerosis (4), which is an inflammation process closely linked to CAD (5,6). The circulating *CRP* level was found to be significantly associated with the risk of CAD (7-10). Aggregated C reactive protein was found to bind to low-density lipoproteins and very low-density lipoprotein (11) in the atherosclerotic plaques (12).

Recent genetic and genome-wide association studies have identified a number of genetic loci that are associated with *CRP* levels (9,13-16). Approximately 40% of *CRP* level variation was determined by genetic factors (10), including the *CRP* rs2794520 polymorphism, which was one of most significant markers in the meta-analysis of the genome-wide association studies among >80,000 European subjects (9).

In association with the previous studies, we hypothesized that the *CRP* rs2794520 polymorphism may influence the risk of CAD. Thus, a case control study was performed to assess the contribution of the *CRP* rs2794520 polymorphism to CAD in the Han Chinese population.

Materials and methods

Study population. A total of 891 unrelated individuals were carefully selected, which included 459 CAD patients (males, 69.9%; age, 59.7±9.48 years; diabetes mellitus, 22.1%; hypertension, 60.3%; body mass, 23.4±3.4 kg/m²; and smokers, 44.5%) and 432 non-CAD patients (males, 54.4%; age, 59.7±9.48 years; diabetes mellitus, 11.5%; hypertension, 49.3%; body mass, 23.3±3.4 kg/m²; and smokers, 31.7%). All were Han Chinese residents of Ningbo city in Eastern China. The standards required were that the involved CAD patients should have one or more major coronary arteries with a diameter stenosis ≥50%, or had a history of a coronary artery bypass surgery or prior angioplasty. The non-CAD patients were diagnosed with a diameter stenosis <50% of the major coronary arteries and without any atherosclerotic vascular disease. The diagnosis was made by at least two cardiologists (JZ and JL). All the samples were recruited between May 2008 and April 2014 from Ningbo Lihuili Hospital (Ningbo, China). Blood samples were collected in 3.2% citrate sodium-treated tubes and subsequently stored at -80°C. The protocol was approved by the Ethical Committee of Ningbo Lihuili Hospital and all the involved individuals provided signed informed consent.

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Table I. Comparison of the genotype and allele frequencies between the cases and controls by gender.

Subjects	rs2794520 group (n)	Genotype, counts TT/TC/CC	P-value χ^2 (df=2)		HWE	Allele, counts		P-value χ^2 (df=1)		OR (95%CI)	Power
						T	C				
All	CAD case (459)	146/230/83			0.70	522	396				
	Non-CAD controls (432)	153/217/62	2.77	0.25	0.31	523	341	2.47	0.12	1.16 (0.96-1.41)	0.474
Male	CAD case (321)	100/159/62			1.00	359	283				
	Non-CAD controls (235)	81/124/30	4.25	0.12	0.13	286	184	2.71	0.10	1.23 (0.96-1.56)	0.314
Female	CAD case (138)	46/71/21			0.60	163	113				
	Non-CAD controls (197)	72/93/32	0.59	0.74	0.88	237	157	0.08	0.78	1.05 (0.76-1.43)	0.209

df, degrees of freedom; HWE, Hardy-Weinberg equilibrium; OR, odds ratio; CI, confidence interval; CAD, coronary artery disease.

Table II. Comparison of the genotype and allele frequencies between the cases and controls by age.

Age, years	rs2794520 group, n	Genotype, counts TT/TC/CC	P-value χ^2 (df=2)		HWE	Allele, counts T/C	P-value χ^2 (df=1)		OR (95%CI)	Power
55≤	CAD case (115)	40/61/14			0.24	141/89				
	Non-CAD controls (159)	57/81/21	0.13	0.94	0.41	195/123	0.000015	1.00	1.00 (0.71-1.42)	0.179
55-65	CAD case (162)	49/83/30			0.75	181/143				
	Non-CAD controls (166)	59/86/21	2.52	0.28	0.25	204/128	2.11	0.15	1.26 (0.92-1.72)	0.209
≥65	CAD case (182)	57/86/39			0.55	200/164				
	Non-CAD controls (107)	37/50/20	0.47	0.79	0.69	124/90	0.49	0.48	1.13 (0.80-1.59)	0.182

df, degrees of freedom; HWE, Hardy-Weinberg equilibrium; OR, odds ratio; CI, confidence interval; CAD, coronary artery disease.

