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Case–Control Study on the Interaction Effects of rs10757278 Polymorphisms at 9p21 Locus and Traditional Risk Factors on Coronary Heart Disease in Xinjiang, China

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Objective: To study the interaction effects of rs10757278 polymorphisms at 9p21 locus and traditional risk factors on coronary heart disease (CHD) in Xinjiang, China.

Methods: This case–control study consecutively enrolled 310 unrelated consecutive CHD patients aged 18–70 years old. All study participants were recruited between January and December 2017 from The Heart Center of The First Affiliated Hospital of Xinjiang Medical University. CHD patients were confirmed by coronary angiography (≥50% diameter stenosis in at least one of the major coronary arteries) according to the American Heart Association criteria for the confirmation of CHD. Healthy subjects were randomly selected from the occupational population, who received physical examination in our hospital and matched to cases on the basis of age (±3 years) and sex, those without medical history of cardiovascular diseases, and 536 subjects were selected as the control group after medical history inquiry, physical examination, cardiac ultrasound, electrocardiogram, and other blood biochemical examinations in the hospital. The occupational stress was evaluated by an effort-reward imbalance questionnaire. An epidemiological survey was conducted to collect clinical data. Chi-squared test, analysis of variance, and binary logistic regression analysis were adopted.

Results: Both the case and the control groups showed significant difference in smoking, drinking, physical activity, hypertension, diabetes mellitus, family history of CHD, and body mass index (BMI) (all $P < 0.05$); prevalence of CHD was not related to occupational stress. There was no significant difference in occupational stress level between the 2 groups ($P > 0.05$); Differences in rs10757278 genotype between the case group and the control groups were statistically significant; binary logistic regression analysis was used to evaluate the risk factors of CHD. After adjustment for age and sex, significant increased risk effects for CHD were found to be associated with smoking [odds ratio (OR) = 2.311; 95% confidence interval (CI): 1.04–2.499; $P < 0.001$], physical exercise (OR = 1.365; 95% CI: 1.137–1.639; $P < 0.001$), hypertension (OR = 4.627; 95% CI: 2.165–10.764; $P < 0.001$), family history of CHD (OR = 4.103; 95% CI: 3.169–6.892; $P < 0.001$), BMI (OR = 2.484; 95% CI: 2.036–3.03; $P < 0.001$), and GG genotype at rs10757278 (OR = 1.978; 95% CI: 1.413–2.769; $P < 0.001$); We noted that a significant interaction association between GG genotype at rs10757278 and CHD differs across categories of smoking, hypertension, family history of CHD, and BMI.

Conclusion: GG genotype at rs10757278 may be a risk factor for CHD. And there are interaction effects between GG genotype of rs10757278 in region 9p21 gene and traditional risk factors.

Key Words: rs10757278, 9p21, traditional risk factors, CHD

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Experts predict that coronary heart disease (CHD) will become one of the primary diseases endangering human health worldwide by 2030.[1] Cardiovascular diseases (CVDs) are the leading cause of death in both men and women of every major ethnic group in the United States, of which CHD is the most prevalent.[2] In 2014, more than 600,000 Americans were estimated to have a new coronary event, and 300,000 had a recurrent event.[3] Between 2013 and 2030, medical costs of CHD are projected to increase by 100%,[4]highlighting a growing health and socioeconomic problem. In China, CHD is the second leading cause of death from CVDs, accounting for about 22% of urban and 13% of rural mortality. This is due to the aging population and the poor control of CHD risk factors, such as the rising incidence of hypertension, hyperlipidemia, obesity, and diabetes,[5] especially hyperlipidemia.[6]

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Some studies have shown that in addition to the traditional risk factors, the risk of CHD can be about 50% due to its own genetic factors.[7] Moreover, except for a small number of diseases caused by genetic factors (such as congenital heart disease) or environmental factors (such as earthquake), most diseases are caused by the synergistic effects of environmental factors and genetic factors.[8] Genetic and environmental factors have been well proved to be associated with the increased risk of CHD, including family history of CHD, hypertension, diabetes, dyslipidemia, advanced age, poor diet, advanced age, and smoking habit.[9] Studies have shown that CHD has the interaction of gene–gene[10] and gene–environment,[11] and the relationship between gene polymorphism and CHD has been paid more and more attention by scholars.

