Association of NF-κB1 gene polymorphisms with coronary artery disease in a Han Chinese population

X.L. Guo, X.C. Liu, G.B. Su, C.Y. Zhou and Q.T. Cui

Department of Cardiothoracic Surgery,
First Affiliated Hospital of Xinxiang Medical University, Xinxiang, Henan, China

Corresponding author: Q.T. Cui
E-mail: cuiqingtao1978@163.com

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ABSTRACT. Nuclear factor (NF)-κB is a transcription factor that controls cell proliferation, differentiation, and immunity. Activated NF-κB1 is associated with the pathogenesis of coronary artery disease (CAD) and genetic polymorphisms in NF-κB1 have a plausible role in modulating the risk of CAD. To identify markers that contribute to the genetic susceptibility to CAD, we examined the potential association between CAD and single nucleotide polymorphisms (SNPs; rs28362491, rs230531, rs230528, rs1005819, rs4648055, rs3774964, and rs3774968) in the NF-κB1 gene using SNaPshot SNP genotyping assay. Participants included 361 patients with CAD and 385 healthy controls. The genotype and allele frequencies of the rs28362491 (promoter region) polymorphism in the CAD patients were significantly different from those in the healthy controls. The frequency of the D allele was significantly higher in CAD patients than

in the healthy controls (P = 0.005 after Bonferroni correction). Strong linkage disequilibrium was observed in one block (D' > 0.9). Haplotype analysis revealed that haplotypes in block 1 of the NF-κB1 gene did not display a risk or protective effect (P > 0.05). These data suggest that NF-κB1 gene polymorphisms confer susceptibility to CAD and also support the notion that dysfunction of NF-κB1 is involved in the pathophysiological process of CAD.

Key words: Coronary artery disease; Single nucleotide polymorphisms; Nuclear factor-κB

INTRODUCTION

Coronary artery disease (CAD) is the most common type of heart disease. Health data compiled from more than 190 countries shows that heart disease remains the number one global cause of death with 17.3 million deaths each year. CAD is a complex and multisystem disease and has multifactorial etiologies. Multiple studies have provided evidence that genetic factors, including polymorphisms in several candidate genes, may interact with environmental modulators to promote CAD and increase risk of cardiovascular disease (Salazar et al., 2000; Nordlie et al., 2005).

NF-kappa-B (NF-κB) is a pleiotropic transcription factor present in almost all cell types and the endpoint of a series of signal transduction events that are initiated by a vast array of stimuli related to many biological processes such as inflammation, immunity, differentiation, cell growth, tumorigenesis, and apoptosis (Monaco and Paleolog, 2004). NF-κB is a homo- or hetero-dimeric complex formed by the Rel-like domain-containing proteins RELA/p65, RELB, NF-κB1/p105, NF-κB1/p50, REL and NF-κB2/p52. The most common dimer is the p65/p50 heterodimer (Stirrat, 1990). Atherosclerosis is a chronic inflammatory disease of the arterial wall (Libby, 2012). NF-κB-mediated vascular inflammation plays a critical role in the initiation and progression of atherosclerosis. Therefore, activation of NF-κB may be considered an important contributor to the development of CAD (Yang et al., 2014). The NF-κB1 promoter variant was associated with a higher risk of CAD in three independent prospective studies of generally healthy Caucasians (Vogel et al., 2011), indicating a potential linkage of NF-κB1 gene polymorphism and risk of CAD in the general population.

In this study, we investigated 7 loci (rs28362491, rs230531, rs230528, rs1005819, rs4648055, rs3774964, and rs3774968) in a Chinese population from He'nan province (China) to verify the putative association between NF-κB1 polymorphisms and CAD.

MATERIAL AND METHODS

Subjects

A total of 361 unrelated patients with CAD (mean age = 62.1 ± 6.8 years) were recruited from the First Affiliated Hospital of Xinxiang Medical University. The patients received the following lab examinations: coronary angiography, electrocardiogram (EKG), blood test, and/or stress test. The interview was also performed on the patients. Inclusion criteria were: 1) patients had at least one diseased vessel (≥25% stenosis) in coronary angiograph; 2) patients

