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Original Article

Association of the CDKN2B-AS1 genetic polymorphisms with coronary artery disease

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Abstract: The impacts of the interactions between the susceptibility genes rs10757274 and rs1333049 of coronary artery disease (CAD) and clinical factors on CAD susceptibility of the Han CAD population were investigated. We selected 1061 CAD patients of Han nationality as the subjects. The two SNP sites rs10757274 and rs1333049 were genotyped by polymerase chain reaction (PCR). The differences of the alleles of each SNP site and the corresponding frequencies between the CAD and control group were analyzed. Age, BMI, smoking, hypertension, dyslipidemia and traditional risk factors related to diabetes mellitus were counted statistically, and their correlations with the onset of CAD were analyzed. The relationships between CAD and genes and various clinical factors were explored via multiple stepwise regression analysis. The rs10757274 AG/GG genotypes and the rs1333049CG/CC genotypes correlated with CAD susceptibility of the Han population in Kunming region (rs10757274-GG OR=2.887; rs1333049-CC OR=1.527). The homozygotes of the two genes both increased CAD susceptibility compared with the heterozygotes. Smoking, hypertension, dyslipidemia and diabetes mellitus remarkably increased the incidence of CAD (P<0.05). No significant age or BMI difference was observed between the two groups (P>0.05). The interactions between the single-nucleotide polymorphism of rs10757274 and rs1333049 and clinical factors impacted the susceptibility to CAD.

Keywords: Coronary artery disease, single-nucleotide polymorphism, susceptibility gene, rs10757274, rs1333049

Introduction

Coronary artery disease (CAD) has always been a hotspot of medical research due to its high incidence and mortality [1]. The latest pathogenesis researches show that CAD is a common complex disease caused by the interactions between multiple genes and environmental factors [2, 3]. Besides traditional risk factors such as age, smoking, hypertension, dyslipidemia, diabetes mellitus, etc., the combined effects of susceptibility genes, modifying genes and environmental risk factors affect the initiation and development of CAD [4, 5]. Determining the pathogenic/susceptibility genes of CAD is conductive to confirming the therapeutic targets and early risk stratification. Therefore, geneticists have been devoted to searching for CHD genes in recent two decades. After repeated verification by multiple countries and centers and different populations [6, 7], rs10757274 and rs1333049 were found to be the single-nucleotide polymorphism (SNP) sites most related to CAD. In this study, the Han population was selected as the subjects, and the correlations of the two SNPs and clinical factors with CAD were investigated to explore the impacts of their interactions on CAD among the Han population in the plateau region of Kunming.

Subjects and methods

Case selection

We enrolled 2059 patients who were subjected to coronary angiogram and hospitalized in the Department of Cardiology in our hospitals from Dec 2010 to Dec 2015. And the recruited subjects were divided into the CAD group (n=1061) and the control group (n=998) based on the coronary angiogram results. The relatives within three generations of all the subjects were Han people after inquiry. The protocol was

Table 1. Characteristics of the participants

Characteristics	CAD group (n=1061)	Control (n=998)	*P* value
Age ($\bar{x}$±s, year)	58.45±10.29	57.70±11.75	0.112
BMI ($\bar{x}$±s, kg/m^2)	25.50±3.28	25.90±3.40	0.344
Smoking	679 (64.0)	386 (38.7)	<0.000
Hypertension	509 (48.0)	352 (35.3)	<0.001
Diabetes	331 (31.2)	349 (23.3)	0.038
Hyperlipidemia	552 (52.0)	392 (39.3)	<0.001

Table 2. Genotype and allele distributions of rs10757274 in patients with CAD and control participants

rs10757274	Genotype (n, %)			Allele (Frequency)	
	AA	AG	GG	A	G
CAD group	340 (32.0)	488 (46.0)*	234 (22.1)*	1168 (55.0)	954 (45.0)*
Control	359 (36.0)	362 (36.3)	297 (29.7)	1080 (54.1)	956 (45.9)

Note: *P<0.05.