The rs10757278 locus in region 9p21 has been proved to be correlated with the incidence of CHD in the Han population in the low-altitude plain area of China,[12] while the relevant studies on the population in the minority areas of northwest China have been rarely reported. This study aimed to explore the independent and interaction effects of rs10757278 polymorphisms at 9p21 locus and environmental factors on the risk of CHD in Xinjiang occupational population, to provide theoretical basis for prevention of CHD in population with rs10757278 gene.

SUBJECTS AND METHODS

Subjects

According to the calculation formula of sample size in this case–control study $N = \frac{(Z_\alpha \times \sqrt{2 \times \bar{P}(1-\bar{P})} + Z_\beta \times \sqrt{P_1(1-P_1)+P_0(1-P_0)})^2}{(P_1-P_0)^2}$. The exposure rate = 20.5%,[13] OR = 2.179,[14] α = 0.05 (bilateral), Z_α = 1.96, Z_β = 1.28, and N = 176. To prevent the research process does not cooperate with the investigation or the questionnaire does not meet the requirements, 310,536 people finally met the requirements. All study participants were recruited between January and December 2017 from the Heart Center of The First Affiliated Hospital of Xinjiang Medical University. The diagnosis of CHD was based on the coronary artery angiography and the clinical manifestation. CHD patients were confirmed by coronary angiography (≥50% diameter stenosis in at least one of the major coronary arteries) according to the American Heart Association criteria for the confirmation of CHD. Healthy subjects were randomly selected from the occupational population, who received physical examination in our hospital and matched to cases on the basis of age (±3 years) and sex. Finally, 536 subjects who had no medical history of CVDs were selected as the control group after medical history inquiry, physical examination, cardiac ultrasound, electrocardiogram, and other blood biochemical examinations in the hospital. All subjects used in this study were employees aged from 18 to 70 years, who had been working for more than 1 year and had no history of significant concomitant diseases, including cardiomyopathy, bleeding disorders, renal failure, thyroid disease, pulmonary hypertension, and malignancy.

This study protocol that conformed to the Declaration of Helsinki was approved by the Ethics Committee of Xinjiang Medical University, and signed informed consents were obtained from all participants. All authors had no access to information that could identify individual participants after data collection.

Methods

Questionnaire Survey

An epidemiological survey questionnaire was used to collect data from the patients in addition to the review of clinical data. The face-to-face interviews were conducted between a specially trained researcher and each patient at the Hospital Inpatient Department and Physical Examination Center. To reduce potential information bias, the researcher was required to sit together with each patient and explain the questions to them. Finally, the interviewers checked the questionnaires and asked the patients whether there were any inconsistencies in the answers provided. Best efforts were made to ensure the authenticity and integrity of the data collected on occupational features. The data of demographic characteristics (eg, sex, ethnic group, marital status, educational levels, and length of service), lifestyle (eg, smoking, drinking, dietary habit, and physical exercises), history of illness (hypertension and diabetes), family history of illness (hypertension, CHD, and diabetes), and occupational stress were collected. Diabetes mellitus was diagnosed if the fasting plasma glucose ≥7.0 mmol/L and (or) 2-hour postprandial glucose (2hPG) was ≥11.1 mmol/L, or if the diagnosis had been previously confirmed. Hypertension was diagnosed if the systolic blood pressure ≥140 mm Hg and (or) diastolic blood pressure ≥90 mm Hg, or if the diagnosis had been previously confirmed. The patients were stratified by body mass index (BMI) into normal (≤24), overweight (>24 and ≤28), and obese groups (>28). Smoking habits were defined as continuous or accumulating smoking for 6 months and above and for at least one cigarette daily. Drinking habit was defined as alcohol consumption of at least on time per week, regardless of the type of wine. Occupational stress was evaluated by effort-reward imbalance (ERI) questionnaire (Chinese version).[15] For the items evaluating efforts and reward factors, a Likert 5-point scale was used, and 1–5 were assigned. For the items evaluating the burden factor, a Likert 4-point scale was used, and 1–4 were assigned. The score for each factor was the sum of the scores of all relevant items. The total score of ERI was summation of the scores for each factor. ERI ratio ≥1.00 was defined as the high occupational stress group due to ERI; ERI ratio <1.00 was defined as the low occupational stress group.[16]