with stable angina pectoris (SAP) had a long-term and stable effort angina that had lasted for at least three months and a positive stress test; 3) patients with unstable angina pectoris had either angina with a progressive crescendo pattern or angina that occurred at rest without a recent myocardial infarction; 4) patients presented with transient ST-T segment depression and T-wave inversion without significantly elevated levels of cardiac enzymes; and 5) patients with acute myocardial infarction presented with typical angina associated with ST-segment elevations in EKG, and the frequency of occurrence of elevated levels of creatine kinase and troponin-I in serum were more than 3. Exclusion criteria were non-cardiac diseases including acute or chronic infections, malignancies, autoimmune diseases, hyperthyroidism, or medication with immunosuppressive agents. The control group consisted of 385 unrelated healthy subjects (mean age = 61.8 ± 6.2 years) who underwent health examinations in the Medical Examination Center of the First Affiliated Hospital of the Xin'xiang Medical College (China). The patients with angina-like symptoms and a suspected diagnosis of CAD were excluded by coronary angiogram. The subjects with diabetes mellitus or hyperlipidemia were excluded. All participants were from a non-genetically-related Chinese Han population in He'nan Province (China). The study was performed according to the Guidelines of the Medical Ethical Committee of Xin'xiang Medical College (Xinxiang, China). Written informed consent was obtained from all the participants recruited in this study.

Genotyping

Peripheral venous blood was drawn into a sterile tube containing ethylenediamine tetraacetic acid. Plasma samples were stored at -20°C. Genomic DNA was extracted from frozen peripheral blood samples using a QIAmp Blood Mini Kit (Qiagen Inc., Valencia, CA, USA) according to the manufacturer protocols. Genotyping was performed using SNaPshot single nucleotide polymorphism (SNP) genotyping assay (Genesky, Shanghai, China). The touch-down polymerase chain reaction was carried out in a 20-μL reaction mixture. Next, the SNaPshot multiplex single-base extension reaction was performed in a 10-μL reaction volume. After purification using 1 U SAP for 60 min at 37°C followed by 15 min at 75°C, the extension reaction product was separated using capillary electrophoresis (ABI 3130XL Genetic Analyzer, Applied Biosystems Co Ltd., USA) and the results were analyzed using the GeneMapper 4.1 software (Applied Biosystems Co Ltd.). Fifty samples were selected randomly to repeat genotyping for quality control and the repeat accuracy was 100%.

Statistical analysis

The allele and genotype frequencies for each individual polymorphism were compared, and Hardy-Weinberg equilibrium (HWE) was evaluated using the chi-square test. Pearson's chi-squared (χ^2) test was used to evaluate the differences in the distribution of the categorical variables, including the known risk factors and frequencies of the NF-κB1 genotypes, alleles, and haplotypes. A logistic regression analysis was conducted to calculate the adjusted odds ratios and 95% confidence intervals for associations between the genetic polymorphisms and CAD risk. Pair-wise linkage disequilibrium (LD) statistics (D' and r^2) and haplotype frequency were calculated, and haplotype blocks were constructed using Haploview 4.0. To ensure that the LD blocks most closely reflect the population level LD patterns, definition of the blocks was based on the control samples alone. All data were analyzed using the SPSS 20.0 software (SPSS Inc., Chicago, IL, USA).

RESULTS

No significant deviation from HWE was found in cases or controls for any of the SNPs. LD analysis of the patients and controls revealed that the six downstream SNPs are located in a haplotype block (Figures 1 and 2). The genotype distribution, allelic frequencies, and haplotypes in the patients with CAD and healthy controls are shown in Tables 1 and 2.

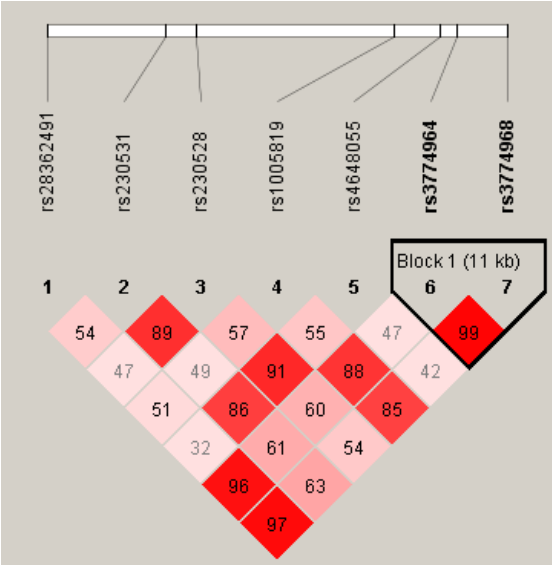

Figure 1. LD plot for the 7 SNPs in the NF-κB1 gene. Values in squares are the pair-wise calculation for r^2.

