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Independent Association of 9p21 Locus and Subclinical Atherosclerosis

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Abstract

Objective: Chromosome 9p21 has recently been shown to be a risk region for coronary artery disease. Since Carotid Intima-Media Thickness (IMT) and Left Ventricular Hypertrophy (LVH) are independent predicators for CAD. The association between 9p21 and these phenotypes were investigated.

Methods: 361 Han Chinese elderly in Beijing were included. These subjects underwent the following procedures: (1) Personal medical history and physical examination; (2) Routine blood chemistry and urine analysis; (3) Left ventricular mass index was obtained by echocardiography, and ultrasound evaluation of carotid intima-media thickness. We tested the genotypes of six single nucleotide polymorphisms on chromosome 9p21 (rs2383206, rs10965234, rs10965235, rs10757277, rs10811656 and rs1333047).

Results: For the SNP rs2383206, Genotypes with allele A (AA/AG) was significantly associated with higher CCA IMT (p=0.003). For the SNP rs1075277, Genotypes with allele A (AA/AG) was significantly associated with higher CCA IMT as well (p=0.021). This phenomenon was not observed in ICA IMT and LVH. After adjustment for age, sex, BMI, SBP, DBP, TC, FPG, general linear regression analysis demonstrated that age (β=0.145, p=0.009) and rs1075277 (β=-0.115, p=0.037) were independently associated with CCA IMT. But there was no association between rs2383206 with CCA IMT.

Conclusions: In a cross-sectional study of Han Chinese elderly in Beijing, chromosome 9p21 locus showed a significant association with carotid atherosclerosis, especially CCA IMT. However, there was no association between 9p21and LVH.

Keywords: Single Nucleotide Polymorphism (SNP); 9p21; Intima-media thickness (IMT); Left Ventricular Hypertrophy (LVH)

Introduction

In 2007, three chip-based Genome-Wide Association Studies (GWAS) simultaneously revealed the significant association between common variants on chromosome 9p21 and Coronary Artery Disease (CAD) [1-3]. The initial discovery of Chromosome 9p21 was made in CAD and myocardial infarction cohorts of predominant individuals of European descent. Also the locus has now been replicated in many ethnicities, such as Korean, Japanese, Chinese, Pakistani and US Hispanic populations. Atherosclerosis is considered as the pathology underlying the majority of CAD, and current evidence suggests that the 9p21 genotype may influence the risk for CAD through molecular modulation of the atherosclerotic process [4]. Carotid Intima-Media Thickness (IMT) and Left Ventricular Hypertrophy (LVH) have been shown to be independent predicators for cardiovascular events [5,6]. However, only few studies have investigated the association of this newly identified risk 9p21 locus with carotid atherosclerosis and LVH. The six SNPs we choose were placed in two known genes (*CDKN2A* and *CDKN2B*), which coded for 3 proteins (p16INK4a, ARF, and p15INK4b) those were expressed at high levels in a wide range of cell types, including endothelial and inflammatory cells. Those cells played important roles in the process of atherosclerosis. We conducted a study using Han Chinese older population resided in Beijing to evaluate the risk of chromosome 9p21 for subclinical atherosclerotic phenotypes, including IMT and LVH.

Material and Methods

Study population and clinical evaluation

The cross sectional analysis includes 361 participants aged from 60 years to 94 years who lived in Beijing from 2009 to 2011. All participants were outpatients from the department of gerontology of Peking University People's Hospital. Exclusion criteria: (1) Secondary hypertension or accelerated malignant hypertension; (2) Valvular heart disease or cardiomyopathy; (3) Coronary heart disease; (4) Thyroid disorder; (5) Acute infection or carcinomas; (6) Severe anemia.

Each participant signed an informed consent and was required to answer a questionnaire which included basic demographic, social economic information and past medical history. A complete physical examination including height, weight, heart rate and blood pressure was done and blood chemistry including Fasting Blood Glucose (FBG) and lipid profile were analyzed. Body Mass Index (BMI) was calculated using standard methods.

Ultrasound examination of the carotid arteries and echocardiography

The bilateral Common Carotid Arteries (CCAs) were measured using Philips HD 11 ultrasonography system with a 7.5-MHz transducer. The far wall of carotid IMT was visualized bilaterally and IMT was measured at plaque-free area of CCA (10-20 mm proximal to the tip of the flow divider), Bif (tip of the flow divider and extending 10 mm proximally) and ICA (proximal 10 mm above the bulb) separately. Three measurements were made on each side and the average measurement was used as the IMT. Two-dimensional and guided

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M-mode echocardiograms were performed on each subject by a single cardiologist using a commercially available machine (Acusonll28xp/10, USA) with a 2.5/2.0 MHz transducer. Measurements for M-mode guided calculations of LV mass were taken at or just below the tip of the mitral valve with a paper speed of 50 mm/sec. Left ventricular mass index (LVMI) was calculated as follows. LVH was diagnosed by using the criteria of LVMI greater than 49.2 g/m$^{2.7}$ for men and 46.7g/m$^{2.7}$ for women [7,8].