Yang et al[17,18] have proved that ERI model can better diagnose and interpret psychosocial factors in the workplace.

TABLE 1. Polymerase Chain Reaction Direct-Sequencing Primer Information

Name	Primer Sequences (5′–3′)	Size (bp)
rs10757278-F	AAGGGCATT AAGAAAGGGATGG	400
rs10757278-R	TATGAATGATAGCTCAACTAG	

TABLE 2. Comparison of Demographic Characteristics Between the Case Group and Control Group

Variable	Control Group (n = 536)	Case Group (n = 310)	*P*
Age			0.361
≤40	62 (11.6)	28 (9.0)	
41–50	186 (34.7)	102 (32.9)	
≥51	288 (53.7)	180 (58.1)	
Gender			0.354
Male	416 (77.6)	249 (80.3)	
Female	120 (22.4)	61 (19.7)	
Ethnic group			0.576
Han	339 (63.2)	202 (65.2)	
Uygur	197 (36.8)	108 (34.8)	
Education levels			0.275
Junior college and below	242 (45.1)	152 (49.0)	
Undergraduate and above	294 (54.9)	158 (51.0)	
Length of service			0.414
<20	138 (25.7)	72 (23.2)	
≥20	398 (74.3)	238 (76.8)	
Marital status			0.878
Single	138 (25.7)	75 (24.2)	
Married	377 (70.3)	223 (71.9)	
Divorced or widowed	21 (3.9)	12 (3.9)	

In general, the Cronbach alpha coefficient should be above 0.7,[19] >0.8 means excellent internal consistency, 0.6–0.8 means good, while <0.6 means poor.[20] Studies have confirmed that Cronbach alpha coefficients of ERI questionnaire (Chinese version) for all dimensions (efforts, reward, and burden) and total scales are 0.885, 0.897, 0.787, 0.913, and 0.902, respectively, higher than the recommended value of 0.70, indicating that the scale measurement results are reliable. Model fitting indexes Goodness-of-Fit Index, ≥0.85, Tucker-Lewis Index (Non-normed Fit Index) ≥ 0.85, Comparative Fit Index ≥ 0.85, root mean square error of approximation ≤ 0.08, $\chi^2/df \leq 5$ were well fitted.[21] Goodness-of-Fit Index = 0.900, Tucker-Lewis Index = 0.834, Comparative Fit Index = 0.912, root mean square error of approximation = 0.079, $\chi^2/df = 4.652$, indicating that the model fits well. In conclusion, ERI questionnaire (Chinese version) has high reliability and validity in the Chinese population.[22]

The medical records were reviewed, and the laboratory test data of hypertension and diabetes mellitus as well as the

TABLE 3. Comparison of Life Styles Between the Case and Control Groups

Variable	Control Group (n = 536)	Case Group (n = 310)	χ^2	*P*
Smoking				
Yes	121 (22.6)	250 (80.6)	268.993	<0.05
No	415 (77.4)	60 (19.4)		
Drinking				
Yes	226 (42.2)	162 (52.3)	8.06	<0.05
No	310 (57.8)	148 (47.7)		
Physical activity				
No activity	226 (42.2)	148 (47.7)	41.62	<0.05
<3	123 (22.9)	19 (6.1)		
≥3	74 (13.8)	50 (16.1)		
Irregular	113 (21.1)	93 (30.1)		
Sleep quality				
Poor	31 (5.8)	41 (13.2)	15.768	<0.05
General	394 (73.5)	221 (71.3)		
Good	111 (20.7)	48 (15.5)		
Diets				
Light	76 (14.2)	42 (13.5)	3.955	0.138
General	342 (63.8)	181 (58.4)		
Heavy	118 (22.0)	87 (28.1)		