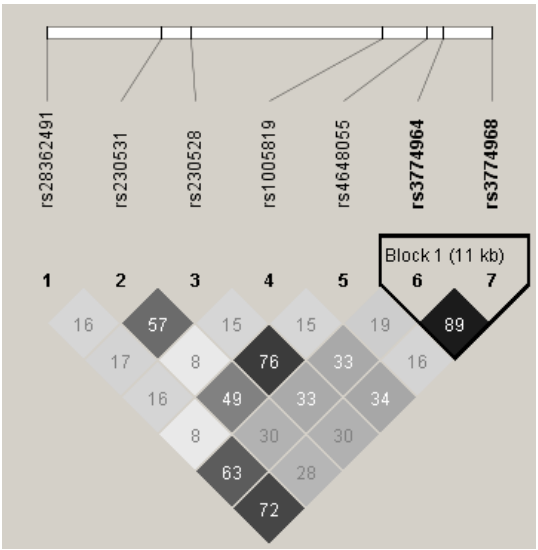

Figure 2. LD plot for the 7 SNPs in NF-κB1 gene in controls. Values in squares are the pair-wise calculation for D'.

Table 1. Genotype and allele frequencies of NF-κB1 polymorphisms in the controls and patients with CAD.

Variable	MAF	CAD (N = 361)		Controls (N = 385)		HWE P value	P value[a]	OR, 95%CI
		No.	%	No.	%			
rs28362491	0.461					0.179	0.001	
SS		109	30.2	72	18.7		0.000281	1.880, 1.337-2.644
SD		167	46.3	211	54.8		0.020	0.710, 0.532-0.947
DD		85	23.5	102	26.5		0.353	0.854, 0.613-1.191
S allele		385	53.3	355	46.1		0.005	1.336, 1.089-1.637
D allele		337	46.7	415	53.9			
rs230531	0.381					0.519	0.442	
TT		150	41.6	144	37.4		0.247	1.190, 0.887-1.596
CT		161	44.6	189	49.1		0.219	0.835, 0.626-1.114
CC		50	13.9	52	13.5		0.891	1.030, 0.678-1.564
T allele		461	63.9	477	61.9		0.447	1.085, 0.879-1.339
C allele		261	36.1	293	38.1			
rs230528	0.468					0.265	0.343	
CC		89	24.7	112	29.1		0.173	0.798, 0.576-1.104
CA		191	52.9	186	48.3		0.210	1.202, 0.902-1.602
AA		81	22.4	87	22.6		0.958	0.991, 0.703-1.397
C allele		369	51.1	410	53.2		0.409	0.918, 0.749-1.125
A allele		353	48.9	360	46.8			
rs1005819	0.339					0.360	0.982	
CC		153	42.4	163	42.3		0.990	1.002, 0.749-1.340
CT		170	47.1	183	47.5		0.904	0.982, 0.737-1.310
TT		38	10.5	39	10.1		0.859	1.044, 0.651-1.673
C allele		476	65.9	509	66.1		0.943	0.992, 0.801-1.229
T allele		246	34.1	261	33.9			
rs4648055	0.486					0.269	0.833	
AA		96	26.6	110	28.6		0.546	0.906, 0.657-1.249
GA		170	47.1	176	45.7		0.706	1.057, 0.792-1.410
GG		95	26.3	99	25.7		0.852	1.032, 0.744-1.431
C allele		362	50.1	396	51.4		0.618	0.950, 0.775-1.164
A allele		360	49.9	374	48.6			
rs3774964	0.451					0.123	0.065	
AA		111	30.7	108	28.1		0.419	1.139, 0.831-1.561
GA		165	45.7	207	53.8		0.028	0.724, 0.543-0.966
GG		85	23.5	70	18.2		0.072	1.386, 0.972-1.977
C allele		387	53.6	423	54.9		0.605	0.948, 0.773-1.162
A allele		335	46.4	347	45.1			
rs3774968	0.477					0.179	0.065	
AA		109	30.2	96	24.9		0.108	1.302, 0.943-1.797
GA		167	46.3	211	54.8		0.020	0.710, 0.532-0.947
GG		85	23.5	78	20.3		0.278	1.212, 0.856-1.716
C allele		385	53.3	403	52.3		0.703	1.040, 0.849-1.275
A allele		337	46.7	367	47.7			

[a]P value was calculated by 2 x 3 and 2 x 2 chi-squared tests based on codominant, dominant for the rare allele, and heterosis and recessive for the rare allele models of inheritance. Alpha value is adjusted by Bonferroni correction and statistically significant results ($P < 0.003$).

