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A novel major histocompatibility complex locus confers the risk of premature coronary artery disease in a Chinese Han population

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Abstract Several novel loci have been proved to be associated with coronary artery disease and/or myocardial infarction risk by genome-wide association studies, however, the available coronary artery disease risk variants explain only a small proportion of the predicted genetic heritability of the disease. Recently, a novel coronary artery disease locus on chromosome 6p21.3 in the major histocompatibility complex was identified in an European population. We hereby investigated whether this single nucleotide polymorphisms (rs3869109) confers the risk of premature coronary artery disease in a Chinese Han population. A total of 422 patients were studied including 210 cases with coronary stenosis ≥50 % or previous myocardial infarction (male <55 years and female <65 years) and 212 controls without documented coronary artery disease. Ligase detection reaction was performed to detect rs3869109. The 3 genotypes AA, AG, and GG were present in rs3869109. There were significant differences between the control and premature coronary artery disease groups in the frequencies of the rs3869109 variants and alleles (all $P < 0.05$). The distribution of 3 genotypes and alleles at rs3869109 does not differ between women and men (all $P > 0.05$). There was a significant association between rs3869109 genotypes and the severity of premature coronary artery disease ($P = 0.038$). Multivariate logistic regression showed that carriers with AG and GG genotypes at rs3869109 have a higher risk of premature coronary artery disease than carriers of AA genotype (odds ratio [OR] 1.997, 95 % CI: 1.166–3.419, $P = 0.012$; OR 1.695, 95 % CI: 1.044–2.752, $P = 0.033$; respectively). Our results indicate that the rs3869109 variants are associated with premature coronary artery disease in a Chinese Han population, suggesting this genetic risk marker is useful in early coronary artery disease risk prediction.

Keywords Coronary artery disease · Single nucleotide polymorphism · Genetic · Gene · Major histocompatibility complex

Introduction

Coronary artery disease (CAD) is one of the leading causes of death and disability in most developing countries including China [1]. Genetic factors have been defined as important risk contributors for the pathogenesis of CAD [2–4]. With the recent development in high-density genotyping arrays, association studies between the whole-genome assessment of variants and some complex diseases become possible [5], which opens entirely new scientific directions and may ultimately lead to new interventions [6].

Despite several novel loci have been proved to be associated with CAD and/or myocardial infarction (MI) risk by genome-wide association studies (GWAS) [2, 3, 7, 8], these available risk variants explain only a small proportion of the predicted genetic heritability of CAD, suggesting other novel important genomic loci and biological processes for disease aetiology remain to be discovered. Besides the genetic background, CAD has also been regarded as a chronic inflammatory disease [9], in which

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infections and immunity have been suggested to play a role and inflammatory role in metabolic syndrome and over-nutrition-related diseases have also been raised [10, 11]. A link between CAD genetic susceptibility and the response to inflammatory signalling in a vascular cell type has been established [12].

Major histocompatibility complex (MHC) genes regulate innate and adaptive immunity and chronic infection [13], which in turn has been linked to the initiation and propagation of atherosclerosis [14, 15]. Furthermore, there exists genetic basis of MHC-linked genes in the initiation of smoking [16, 17]. Interestingly, human MHC region harbors genes that protect from and predispose to CAD [17]. Recently, the single nucleotide polymorphism (SNP), rs3869109, a novel CAD locus on chromosome 6p21.3 in the MHC, has been reported [18]. In the study by Davies et al. [18], the authors performed a discovery meta-analysis of 5 GWAS involving 13,949 subjects (7,123 cases, 6,826 controls) of Europeans ethnicity imputed at approximately 5 million SNPs using pilot 1000 Genomes based haplotypes, and found a discovery P of 3.3×10^{-7}, replication P of 5.3×10^{-4} and combined P of 1.12×10^{-9}, but the path to clinical translation of risk estimates for common variants found in GWAS remains unclear [19].

Also, it has recently been shown that CAD in young adults of pediatric cancer survivors has a poor long-term prognosis which means that clinicians need to be aware of screening and treatment strategies for primary and secondary prevention of cardiovascular complications in this group [20]. Since genotype information is fixed, it is therefore useful in early CAD risk prediction and individualised prevention if genetic risk markers could be replicated in different ancestries. This study was designed to determine whether rs3869109 on chromosome 6p21 confers risk of premature CAD in a Chinese Han population. Our study results for the first time confirmed an association of rs3869109 variants with risk of premature CAD in a Chinese Han population.