TABLE 4. Comparison of Clinical Indicators Between the Case and Control Groups

Variable	Control Group (n = 536)	Case Group (n = 310)	χ^2	*P*
Hypertension				<0.05
Yes	15 (2.8)	32 (10.3)	21.191	
No	521 (97.2)	278 (89.7)		
DM				<0.05
Yes	9 (1.7)	78 (25.2)	117.385	
No	527 (98.3)	232 (74.8)		
Family history of CHD				<0.05
Yes	78 (14.6)	159 (51.3)	131.451	
No	458 (85.4)	151 (48.7)		
BMI				<0.05
Normal	270 (50.4)	70 (22.6)	86.570	
Overweight	197 (36.8)	130 (41.9)		
Obesity	69 (12.8)	110 (35.5)		

results of coronary angiography were extracted. All data associated with the conventional risk factors of CHD were collected. The medical records were written by a specially trained cardiovascular physician.

Gene Analysis

Venous blood was collected on an empty stomach peripheral 2 mL, thoroughly incorporated into −20°C after keep waiting for inspection DNA from chromosomes. Log in the human HapMap plan database, refer to the phase II database, and use the Haploview 4.2 software to find the tag single nucleotide polymorphism. The genotyping success rate was above 95%, so 9p21 region-related gene rs10757278 was selected. The DNA products were digested, and 3 genotypes (GG, AA, and GA) were obtained. polymerase chain reaction direct sequencing primer information was shown in Table 1.

Statistical Analysis

The data were statistically analyzed using SPSS18.0 software, and Hardy–Weinberg equilibrium law was used to detect the population representation of samples. Categorical variables were presented as absolute values and percentages. Distribution differences were examined by the χ^2 test for categorical variables or 2-tailed Student's *t*-test for normally distributed data and the Mann–Whitney *U* test for skewed data.

The odds ratios (ORs) and 95% confidence intervals (95% CIs) were calculated by binary logistic regression analyses (forward selection: likelihood ratio) to assess the risk factors of CHD, as well as the interactions between rs10757278 and environmental factors in CHD risk. Take the presence or the absence of CHD as the dependent variable (1 = CHD patients; 0 = healthy subjects), all the relevant factors as the independent variables (smoking, 1 = yes, 0 = no; physical exercise, 1 = yes, 0 = no; hypertension, 1 = yes, 0 = no; family history of CHD, 1 = yes, 0 = no; BMI, 1 = normal, 2 = overweight, 3 = obesity; occupational stress, 1 = high occupational stress, 0 = low occupational stress). *P*-value < 0.05 (2-tailed) was considered significant.

RESULTS

The distribution of gene polymorphisms in the 9p21 region in different occupational health population and CHD population in Xinjiang was consistent with the Hardy–Weinberg equilibrium law, indicating that the samples were representative of the population (data not shown).

Comparison of Demographic Characteristics Between the Case and Control Groups

Among 846 subjects, 310 were CHD patients, and 536 were healthy subjects. No significant differences were found in terms of age, sex, ethnic group, education levels, length of service, and marital status between the CHD and control groups (all $P > 0.05$). It suggested that the 2 groups have a good match in the demographic characteristics (Table 2).

Comparison of Environmental Factors Between the Case and Control Groups

Table 3 shows comparison of lifestyle between the case and control groups. Increased CHD risk was associated with smoking, drinking, lack of physical activity, and poor sleep quality. In addition, statistically significant differences in the composition of hypertension, diabetes, family history of CHD, and BMI were found between 2 groups (all $P <$ 0.05; Table 4). Differences in occupational stress level

TABLE 5. Comparison of Occupational Stress Between the Case and Control Groups

Subgroup	Control Group (n = 536)	Case Group (n = 310)	χ^2	*P*
High occupational stress group	282 (52.6)	175 (56.5)	1.166	0.28
Low occupational stress group	254 (47.4)	135 (43.5)		

TABLE 6. Comparison of rs10757278 Genotype Between the Case Group and Control Groups

Variable	Control Group (n = 536)	Case Group (n = 310)	χ^2	*P*
rs10757278				
AA	148 (27.6)	73 (23.5)	17.905	<0.05
GG	114 (21.3)	107 (34.5)		
GA	274 (51.1)	130 (42.0)		

between 2 groups were not statistically significant ($P > 0.05$; Table 5).

