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Toll-like receptor 8 polymorphism and coronary artery disease

Zhong Chen · Genshan Ma · Qi Qian · Yuyu Yao · Yi Feng · Chengchun Tang

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Abstract Toll-like receptors (TLRs) play roles in innate and adaptive immune responses. Some TLRs are involved in the pathogenesis of cardiovascular diseases. Coronary artery disease (CAD) has an inflammatory and immunological basis. We investigated whether TLR8 Met1Val and TLR8-129G>C single nucleotide polymorphisms (SNPs rs3764879 and rs3764880) are associated with CAD in the Chinese population. We enrolled 412 consecutive patients (185 with coronary stenosis ≥50% or previous myocardial infarction and 227 controls). Ligase detection reaction was performed to detect SNPs rs3764879 and rs3764880 of TLR8. The SNP at rs3764879 is in complete linkage disequilibrium with rs3764880. No significant difference was found in genotypic or allelic frequencies of these two common SNPs between CAD cases and controls ($P > 0.05$, respectively). No associations existed between these two SNPs and the severity of coronary artery stenosis (All $P > 0.05$). These results do not support an involvement of SNPs rs3764879 and rs3764880 of TLR8 in predisposition to CAD.

Keywords Coronary artery disease · Single nucleotide polymorphisms · Toll-like receptors

Abbreviations

CAD	Coronary artery disease
SAP	Stable angina pectoris
UAP	Unstable angina pectoris
MI	Myocardial infarction
CI	Confidence interval
OR	Odds ratio
SNP	Single nucleotide polymorphism
TLR	Toll-like receptors
LDL-C	Low-density lipoprotein cholesterol
PAMPs	Pathogen-associated molecular patterns
PRRs	Pattern recognition receptors
EV	Enterovirus
DCM	Dilated cardiomyopathy

Introduction

Cardiovascular disease is one of the major causes of death in most countries, including China [1], and the most common form of heart disease is coronary artery disease (CAD) resulting from atherosclerosis. Epidemiological and clinical studies have strongly supported a role for inflammatory, innate immune, and adaptive immune mechanisms in many facets of vascular disease [2, 3]. The atherosclerotic lesions contain large numbers of immune cells, particularly macrophages and T cells, playing a vital role in defense against invasion of infectious agents [4].

The innate or natural immune system is the body's rapid first-line defense against environmental threats, such as microbial infection and physical or chemical injury and controls the adaptive immune response. Several germ line-encoded (i.e., nonclonal) pattern recognition receptors (PRRs) only recognize highly conserved pathogen motifs known as pathogen-associated molecular patterns (PAMPs) [5].

The most studied PRRs are probably the family of Toll-like receptors (TLRs) [4]. TLRs are transmembrane

Z. Chen and G. Ma contributed equally to this paper.

Z. Chen (✉) · G. Ma · Q. Qian · Y. Yao · Y. Feng · C. Tang
Department of Cardiology, The Affiliated ZhongDa Hospital of Southeast University, NO. 87 Dingjiaqiao, Hunan Road, Nanjing 210009, People's Republic of China
e-mail: zhongchen7498@sina.com.cn

proteins with extracellular leucine-rich repeat domains and a cytoplasmic domain having significant homology to interleukin-1 (IL-1) receptor type-I called Toll/IL-1 receptor domain [6, 7].

It is apparent that most infectious microbial agents express multiple PAMPs that act as TLR agonists. It is also obvious from gene deletion experiments that distinct TLRs are required in order to sense these agonists. When components of pathogens are recognized, TLRs orchestrate a specific immune response that may include the production of cytokines and enzyme cascades [8, 9] to kill the invading microorganisms.

Thus far, 13 TLRs (TLR1-TLR13) have been identified in mammalian species, including ten in humans [10, 11]. Of the 13 TLRs, TLRs 3, 7, 8, and 9 are the major PRRs that recognize distinct types of virally derived nucleic acids that enter the endosome through endocytosis. TLR7, TLR8, and TLR9 are the most closely related TLR family members [12].