Methods

Subjects

From Janunary 2008 to December 2010, 422 patients undergoing coronary angiography (CAG) for suspected CAD were enrolled in this study, including 210 patients with documented premature CAD [21] and 212 subjects without coronary stenosis acting as controls. CAD was defined as a significant coronary stenosis (≥50 %) in at least one of the three main coronary arteries or their major branches (branch diameter ≥2 mm) assessed by CAG or having experienced a MI defined according to World Health Organization criteria [22]. All patients having contraindications for heparin, auto-immunologic disease, type 1 diabetes mellitus, congenital heart disease, syndrome X, severe kidney or liver disease or malignant disease were excluded from this study. The study was approved by the Medical Ethics Committee of the Affiliated Zhongda Hospital of Southeast University. Before enrollment, the study information was explained carefully to each patient, and subsequently written informed consent was obtained from all participants. All patients were from the same geographical area and had a similar socioeconomic and ethnic background.

Coronary angiography

All patients underwent elective CAG according to the Judkins technique and images were recorded on CD-R. Patients with premature CAD were grouped according to the number of significantly stenosed vessels as 1-vessel, 2-vessel and 3-vessel diseased groups. For control group, patients had no detectable coronary artery stenosis. The grade of the coronary stenosis was judged by two cardiologists unaware of this study on their consensus opinion.

Determination of classical risk factors

At the time of enrollment, data were collected from each subject, including a complete history of classical cardiovascular risk factors such as hypertension, type 2 diabetes mellitus (T2DM), smoking habit and family history of cardiovascular disease (CVD). Anthropometric and blood pressure measurements were made according to standard protocols. Hypertension was defined as blood pressure ≥140/90 mmHg or the use of antihypertensive medications. Subjects with a history of T2DM, those receiving anti-diabetic medications, and those with a confirmed fasting blood sugar (FBS) concentration >126 mg/dL (7.0 mmol/L) were considered to have T2DM. Subjects who smoked at least one cigarette per day at the time of enrollment were considered as smokers, as were those who had smoked in the month before the study.

DNA extraction and genotyping

DNA was obtained from blood cells by using a whole-blood genome DNA extraction reagent kit [Axygene Biotechnology (Hangzhou) Limited (Hangzhou City, China)], following the manufacturers' instructions. The SNP rs3869109 were genotyped by ligase detection reaction using TaqMan genotyping assays on an ABI Prism 377 Sequence Detection System according to the manufacturer's instructions (Applied Biosystems, Foster City, CA). A successful genotyping rate of over 96 % was achieved

for the entire SNP test. Random duplicate samples and sequencing technique were used for quality control in the genotyping and the concordance was 100 %.

Statistical analysis

The statistical software package SPSS 15.0 was used for statistical calculations. Continuous data were expressed as mean ± SD and Student *t* test was employed to analyze differences between 2 study groups. Categorical variables were analyzed by χ^2 test. Allele and genotype frequencies in controls were compared with values predicted by Hardy–Weinberg equilibrium using the χ^2 test. Distribution of genotypes at rs3869109 among different groups according to the severity of CAD were determined by χ^2 test. Relative risks of CAD associated with each genotype and main classical risk factors were calculated by multivariate regression analysis and confounding factors were adjusted. For all statistical tests, a *P* value of <0.05 was considered to be statistically significant.

Results

Basic characteristics of the study population

Four hundred and twenty-two patients were recruited to participate in this study, and Table 1 summarizes the basic characteristics of the study population. 210 patients were diagnosed as premature CAD and 212 persons without detectable coronary stenosis were enrolled as controls. There was a higher prevalence of hypertension, T2DM and smokers in patients with premature CAD compared to that in controls (all $P < 0.05$). There were no significant differences in baseline according to average age, ratio of family history of CVD and composition of gender between patients with premature CAD and controls (all $P > 0.05$).