Single-nucleotide polymorphism (SNP) genotyping. The genomic DNA was isolated from peripheral blood lymphocytes using a conventional phenol/chloroform method, and subsequently DNA was quantified using the PicoGreen® double strand (dsDNA) DNA Quantification kit (Molecular Probes, Inc., Eugene, OR, USA). Amplification of the genomic DNA was performed on the ABI Gene Amp® PCR System 9700 Dual 384-Well Sample Block Module (Applied Biosystems, Foster City, CA, USA) for the quantitative polymerase chain reaction (qPCR) analysis. The surrounding DNA sequence of the tested polymorphism was downloaded from the NCBI dbSNP. Online program (<http://www6.appliedbiosystems.com/support/techtools/calc/>) was used to design the primers. The sequences of the primers are as follows: Forward, 5'-GCGGGCAGGGCGGCCTGTGTGTATGAAGGGCATAGGAC-3'; and 5'-GATTACCGCTGTGTGTATGAAGGCATAGGAT-3'; and reverse, 5'-CAGGCCTCATTCACTGTGAC-3'. PCR amplification procedures consisted of an initial denaturation at 95°C for 30 sec to activate the enzyme activity, a 40-cycle denaturation (95°C for 30 sec, annealing stage at 59°C for 30 sec, and an extension at 72°C for another 30 sec), and a final extension for 5 min at 72°C. The amplified DNA was held at 4°C. DNA amplification for genotyping was performed on the SEQUENOM® Mass-ARRAY iPLEX® platform according to the manufacturer's instructions (17).

Data analysis. Arlequin program (version 3.5) was used to estimate the Hardy-Weinberg equilibrium (HWE) (18). Clump 16 software with 10,000 Monte Carlo simulations was used to compare the frequency of the genotype and allele between cases and controls (19). Odds ratio (OR) with 95% confidential interval (CI) was calculated using an online program (<http://faculty.vassar.edu/lowry/odds2x2.html>). The power of the study was assessed by Power and Sample Size Calculation software (v3.0.43) (20). χ^2 analysis was used to compare the severity of CAD and the rs2794520 polymorphism (21). A two-sided P-value <0.05 was considered to indicate a statistically significant difference.

Results

Association between the SNP and CAD. In the present study, no significant association was found between rs2794520 and CAD (P=0.12; OR, 1.16; 95% CI, 0.96-1.41; Table I). As gender and age are two important factors in the risk of CAD, a subgroup analyses was further performed by age or gender; however, no positive results were revealed in all the tests (P>0.05; Tables I and II). Additional subgroup analysis by age and gender showed a negative result in rs2794520 with CAD (P>0.05; Table III). Under the dominant model, a significant result occurred between male CAD patients and controls

Table III. Comparison of the genotype and allele frequencies between the cases and controls by age and gender.

Age, years	Gender	rs2794520 group (n)	Genotype, counts TT/TC/CC	χ^2	P-value (df=2)	HWE	Allele, counts T/C	χ^2	P-value (df=1)	OR (95%CI)	Power
≤55	Male	CAD case (85)	30/44/11	0.75	0.69	0.50	104/66	0.15	0.70	1.09 (0.71-1.66)	0.136
		Non-CAD controls (99)	35/55/9				125/73				
	Female	CAD case (30)	10/17/3	2.01	0.37	0.44	37/23	0.18	0.67	0.87 (0.46-1.64)	0.089
		Non-CAD controls (60)	22/26/12				70/50				
55-65	Male	CAD case (118)	35/62/21	2.23	0.33	0.58	132/104	1.51	0.22	1.30 (0.86-1.96)	0.137
		Non-CAD controls (78)	27/43/8				97/59				
	Female	CAD case (44)	14/21/9	0.75	0.69	1.00	49/39	0.63	0.43	1.23 (0.74-2.07)	0.107
		Non-CAD controls (88)	32/43/13				107/69				
≥65	Male	CAD case (118)	35/53/30	0.27	0.88	0.27	123/113	0.29	0.59	1.13 (0.72-1.77)	0.125
		Non-CAD controls (58)	19/26/13				64/52				
	Female	CAD case (64)	22/33/9	0.08	0.96	1.00	77/51	0.03	0.87	1.05 (0.61-1.79)	0.101
		Non-CAD controls (49)	18/24/7				60/38				

df, degrees of freedom; HWE, Hardy-Weinberg equilibrium; OR, odds ratio; CI, confidence interval; CAD, coronary artery disease.

Table IV. Comparison of the genotype distribution between the cases and controls in the recessive and dominant models.