LVMI (g/m$^{2.7}$) = {1.04×[(IVST+LVEDD+ PWT)^3-LVEDD^3]-13.6}/height$^{2.7}$

(Left Ventricular Internal Dimension at End-Diastole (LVEDD), Intraventricular Septal Thickness (IVST), Posterior Wall Thickness (PWT)).

Genotyping

Genome DNA was isolated from whole blood using a commercial available DNA isolation kit (Invitrogen Inc.) Six SNPs (Table 1) were selected in this study. One pair of primers was constructed to cover the site of rs2383206, rs10965234, rs10965235. The sequence of upstream primer is: 5'-GGCCCGATGATTTTCAGTTA -3', downstream primer is: 5'-TAAGCCACCAAGGAAGAGGA -3'. The other pair of primers was constructed to cover the site of rs10757277, rs10811656, rs1333047. The sequence of upstream primer is: 5'-CAAACAGCCAATTTGTGGAG-3', the downstream primer is: 5'-GCCAGGACTACCTCTAGTTCCA-3'. Polymerase Chain Reaction (PCR) was performed in a final volume of 50 uL which contained 50 ng of genomic DNA, 125 ng of each primer, and premixed PCR reaction mixture (Tian Gen Biotech. Inc.). Cycling conditions consisted of an initial 5 min at 94°C followed by 30 cycles of 30 s at 94°C, 45 s at 60°C, 60 s at 72°C, and finally, 10 min at 72°C. DNA sequencing was performed in DNA sequence (ABI 3730XL, Applied Biosystems).

Statistical analysis

All data analyses were performed using SPSS software package (Version 19.0 for windows, Chicago, IL). Data are expressed as the mean ± Standard Deviation (SD). Differences were tested among groups by one-way ANOVA test and qualitative variables difference by χ^2 analysis. A forward stepwise multiple linear regression analysis was used to determine the factors independently associated with carotid IMT. A p value less than 0.05 was considered to be significant.

Results

General characteristics

In this cross sectional analysis, 361 Han Chinese elderly participants were included. The clinical general characteristics data was shown in (Table 2). The mean and SD of age was 78.0 ± 7.3 years (range from 60–94 years). Men accounted for 68.7% of the total population. Hypertension patients accounted for 78.9% and diabetes for 40.9%. The genotype distributions of the six selected SNPs (rs2383206, rs10965234, rs10965235, rs10757277, rs10811656 and rs1333047) did not deviate from Hardy-Weinberg Equilibrium (HWE).

Association of chromosome 9p21 SNPs with carotid IMT and LVMI

For the SNP rs2383206, Genotypes with allele A (AA/AG) was significantly associated with higher CCA IMT (p=0.003). There was no significant difference in ICA IMT and LVH among different genotypes. For the SNP rs1075277, Genotypes with allele A (AA/AG) was significantly associated with higher CCA IMT (p=0.021). There was no significant difference in ICA IMT and LVH among different genotypes (Table 3).

SNP	rs2383206	rs10965234	rs10965235	rs10757277	rs10811656	rs1333047
allele	G/A	G/T	A/C	A/G	C/T	A/T

Table 1: SNPs in 9p21 locus.

Characteristic	All participants
	n(%) or mean ± SD
Age, year	78.0 ± 7.3
Male gender	248 (68.7%)
Hypertension	285 (78.9%)
Diabetes	148 (40.9%)
Antihypertensive medications	140 (38.7%)
Lipids lowering drugs	34 (9.4%)
BMI (Kg/m^2)	24.3 ± 3.52
SBP (mmHg)	132.3 ± 17.1
DBP (mmHg)	73.8 ± 9.3
FPG (mmol/l	5.5 ± 1.6
Scr (μmol/l)	91.0 ± 34.0
TC (mmol/l)	4.3 ± 0.9
LDL-C (mmol/l)	2.6 ± 0.8
HDL-C (mmol/l)	1.1 ± 0.3
TG (mmol/l)	1.3 ± 0.6

Table 2: General characteristics of the study participants.

Linear regression model of CCA IMT

After adjustment for age, sex, BMI, SBP, DBP, TC, FPG, general linear regression analysis demonstrated that age (β=0.145, p=0.009) and rs1075277 (β=-0.115, p=0.037) were independently associated with CCA IMT (Table 4), there was no association between rs2383206 and CCA IMT. Also, there was no relationship between rs2383206 or rs1075277 and LVMI.