Comparison of rs10757278 Genotype Between the Case and Control Groups

The genotype of rs10757278 was statistically different between the case and control groups ($P < 0.05$; Table 6).

Logistic Regression Analysis on the Risk Factors of CHD

Binary logistic regression analysis was used to evaluate the risk factors of CHD. After adjustment for age and sex, significant increased risk effects for CHD were found to be associated with smoking (OR = 2.311; 95% CI: 1.04–2.499; $P < 0.001$), physical exercise (OR = 1.365; 95% CI: 1.137–1.639; $P < 0.001$), hypertension (OR = 4.627; 95% CI: 2.165–10.764; $P < 0.001$), family history of CHD (OR = 4.103; 95% CI: 3.169–6.892; $P < 0.001$), BMI (OR = 2.484; 95% CI: 2.036–3.03; $P < 0.001$), and GG genotype at rs10757278 (OR = 1.978; 95% CI: 1.413–2.769; $P < 0.001$). Thus, a total of 6 factors were selected for the evaluation the risk factors of CHD (Table 7).

Interactions Between rs10757278 and Environmental Factors in CHD Risk

Table 8 shows interactions between GG genotype at rs10757278 and environmental factors in CHD risk. After adjustment for age and sex, we noted that a significant interaction between GG genotype at rs10757278 and smoking, hypertension, family history of CHD, and BMI is associated with the CHD.

DISCUSSION

CHD is caused by a combination of genetic and environmental factors, especially complex diseases. Xinjiang is a high prevalence area of cardiovascular disease in China. The prevalence of CHD varies with ethnic, sex, and geographical factors.[23] Therefore, this study was conducted to provide theoretical basis for prevention of CHD in population with rs10757278 gene.

By analyzing the risk factors of CHD, this study found that smoking, physical exercise, hypertension, family history of CHD, BMI, and GG genotype at rs10757278 were risk factors of CHD. GHATGE found[24] that some unhealthy behavioral factors can also increase the risk of CHD. For example, smoking can accelerate atherosclerosis by affecting endothelial cells. Rona et al[25] reported that smokers had a higher risk of cardiovascular disease, a longer duration than nonsmokers, and were the most important risk factor for myocardial infarction in young- and middle-aged population. A lot of studies have identified appropriate intensity exercise can effectively reduce the risk of hypertension, CHD, and other CVDs.[26] Hypertension is an independent risk factor for CHD. Long-term high blood pressure will impact the intima of blood vessels and cause damage to the intima. Lipids in the blood will deposit on the arterial wall and form atherosclerotic plaques, leading to vascular stenosis. Nishitani and Sakakibar[27] reported that BMI may be more likely to lead to CVDs such as hypertension.

Genome-wide association studies found that the CHD susceptibility gene was located on chromosome 9p21, which was further confirmed in different populations in 2007.[28–30] Akan et al[31] found that GG genotype in rs10757278 indicated a significant 4-fold increased risk of CAD ($P < 0.0001$). Shendy et al[32] studied the association of rs10757278 and rs2383206 single nucleotide polymorphism on Chr9p21 with the incidence of CAD in the presence and absence of type 2 diabetes (T2D) in Egyptians and showed that rs10757278/