Table 3. Genotype and allele distributions of rs1333049 in patients with CAD and control participants

rs1333049	Genotype (n, %)			Allele (Frequency)	
	GG	CG	CC	C	G
CAD group	233 (22.0)	424 (40.0)*	403 (38.0)*	1230 (58.0)	890 (42.0)*
Control	205 (20.5)	489 (49.0)	304 (30.5)	1097 (55.0)	899 (45.0)

Note: *P<0.05.

Table 4. Multiple logistic regression analysis for CAD patients and control subjects

SNP$_s$	OR value	P
rs10757274-AG	1.430	0.011
rs10757274-GG	2.887	0.000
rs1333049-CG	2.602	0.047
rs1333049-CC	1.527	0.011
Smoking	2.570	0.000
Hypertension	10.831	0.005
Hyperlipidemia	6.608	0.001
Diabetes	25.442	0.001

Note: OR, odds ratio.

approved by the Ethics Committee of our hospital, and the informed consent was signed by both the patients and controls.

The inclusion criteria of the CAD group: (1) among the left main coronary artery, left anterior descending branch, circumflex branch and right coronary artery and its main branches, at least one had a stenosis ≥50% based on the coronary angiogram results; (2) patients who underwent coronary artery bypass surgery or percutaneous coronary intervention. The exclusion criteria: patients with cancers, other heart diseases or multiple organ failure.

Case data

Personal information of all subjects was recorded in details after hospitalization like age, gender, various risk factors of cardiovascular diseases including the history of premature acute myocardial infarction (male <55 years, female <65 years), smoking history, family history of premature CAD, hypertension, diabetes mellitus, hyperlipemia and drug use. The body mass index (BMI) was calculated. The blood pressure level was measured. And fasting blood-glucose, triglyceride, total cholesterol, high and low density lipoprotein cholesterol, uric acid, myocardial enzyme and troponin I were detected by laboratory examination.

Sample collection

EDTA anticoagulant tubes were used to collect venous blood (2 mL) from each subject and allocated into EP tubes (0.5 mL for each). Then these samples were stored at -80°C.

Methods

SNP genotyping

High-throughput multiple PCR based on single nucleotide primer extension was adopted for SNP site genotyping, which was carried out with the SNP Stream genotyping system. The primers were designed online at www.autoprimer.tom and synthesized by Shanghai Yingjun Biotechnology. dNTP was bought from TaKaRa. PCR buffer, $MgCl_2$ and Taq enzyme were purchased from Roche. The other reagents were bought from Beckman coulter.

Detection of the SNP sites

The collected samples were successively subjected to DNA extraction, determination of SNP sites, primer design, PCR and purification, single nucleotide primer extension, hybridization and imaging and high-resolution melting analysis to perform SNP site detection.

Statistics

The data were processed with SPSS17.0 (two-tailed test). P<0.05 indicated significant difference. Measurement data were subjected to normality test and the data consistent with the normal distribution were shown as $\bar{x}\pm s$. The comparison between two independent samples was performed by t-test. The differences of allele and genotype frequency between the CAD and control group were analyzed with Chi-square test. Multiple-factor analysis was performed with multiple stepwise regression analysis. The correlation analysis between variables was carried out with Spearman correlation analysis.

Results

Comparison of the general clinical data

The comparison of the general clinical data between the CAD and control group is shown in **Table 1**. Compared with the control group, the CAD group had a much higher number of smokers (P<0.001), a markedly higher rate of hypertension and hyperlipemia (P<0.001) and a relatively higher rate of diabetes (P<0.05). No significant difference of age or BMI was found between the two groups (P>0.05).

Contrast of genotyping of the two SNP sites and the allele frequencies

Compared with the control group, the distribution frequencies of both the rs10757274 AG/GG genotype and the rs1333049 CG/CC genotype were higher in the CAD group (P<0.05). The risk alleles of rs10757274 and rs1333049 were G and C respectively. See **Tables 2** and **3**.