Discussion

In this study, we investigated the relationship between SNPs at chromosome 9p21 among carotid IMT and LVH. We found: (1) the rs1075277 variant on chromosome 9p21 was independently associated with CCA IMT; (2) there was no association of chromosome 9p21 with LVH. Given that atherosclerosis is a common pathogenesis underlying the majority of CAD and stroke, our findings provided additional insight to understand the mechanistic basis of 9p21 on these clinical consequences.

Different atherosclerosis phenotypes have been used for evaluating the association of 9p21 in the carotid artery, including IMT, stenosis and plaque [9-11]. Up to the present, there is no firm association by using carotid IMT established. In this study, genotypes with allele A of rs2383206 and genotypes with allele A of rs10757277 variants were associated with the thickening of CCA IMT and the rs1075277 variant was independently associated with CCA IMT. Consistent with our findings, the Taiwan study of included stroke- and myocardial infarction-free participants and found the rs1333040 and rs1333049 variant had a significant association with carotid IMT [10]. While, the study enrolling 1425 members of 248 Caucasian families ascertained through a hypertensive proband failed to provide the evidence of association between four 9p21 SNPs (rs1333049, rs7044859, rs496892 and rs7865618) and CCA IMT [12]. Another study from France found no evidence of association between rs1333049 variant of 9p21 and carotid IMT [11]. The difference might result from different study population and different race ethnicity partially.

	Genotype			p value
rs2383206	**AA (n=113)**	**AG (n=188)**	**GG (n=60)**	
CCA IMT	0.94 ± 0.22	0.95 ± 0.21	0.86 ± 0.12	0.003
ICA IMT	0.87 ± 0.21	0.85 ± 0.14	0.83 ± 0.18	0.508
LVMI	45.96 ± 14.92	47.92 ± 11.51	45.03 ± 11.83	0.283
LVH	35/111 (31.8%)	77/185 (41.6%)	22/59 (37.2%)	0.243
rs10757277	AA(n=108)	AG(n=181)	GG(n=69)	
CCA IMT	0.94 ± 0.22	0.95 ± 0.22	0.87 ± 0.12	0.021
ICA IMT	0.87 ± 0.21	0.86 ± 0.16	0.83 ± 0.18	0.449
LVMI	46.82 ± 14.91	47.27 ± 11.81	45.73 ± 11.61	0.697
LVH	37/105 (35.2%)	70/179 (39.1%)	27/69 (39.1%)	0.790

Table 3: Association of chromosome 9p21 SNPs with carotid IMT and LVMI.

	Unstandardized Coefficients	Standardized Coefficients	p value
age	0.004	0.145	0.009
rs1075277	-0.034	-0.115	0.037

Table 4: Linear regression model of CCA IMT.

Current molecular evidence showed the six SNPs we choose were placed in two known genes (*CDKN2A* and *CDKN2B*), which coded for 3 proteins (p16INK4a, ARF, and p15INK4b) those were expressed at high levels in a wide range of cell types, including endothelial and inflammatory cells. All 3 proteins were inhibitors of cyclin-dependent kinases controlling cell proliferation, cell aging, and apoptosis functions those were all potentially relevant to the atherosclerotic process [13,14]. Atherosclerosis could be viewed as a gradual process from thickening of IMT to plaque formation. Our results demonstrated the relationship between 9p21 and partial carotid atherosclerotic phenotypes, and indicated the contribution of the risk gene variants in early stages of atherosclerosis.

The initial discovery of Chromosome 9p21 was made in CAD and myocardial infarction cohorts of predominantly individuals of European descent [15,16]. Also the locus has now been replicated in many ethnicities, such as Korean, Japanese, Chinese, Pakistani and US Hispanic populations [17]. In the present study, we found no association of chromosome 9p21 genotype with cardiovascular structure measured by LVH in elderly. Our findings meant that although 9p21 genotype conferred an increased risk of developing coronary artery disease among healthy individuals, it did not predict abnormalities in cardiovascular structure. Similar conclusion was found in the Heart and Soul Study performed in Caucasian individuals in USA [18].

The main strength of the present study is the collection of subclinical detailed phenotypic information derived from carotid ultrasonography and echocardiography in elderly. However, several important limitations should be considered in the interpretation for the results. First, our study population was restricted to Han Chinese individuals, predominantly men. Our results may not therefore extrapolate automatically to women or other racial groups. Second, our sample size was inadequate to test the prognostic significance of the 9p21 polymorphism with respect to subsequent adverse cardiovascular events. Third, our findings were restricted to individuals with age over 60 years, and could not be extrapolated to community population.

To conclude, the association of genotypes with allele A of rs1075277 with carotid IMT development suggested that the association between genetic variability at chromosome 9p21 and CAD might involve carotid IMT development or its mechanism. What's more, we found no association of chromosome 9p21 with LVH in a cross-sectional study of Han Chinese elderly in Beijing.

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