TABLE 7. Logistic Regression Analysis on the Risk Factors of CHD

Influencing Factor	B-Value	S_b	Wald χ^2	*P*	OR	95% CI
Age*	0.272	0.096	8.003	0.005	1.312	1.087–1.584
Gender*	0.198	0.1	3.931	0.047	1.219	1.002–1.483
Smoking	0.792	0.148	27.137	<0.001	2.311	1.04–2.499
Physical exercise	0.311	0.093	11.159	<0.001	1.365	1.137–1.639
Hypertension	1.574	0.409	14.804	<0.001	4.627	2.165–10.764
Family history of CHD	1.542	0.198	60.497	<0.001	4.103	3.169–6.892
BMI	0.91	0.101	80.392	<0.001	2.484	2.036–3.03
rs10757278						
AA	0.039	0.178	0.047	0.828	1.04	0.733–1.475
GG	0.682	0.172	15.799	<0.001	1.978	1.413–2.769
GA	—	—	—	—	1.00 (ref)	—

*Drinking, sleep quality, diets, DM, and occupational stress were not statistically significant after adjustment for age and sex.
DM, diabetes mellitus.

TABLE 8. Interactions Between GA Genotype at rs10757278 and Environmental Factors in CHD Risk

Influencing Factor	B-Value	S_b	Wald χ^2	*P*	OR	95% CI
GG* smoking	1.41	0.227	38.684	<0.001	4.094	2.626–6.384
GG* physical exercise	0.016	0.021	0.59	0.442	1.016	0.976–1.058
GG* hypertension	1.535	0.705	4.744	0.029	4.641	1.166–18.473
GG* family history of CHD	1.414	0.31	20.866	<0.001	4.114	2.242–7.547
GG* BMI	1.768	0.167	25.872	<0.001	3.769	2.976–3.671
GG* occupational stress	−0.204	0.232	0.773	0.379	0.816	0.518–1.285

*There was no significant interaction between GG genotype at rs10757278 and drinking, sleep quality, diets, and DM after adjustment for age and sex.
DM, diabetes mellitus.

rs2383206-G allele increased the risk for CAD in Egyptians. Liu et al[33] found rs10757278-G increased the risk of CAD in patients indicated by an OR = 1.242 (95% CI = 1.04–1.49). Ivanova et al[34] used case–control to study the relationship between rs10757278 (9p21) and myocardial infarction and found that GG of rs10757278 is associated with an increased sudden cardiac death risk in men. Duan et al[12] studied the correlation between rs10757278 single-nucleotide polymorphism on chromosome 9p21 and CHD in Han population in Kunming plateau region and found that GG genotype was a risk genotype of CHD. Our study found GG genotype at rs10757278 associated with risk of CHD increased significantly in Xinjiang, consistent with the above study. Thus, we further conducted gene–environment interaction analysis because CHD is caused by a combination of genetic and environmental factors.

Table 8 shows interactions between GG genotype at rs10757278 and environmental factors on CHD risk. We noted that a significant interaction between GG genotype at rs10757278 and smoking, hypertension, family history of CHD, and BMI is associated with the CHD. Current smokers with GG genotype have the highest CHD risk, compared to never smokers with GG genotype. Similarly, hypertension, family history of CHD, and BMI were the same results. Therefore, we suggest that people carrying GG gene should quit smoking, pay attention to the stability of blood pressure, and the occurrence of obesity.

In conclusion, GG genotype at rs10757278 is a risk factor for CHD. And there is an interaction between GG genotype of rs10757278 in region 9p21 gene and traditional risk factors.

Limitations

Our study was limited in some ways. First, this was a case–control study that analyzed the interaction effects of rs10757278 polymorphisms at 9p21 locus and traditional risk factors on CHD, through which no causal relationship could be determined. Second, only one of single gene loci was selected to analyze gene–environment effect on CHD. However, CHD is a polygenic disease with intergene interactions, so this study is bound to be affected by other genes.

Future Directions

There are differences between cardiovascular disease and 9p21 region gene in different countries and the ethnic groups. Our study confirmed the existence of the correlation between cardiovascular disease and 9p21 region gene in Xinjiang, China. In the future, we will first consider conducting cohort studies to discover causation; second, further studies need to select more genes to verify the influence of gene environment on CHD. Finally, the statistically significant interaction positive results in this study can further analyze the molecular mechanisms involved.

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