After a long time of neglect, recently, it was reported that TLR8 activated under specific conditions opened up new fields of investigation [13]. Triantafilou et al. [14] reported that human cardiac inflammatory responses triggered by coxsackie B viruses are mainly TLR8-dependent. It has been shown that TLR8 expression may be involved in the immune response to enterovirus (EV) replication in EV-associated dilated cardiomyopathy (DCM). In addition, TLR8 may provide important prognostic information in patients with EV-associated DCM [15, 16]. Most importantly, these studies are deliberately ethnically matched to avoid genetic diversity, and an ethnic difference exists in TLR8 polymorphisms [11].

Gene variants of TLR8 have overt relevance to viral infection diseases, but few polymorphisms have been reported in the Chinese population. To date, no information have been published on what form of TLR8 protein is expressed in people with these single nucleotide polymorphisms (SNPs) and whether this changes the TLR8 gene expression or protein functional level. Also, there are no published animal models or in vitro data which describe the association between TLR8 SNPs and CAD. Whether a causal relationship exists between SNPs rs3764879 and rs3764880 of TLR8, a mediator of inflammatory process and infectious diseases, and CAD is still unknown. The aim of this study was to determine whether these two common SNPs of TLR8 are associated with CAD in a Chinese population.

Methods

Subjects

The study cohort comprised 412 Chinese subjects age 31–86 years, consecutively enrolled to participate in this study due to symptoms of chest discomfort. CAD was present in 185 patients, 74 of whom were diagnosed with stable angina pectoris (SAP), 75 with unstable angina pectoris (UAP), and 36 with myocardial infarction (MI). Exclusion criteria were contraindications to heparin, and patients with cardiomyopathy, auto-immunologic disease, severe kidney or liver disease, or malignant disease. Smoking habits and family history of CAD were identified by the information obtained in patients' questionnaire. The study was approved by the Ethics Committee of the affiliated ZhongDa Hospital of Southeast University, and all participants gave informed consent to participate.

Coronary angiography

All patients underwent elective coronary angiography according to the Judkins technique. Images were recorded on CD-R. CAD was verified by angiography with $\geq$50% luminal narrowing in at least one main coronary artery or a definite diagnosis of acute MI defined by WHO criteria. CAD patients were grouped according to the number of significantly stenosed vessels as 1-vessel, 2-vessel, and 3-vessel disease groups. Patients without detectable coronary stenosis were considered controls. Two cardiologists unaware of this study on their consensus opinion judged the grade of the coronary stenosis.

DNA extraction and genotyping

Peripheral venous blood was drawn from each participant. Genomic DNA was extracted using the QIAamp DNA Blood kit (Qiagen, Valencia, CA, USA). The SNPs rs3764879 and rs3764880 of TLR8 were genotyped by ligase detection reaction [17, 18] using TaqMan genotyping assays on an ABI Prism 377 sequence detection system according to the manufacturer's instructions (Applied Biosystems, Foster City, CA, USA).

Statistical analysis

The statistical software package SPSS 15.0 was used for statistical calculations. Results are presented as mean $\pm$ SD for continuous variables and as proportions for categorical variables. Age and body mass index (BMI, kg/m^2) were used as continuous variables. Hypertension, diabetes mellitus, smoking, family history of CAD, and sex were taken as categorical variables. Allele and genotype frequencies among CAD cases and controls were compared with values predicted by Hardy–Weinberg equilibrium using the χ^2-test. For each odds ratio (OR), we calculated 95% confidence intervals (CIs). Two-tailed P values < 0.05 were considered significant.

Results

Basic characteristics of the study population

This study cohort included 412 persons. Of these, 185 with CAD were considered the study group, and 227 without coronary stenosis were enrolled as controls. Table 1 summarizes the basic characteristics of the study population. No significant differences existed in baseline parameters according to composition of sex, ratio of hypertension, and mean values of BMI between CAD cases and controls. A higher prevalence of hypercholesterolemia, diabetes mellitus, smokers, family history of CAD and left ventricular hypertrophy was found in patients with CAD. The CAD patients were also older than the controls.

Table 2 shows that no differences existed between the control and CAD groups in the frequencies of the rs3764880 variants and alleles ($P > 0.05$). As to the distribution of genotypes GG, AG, and AA, both groups were in Hardy–Weinberg equilibrium. Frequencies of the genotypes and alleles at rs3764879 are the same as those in rs3764880. Table 2 also shows the evaluation of frequency of the genotypes and alleles according to sex. The distribution of three genotypes at rs3764879 and rs3764880 variants between CAD and controls does not differ between women and men ($P > 0.05$, respectively).