Table 1 Baseline characteristics of the study population

	Controls	Premature CAD
Numbers, *n*	212	210
Age, years	51.34 ± 7.03	52.20 ± 6.70
Male, *n* (%)	108 (50.94)	122 (58.09)
Hypertension, *n* (%)	103 (48.58)	136 (64.76)†
Type 2 diabetes mellitus, *n* (%)	31 (14.62)	50 (23.81)*
Smokers, *n* (%)	71 (33.49)	111 (52.86)†
Family history of CVD, *n* (%)	42 (19.81)	58 (27.62)

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

CAD coronary artery disease, *CVD* cardiovascular disease

* $P < 0.05$ versus controls; † $P < 0.01$ versus controls

Table 2 Genotype and allele distribution at rs3869109 in patients with premature CAD and controls

	Controls, *n* (%)	Premature CAD, *n* (%)	Male, *n* (%)	Female, *n* (%)
Genotypes				
AA	67/31.60	52/24.76	59/25.65	60/31.25
AG	105/49.53	96/45.71	116/50.43	85/44.27
GG	40/18.87	62/29.52	55/23.91	47/24.48
P	0.030		0.366	
Alleles				
A	239/56.37	200/47.62	234/50.87	205/53.39
G	185/43.63	220/52.38	226/49.13	179/46.61
P	0.011		0.466	

P is the significance level of comparison between CAD and controls, or males and females. The χ^2 test for genotypes and alleles were used, *n* number of individuals, with percentage of the total group in parenthesis, *CAD* coronary artery disease

Genotype and allele distribution at rs3869109 in patients with premature CAD and controls and analysis according to gender

Three genotypes were detected in rs3869109 (AA, AG and GG) and there was no deviation from Hardy–Weinberg equilibrium in the control group ($P > 0.05$). Table 2 shows that there were significant differences between the control and premature CAD groups in the frequencies of the rs3869109 variants and alleles. There are lower frequencies of genotypes AA, AG and higher frequency of GG in premature CAD group than those in the control group ($P < 0.05$). Patients with premature CAD also have lower frequency of allele A and higher frequency of allele G in premature CAD group than those in the control group (all $P < 0.05$). The distribution of 3 genotypes and alleles at rs3869109 does not differ between women and men (all $P > 0.05$).

Association between genotypes at rs3869109 and the severity of CAD

The associations between the distribution of genotypes AA, AG, and GG at rs3869109 and the number of diseased coronary arteries were analyzed and there was significant association between rs3869109 genotypes and the number of diseased coronary vessels ($P < 0.05$) (Table 3).

Multivariate regression analysis of association between classicall risk factors, genotypes at rs3869109 and premature CAD

The OR values are displayed in Table 4. After adjustment for gender, hypertension, T2DM, smoking, family history

Table 3 Distribution of genotypes at rs3869109 among different groups according to the severity of premature CAD

	Number of vessels involved			*P* value
	One	Two	Three	
AA, *n* (%)	19/9.05	18/8.57	15/7.14	
AG, *n* (%)	37/17.62	23/10.95	36/17.14	
GG, *n* (%)	26/12.38	26/12.38	10/4.76	0.038

CAD coronary artery disease

Table 4 Logistic regression analysis of association between classical risk factors, rs3869109 genotypes and premature CAD

		B	*P*	OR	95 % CI
rs3869109	AA			1.00 (reference)	
	AG	0.692	0.012	1.997	1.166–3.419
	GG	0.528	0.033	1.695	1.044–2.752
Male		0.361	0.285	1.434	0.740–2.778
Hypertension		0.739	0.005	2.094	1.248–3.514
Type 2 diabetes mellitus		0.606	0.106	1.832	0.877–3.825
Smoking		1.144	0.000	3.139	1.808–5.448
Family history of CVD		0.073	0.810	1.076	0.592–1.954

OR odds ratio, *CI* confidence interval, *CAD* coronary artery disease, *CVD* cardiovascular disease

of CVD and genotypes at rs3869109, hypertension (OR: 2.094, 95 % CI: 1.248–3.514, $P = 0.005$), smoking(OR: 3.139, 95 % CI: 1.808–5.448, $P = 0.000$), heterozygous AG (OR: 1.997, 95 % CI: 1.166–3.419, $P = 0.012$) and homozygotes GG (OR: 1.695, 95 % CI: 1.044–2.752, $P = 0.033$) of rs3869109 were independent predictors of premature CAD.

Discussion

To our knowledge, this study represents the first report in a sample of patients from the Southeast region of China, we confirm an association of rs3869109 variants with risk of premature CAD. This study's results further support the findings by Davies et al. [18], who performed a discovery meta-analysis and found the rs3869109 variants on chromosome 6p21 to be a novel CAD locus.