Correlation of the two SNPs and clinical factors with CAD explored by multiple stepwise regression analysis

The correlation of the two SNPs (rs10757274 and rs1333049) and clinical factors with CAD was explored by multiple stepwise regression analysis with R software. And the results indicated smoking, hypertension and hyperlipemia remarkably correlated to CAD (P<0.05). The rs10757274 AG/GG genotype and the rs1333049 CG/CC genotype were proven to be related to the susceptibility to CAD. See **Table 4**.

Discussion

Genomics most successfully dominates the life science field in recent years [8], and grave progress has also been made in the genomic research of cardiovascular diseases. Many risk genetic loci related to CAD have been successfully discovered by the genome-wide association study [9-11]. Most of these loci were situated at the chromosomal region 9p21.3 [12]. And this has been repeatedly verified in multiple populations including Caucasians such as Americans, Britons, Swedes, etc. and Asians like Japanese, South Koreans, Chinese, etc. [13-15]. In Xinjiang Uygur Autonomous Region and plain areas such as Hunan and Guangdong Provinces of China, relevant researches were also carried out with CAD patients who were of different nationalities and from different living environments [16, 17]. In this study, we found that, together with traditional risk factors of CAD, rs10757274 and rs1333049, the two SNP sites proven to be mostly related to CAD in previous studies were selected for multiple stepwise regression analysis, and the impacts of their interaction on CAD susceptibility were explored.

Statistics of the clinical risk factors in this study: compared with the control group, the CAD group had a much higher number of smokers (P<0.001), a markedly higher rate of hypertension and hyperlipemia (P<0.001) and a relatively higher rate of diabetes (P<0.05). No significant difference of age or BMI was found between the two groups (P>0.05). The intergroup comparison suggested a close correlation between traditional risk factors with CAD.

The results of the pathogenic/susceptibility gene study showed that with the dominant genetic model, the rs10757274 AG/GG genotypes increased the risk of CAD by 71% compared with the AA genotype. The G allele increased the incidence of CAD by 87% compared with the A allele with G as the risk allele of rs10757274. Meanwhile, the rs1333049

CG/CC genotypes increased the risk of CAD by 65% compared with the GG genotype. The C allele increased the incidence of CAD by 70% compared with the G allele with C as the risk allele of rs1333049. These results corroborated altogether that both rs10757274 and rs1333049 were also the susceptibility genes of CAD among the Han population in Kunming region, and rs10757274-G and rs1333049-C were indicated as the susceptibility loci of CAD, consistent with previous studies [8].

The correlation of rs10757274 and rs1333049 and clinical factors with CAD was analyzed by multiple stepwise regression analysis using R software. And the results were as follows: rs10757274-GG OR=2.887; rs1333049-CC OR=1.527; smoking OR=2.570; hypertension OR=10.831; hyperlipemia OR=6.608; diabetes OR=25.442. Among these factors, hyperlipemia had the highest correlation. The genetic factors had remarkably lower correlation coefficients compared with traditional risk factors, partly because of the fact that CAD is a polygenetic disease which is hardly triggered by the change of only one genetic locus.

At present, the genomic research remains at the experimental stage [9]. The clinical transformation requires continually collecting data of large-sample studies on different populations so as to further explore the CAD-related pathogenic/susceptibility genes and establish a SNP database associated with cardiovascular risk. The prediction of CAD risk now predominantly relies on traditional cardiovascular risk factors. However, the predictive ability could be substantially enhanced once genetic risk factors and traditional cardiovascular risk factors are combined. In this study, we specifically analyzed two genetic loci of the Han CAD patients in the plateau region using statistical methods. Despite a comparatively limited study population and a relatively small case number, this research may be accepted as a meaningful exploration in search of CAD-related susceptibility genes.

Disclosure of conflict of interest

None.

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