The associations between the distribution of genotypes AA, AG, and GG at rs3764879 and rs3764880 and the number of diseased coronary arteries were analyzed. No associations were found between these two SNPs and the severity of CAD ($P > 0.05$) (Table 3). Analysis of clinical phenotypic subgroups of CAD indicated that no associations existed in the distribution of genotypes at rs3764879 and rs3764880 variants among the SAP, UAP, and MI groups ($P > 0.05$, respectively) (Table 4).

Table 1 Baseline characteristics of the study population

	Control	CAD
Numbers, *n*	227	185
Sex, male (%)	105 (46.3)	93 (47.0)
Age (years)	54.59 ± 9.83	59.98 ± 11.42†
BMI (kg/m^2)	24.74 ± 4.15	25.10 ± 0.18
Hypertension, *n* (%)	130 (57.3)	100 (54.1)
Diabetes mellitus, *n* (%)	25 (11.0)	67 (36.2)†
Smokers, *n* (%)	62 (27.3)	99 (53.5)†
Hypercholesterolemia, *n* (%)	61 (26.9)	68 (36.8)*
Family history of CAD, *n* (%)	68 (30.0)	80 (43.2)†
Left ventricular hypertrophy, *n* (%)	51 (22.5)	136 (73.5)†

CAD coronary artery disease

Data are mean ± SD, or number (%), as appropriate

*$P < 0.05$ versus data of controls, $^{\dagger}P < 0.01$ versus data of controls

Table 2 Genotype and allele distribution at Rs3764879 and Rs3764880 in CAD cases and controls and genotyping according to sex

	Control (*n*/%)	CAD (*n*/%)	OR (95% CI)
Total			
GG	178/78.4	144/77.8	
AG	29/12.8	28/15.2	1.19 (0.68–2.10)
AA	20/8.8	13/7.0	0.80 (0.39–1.67)
P	0.66		
Relative frequencies of alleles			
Allele G	385/84.8	316/85.4	
Allele A	69/15.2	54/14.6	0.95 (0.65–1.4)
P	0.809		
Female			
GG	95/77.9	66/71.7	
AG	20/16.4	21/22.9	1.51 (0.76–3.01)
AA	7/5.7	5/5.4	1.03 (0.31–3.38)
P	0.50		
Relative frequencies of alleles			
Allele G	210/86.1	153/83.2	
Allele A	34/13.9	31/16.8	
P	0.41		1.25 (0.74–1.25)
Male			
GG	83/79.0	78/83.9	
AG	9/8.6	7/7.5	0.83 (0.29–2.33)
AA	13/12.4	8/8.6	0.66 (0.26–1.67)
P	0.65		
Relative frequencies of alleles			
Allele G	175/83.3	163/87.6	
Allele A	35/16.7	23/12.4	0.71 (0.40–1.25)
P	0.23		

n, number of individuals, with percentage of the total group in parenthesis; OR, odds ratio; 95% CI, 95% confidence interval; CAD, coronary artery disease

P is the significance level of comparison between CAD cases and controls. The χ^2 test for genotypes and likelihood ratio test were used for alleles in the analysis

Discussion

The results of our present study do not support an involvement of the two common SNPs rs3764879 and rs3764880 of TLR8 in the predisposition to CAD in a Chinese population.

It has been proven that the TLR8 gene is mapped to Xp22.3-p22.2, approximately 16 kb posterior to the TLR7 gene, spans 15.5 kb harboring three exons and encodes two splicing variants [19, 20]. In mammals, TLR8 is implicated in the detection of single-stranded viruses. Human TLR8 is expressed on monocytes and myeloid-derived mDCs, responsible for the priming of antigen specific immune responses [21, 22].