Recently, GWAS have successful identified several genetic variants associated with CAD [2, 3, 7, 8]. Therefore, the identification of novel genetic variants in assessing early risk of CAD is attracting increasing interest in medical fields throughout the world. Depending on the genetic information acquired, certain gene variants may influence the risk of CAD, use of these data may help to individualise early risk scanning and personalized preventive approaches. Aggressive application of nationally recommended prevention activities for CVD would potentially add millions of quality adjusted life-years to the adult population and improve the average lifespan by at least 1.3 years [23].

The MHC on chromosome 6 is associated with susceptibility to more common diseases than any other region of the human genome, including almost all disorders classified as autoimmune [24]. Relevant to the immunological origins of atherosclerosis, the observed association location at rs3869109 spans a large region containing numerous genes with known functions in immune mediated processes, related to many key participants in atherosclerosis [15]. The genetic risk marker of CAD at rs3869109 [18] has potential application value if replicated in different ancestries.

With a limited number of available data, this is the first study done in a Chinese population analyzing the effect of a novel locus on chromosome 6p21.3 on risk of premature CAD. In the present study, we evaluated the genetic effect of this SNP on risk of premature CAD, using age- and gender-matched controls to maximize the precision and identify true association. In our analysis, significant associations were observed between rs3869109 variants, its alleles and premature CAD, which further support the previously described association of rs3869109 with CAD [18]. In the present study, there are lower frequencies of genotypes AA, AG and higher frequency of GG in premature CAD group than those in the control group, and the prevalence of the G allele was 52.38 % in patients with premature CAD and 43.63 % in controls, which was lower than that found in European (55 %) [18]. The reason may partly be ethnicity-related factors contributing to the geographic/ethnic genetic polymorphism prevalence.

Epidemiological data showed that the risk of CAD differs between female and male patients, which may be a result of exposure to different risk factors involved in the disease process and hormonal differences [25, 26]. However, to our surprise, in our study, no significant difference was found in genotypic or allelic frequencies of rs3869109 between females and males when analyzed within subgroups divided by gender.

To further establish whether rs3869109 is associated with the severity of CAD, we also explored the correlation between its 3 genotypes and the severity of coronary lesions. A positive association was detected, according to the number of coronary arteries with obvious stenosis. Hypertension, T2DM and smoking are independent risk factors for premature CAD. After adjustment for these co-existing classical risk factors, multivariate regression analysis showed that carriers of heterozygous AG and homozygous GG of rs3869109 confer a higher risk of

premature CAD than carriers of AA genotype, consistent with previous reports in the literature [18]. Taken together, the analyses presented here may suggest that this novel locus can explain not only the susceptibility to premature CAD, but also its association with the severity of premature CAD, and consistently demonstrate that rs3869109 is involved directly in the genetics and aetiology of CAD.

The present study has several strengths and limitations. First, all subjects underwent CAG and this made the enrollment criteria more concise and accurate to test the true association between rs3869109 and premature CAD risk. Secondly, multivariate analysis was used to adjust the confounding factors to determine the significant predictors of premature CAD. Additionally, the fact is that the association between this novel locus and premature CAD has not been previously studied in Asian population and thus our study has its novelty. Our study and results present of course some limitations, because the studied cohort was obtained from a single hospital in the Southeast of China, and all subjects were of yellow ethnicity, the samples might not include all the characteristics of patients from other centers; thus, the results may not be generalizable to other ethnic groups in which disparities in population composition, geographical, and ethnic backgrounds may exist and only the individual clinician can ultimately enable the translation of these important discoveries to systematic implementation in clinical practice [27]. Finally, as generally accepted, CAD is a disease produced by both multiple genes and environmental factors, many other genes could be potential candidates for premature CAD [28, 29].

Conclusion

For the first time, we have confirmed the positive findings on the association of rs3869109 variants on chromosome 6p21with premature CAD in a Chinese Han population. The clinical relevance of the present findings lies in the fact that rs3869109 variants confer risk of CAD in an early stage and this provides genetic information for early risk recognition towards individualised medicine. Functional analyses are warranted to elucidate the biological plausibility of this novel identified genetic locus in the development of atherosclerosis and premature CAD.

Conflict of interest None declared.

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