Table 2. Haplotype in block 1 frequencies and their association with risk of CAD.

Haplotype	Cases [N (%)]	Controls [N (%)]	Statistics			
			χ^2	P*	OR	95%CI
AA	194 (53.740)	202 (52.468)	0.121	0.728	1.052	0.789, 1.403
GG	162 (44.875)	172 (44.675)	0.003	0.956	1.008	0.755, 1.346

*P value is adjusted by Bonferroni correction and statistically significant results ($P < 0.025$).

In this case-control association study, the analysis revealed a strong association between the rs28362491 genotype distribution and CAD ($P = 0.005$). The frequency of the

rs28362491 SS and SD genotypes was significantly lower in CAD patients than in the healthy controls ($P < 0.05$). Significantly more D alleles were found in the patients with CAD. There were no significant differences between the SNPs (rs230531, rs230528, rs1005819, rs4648055, rs3774964, and rs3774968) and CAD occurrence (Table 1).

We performed an association analysis to determine whether the haplotype was associated with risk of CAD (block 1). However, no significant haplotype association was found between CAD patients and healthy controls (Table 2).

DISCUSSION

A key step in linkage and association studies is to identify common risk variants in different populations. To identify markers contributing to genetic susceptibility to CAD, we examined 7 SNPs spanning the coding and non-coding regions of the NF-κB1 gene. We examined the effects of the NF-κB1 SNPs and predicted haplotypes of the LD block of the NF-κB1 gene on the genetic susceptibility to CAD in a Chinese Han population. During the past decade, much evidence has accumulated to support the role of NF-κB in CAD (Baker et al., 2009; Mishra et al., 2013). Our results provide genetic evidence that NF-κB1 is linked to CAD and extends to identify polymorphisms that may affect the development of CAD (Özbilüm et al., 2013; Yang et al., 2014; Arslan et al., 2015).

A common insertion (ins)/deletion (del) (-94 ins/del ATTG rs28362491) polymorphism in the NF-κB1 gene promoter exerts functional effects on the transcription of the NF-κB1 (Chen et al., 2015). Since the 4-bp ins/del polymorphism produces a relatively large sequence change and its location is proximal to binding sites that are important to promoter regulation, the ATTG deletion (D) allele displays significantly reduced promoter activity and is also involved in lowering levels of p50 protein. Yang et al. (2014) used two independent case-control studies including a Han population (633 CAD patients and 616 control subjects) and a Uygur population (437 CAD patients and 356 control subjects). They found that the DD genotype of the rs28362491 SNP in the NF-κB1 gene may be considered a genetic marker of CAD in Han and Uygur women in China. In this case-control association study, the A alleles of the NF-κB1 rs28362491 SNP were strongly associated with a decreased risk of CAD. Our study included 361 CAD subjects and 385 healthy controls, which had sufficient statistical power to prove an epidemiologically relevant impact of hereditary variations among the studied genes. Arslan et al. (2015) found that the genotype frequency of NF-κB1 rs28362491 DD in the CAD group was significantly higher compared to the controls. These NF-κB1 gene variations may induce a change in mRNA secondary structure, which could in turn affect the stability, processing, or subcellular targeting of the mRNA transcript and thereby alter splicing, transcription, and the efficiency of translation. The mechanism by which the rs28362491 SNP affects susceptibility to CAD is that the SNP may affect the function of the NF-κB1 protein rather than its expression. These results indicate that the rs28362491 D allele is a risk factor for CAD.

In conclusion, with a relatively large sample size and a homogeneous sampling population, we found a NF-κB1 gene polymorphism (rs28362491) was associated with CAD. The results of this and similar future studies could help to better understand the molecular mechanisms of CAD, allowing us to devise better treatment strategies.

Conflicts of interest

The authors declare no conflict of interest.

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