Table 3 Distribution of genotypes at Rs3764879 and Rs3764880 among different groups according to the severity of CAD

	Number of vessels involved			*P* value
	One	Two	Three	
GG, *n* (%)	71/38.4	34/18.4	39/21.1	–
AG, *n* (%)	13/7.0	7/3.8	8/4.3	–
AA, *n* (%)	6/3.2	3/1.6	4/2.2	0.997

CAD coronary artery disease

Table 4 Distribution of genotypes at Rs3764879 and Rs3764880 among different clinical phenotypic subgroups of CAD

	Clinical phenotypic subgroups of CAD			*P* value
	SA	UAP	MI	
GG, *n* (%)	51/27.6	62/33.5	31/16.8	–
AG, *n* (%)	16/21.6	9/4.9	3/1.6	–
AA, *n* (%)	7/3.8	4/2.2	2/1.1	0.20

CAD coronary artery disease, *MI* myocardial infarction, *SAP* stable angina pectoris, *UAP* unstable angina pectoris

Recently, Andréoletti et al. [23] detected EV infection markers in 20 (40%) of 50 patients who died suddenly of MI, and demonstrated a significantly higher proportion of active coxsackie viral B infection in patients who died suddenly of acute MI. This study strongly suggests that EV infections may be one of the important environmental and nontraditional risk factors for the development of CAD and its specific phenotype or complication. Furthermore, Triantafilou et al. [14] have reported that coxsackie B viral single-stranded RNA triggers an inflammatory cytokine response through TLR8 and, to a lesser extent, through TLR7 in cultured human cardiac myocytes. These reports suggest that human TLR8-mediated recognition of EV single-stranded RNA may be involved in the antiviral inflammatory response in human CAD. TLR8 polymorphism and its cardiac associations identified in these studies have given rise to interesting questions in terms of the ethnic-specific characteristics and their association with CAD.

To our knowledge, this is the first study analyzing the association of SNPs rs3764879 and rs3764880 of TLR8 with CAD. Compared with other ethnic populations, Han Chinese in Taiwan display a different allele frequency of rs3764879 and rs3764880 than that of Caucasians and African Americans [11]. The frequency of genotypes and alleles of rs3764879 and rs3764880 of the present study in both controls and CAD patients were similar to those in Japanese and Chinese in Taiwan, however, significantly higher than those in Caucasians and African Americans [11]. This further proves the existence of a geographic/ethnic-specific difference in rs3764879 and rs3764880 variants of TLR8. In our study, no significant associations were observed between genotypes and alleles of rs3764879 and rs3764880 variants of TLR8 and CAD. Also in CAD cases, clinical phenotypic subgroup analysis indicated no association between genotypes at rs3764879 and rs3764880 variants and the three subgroups of SAP, UAP, and MI.

Epidemiological data show that the risk of CAD differs between female and male patients. This fact may be a result of exposure to different risk factors involved in the disease process and hormonal differences. In the present study, the distribution of genotypes at rs3764880 and rs3764880 between CAD cases and controls does not differ between women and men. When divided by sex, the sex-specific frequency of genotypes and alleles of rs3764879 and rs3764880 of TLR8 in both controls and CAD cases were still similar to that in the Han Chinese population in Taiwan, significantly different than that in Caucasians and African Americans [11].

To further establish whether SNPs rs3764879 and rs3764880 of TLR8 are associated with the severity of CAD, we explored the correlation between its three genotypes and the severity of coronary lesions. A potential association was not detected, according to the number of coronary arteries with obvious stenosis, which may suggest that this SNP can explain neither the susceptibility to CAD, nor its association with the severity of CAD. The reason may partly be that TLR8 was localized with EV capsid protein VP1 in cardiac myocytes in EV RNA-positive cardiovascular disease [24]. It has been demonstrated that compared with TLR4 and TLR7 signals and cardiac inflammatory cytokine production, CVB3-induced inflammatory response was mainly inhibited by the activation of the TLR8 signal in human cardiac myocytes. TLR8 seems to be a more "efficient sensor" of CVB3 single-strand RNA [24]. As cited above, Chinese subjects displayed an opposite allele frequency of TLR8 Met1Val and TLR8-129G>C compared with Caucasians and African Americans. The reason may partly be ethnicity-related factors contributing to the geographic/ethnic genetic polymorphism prevalence. Another explanation may be that the ligand binding PPRs domain of the protein product of TLR8 recognizes the ssRNA ligand with differential affinity and specificity.

In conclusion, in our sample of patients from the east region of China, we identify that the common SNPs rs3764879 and rs3764880 of TLR8 are not associated with CAD. These results may broaden the knowledge body of TLR polymorphisms and disease-association studies. Additional research to verify these results are necessary.

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