Subjects	rs2794520 group (n)	Recessive			P-value			Dominant			P-value		
		TT+CT	CC	CC	χ^2	(df=1)	OR (95%CI)	Power	TT	CC+CT	χ^2	(df=1)	OR (95%CI)
All	CAD case (459)	376	83						146	313			
	Non-CAD controls (432)	370	62		2.27	0.13	1.32 (0.92-1.89)	0.284	153	279	1.30	0.25	1.18 (0.89-1.55)
Male	CAD case (321)	259	62						100	221			
	Non-CAD controls (235)	205	30		4.21	0.04	1.64 (1.02-2.63)	0.175	81	154	0.68	0.41	1.16 (0.81-1.66)
Female	CAD case (138)	117	21						46	92			
	Non-CAD controls (197)	165	32		0.06	0.80	0.93 (0.51-1.69)	0.146	72	125	0.37	0.54	1.15 (0.73-1.82)

^a df, degrees of freedom; HWE, Hardy-Weinberg equilibrium; OR, odds ratio; CI, confidence interval; CAD, coronary artery disease.

Table V. Comparison of the genotype and allele frequencies between rs2794520 and the severity of CAD patients.

Group	rs2794520 case, n	Genotype, counts TT/TC/CC	χ^2	P-value (df=4)	Allele, counts T/C	χ^2	P-value (df=2)
Single-vessel disease	240	76/123/41			275/205		
Double-vessel disease	110	36/57/17			129/91		
Triple-vessel disease	109	34/50/25	2.53	0.64	118/100	0.98	0.61

df, degrees of freedom; CAD, coronary artery disease.

($P=0.04$; OR, 1.64; 95% CI, 1.02-2.63; Table IV), although the significance was not retained following the correction by the number of statistical tests. No significant association was found for the other genotype analyses (Table IV). The power of each analysis in the study was also calculated (Tables I-IV). These results showed that a moderate power existed in the current association test, indicating that the negative association in the study may be due to a lack of power (power=0.474; Table I). Future study or meta-analysis are required to establish the link of this polymorphism to CAD.

In addition, the association between the severity of CAD and the rs2794520 polymorphism was also examined. All the cases were divided into three groups based on the number of major coronary artery with stenosis $\geq 50\%$. The correlation test revealed no significant association between the severity of CAD and rs2794520 ($P=0.61$; Table IV).

Discussion

CAD has become the leading cause of fatalities in developed and developing countries. The plasma cardiac troponin I (cTnI) level was regarded as a gold standard for the diagnosis of acute myocardial infarction (AMI) worldwide (22). However, the rise of the plasma cTnI level comes after 4-6 h when the clinical events occurred, and this may delay the diagnosis (23). CRP levels had higher sensitivity to cardiovascular events compared to cTnI (24). Increased concentrations of plasma CRP have been found in patients with unstable angina (25). The elevation of CRP was found at the time of hospital admission in patients with myocardial infarction (MI) and history of unstable angina (24). The level of CRP was able to predict cardiovascular events in the healthy population and CAD patients (26,27). A study with 911 typical exertional angina patients indicated that increased CRP levels had a positive correlation with the CAD risk (28). Additionally, a number of studies identified *CRP* polymorphisms as reliable biomarkers of CAD (9). A case-control study indicated that there is a significant association between the *CRP* 1059 G/C polymorphism (rs1800947) and AMI in Italian population (29). In the present study, a case-control study was performed that involved 459 CAD patients and 432 non-CAD patients to assess the association between *CRP* rs2794520 and CAD risks.

There was no association between rs2794520 and CAD ($P=0.12$). As gender and age are two well-known factors that contribute to the CAD risk (30), subgroup analyses were performed by stratifying the samples into gender and age

groups. However, there was no association between the age and gender groups ($P>0.05$). Additionally, the subgroup analyses were performed by different genetic models that comprised of dominant and recessive models. A close significant result was found in the dominant model between male CAD patients and controls ($P=0.04$); however, possible multiple testing may exist in the analysis, and therefore, this result may not be considered significant. Other significant associations based on the genetic models in all the stratifications did not occur ($P>0.05$).

There were certain limitations in the present study. Firstly, the samples of the study were relatively small, and the power was 0.474 in the association between rs2794520 and CAD, which may influence the results of the study. Therefore, studies with larger-scale samples and stronger power are required in future research to confirm the current findings. Secondly, CAD is a complex disease that environmental and genetic status may alter the results of the study, although the cases and controls in the present study were carefully selected with the help of multiple professional doctors, any hidden factors that may influence the results of the current study could not be excluded. More carefully and precisely designed studies are required to strengthen the results of the study. Thirdly, there are 2,587 polymorphisms in the *CRP* based on the information in the dbSNP in PubMed. The present study only focused on one polymorphism that could not represent the whole contribution of *CRP* to CAD. Other *CRP* polymorphisms, such as *CRP* 1059 G/C (rs1800947), were previously found to be associated with the risk of CAD (29). Examining more *CRP* polymorphisms is required to assess their contribution to CAD.

In conclusion, the present study showed that the *CRP* rs2794520 polymorphism had no association with the risk of CAD in the Han Chinese population. Studies with different populations and stronger power are required to further confirm the findings of the study.

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