

Original Article

Association between single nucleotide polymorphisms of polyunsaturated fatty acids metabolic rate-limiting enzyme FADS1 and FADS2 genes and coronary heart disease

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Abstract: Objective: This study aims to investigate the association between the single nucleotide polymorphism (SNPs) of polyunsaturated fatty acids metabolic rate-limiting enzyme FADS1 and FADS2 genes and coronary heart disease (CHD). Methods: 240 patients diagnosed as CHD and 240 healthy controls from the Han population in Northern China were selected. Questionnaires were used to collect the basic information about the patients. PCR was applied to detect the rs174556 SNP of FADS1 and rs174617 SNP of FADS2. The combined effect of two loci and frequency distribution of alleles and genotypes were analyzed. Results: Basic information including hyperlipidemia, hypertension, serum total cholesterol, triglyceride, fasting blood-glucose (FBG) had significant difference between case group and control group ($P < 0.05$). The genotypic frequency distribution of rs174556 was significantly different between case group and control group ($P < 0.05$), while that of rs174617 showed no significant differences between the two groups ($P > 0.05$). Linkage disequilibrium (LD) of the rs174556 SNP of FADS1 and rs174617 SNP of FADS2 was analyzed with genetic software. The result was $D = 0.57$, $r^2 = 0.53$, which indicated that the two loci were located in different regions and exhibited gene interaction. The combined effect analysis of gene SNPs in case group and control group showed that the combined effect of the rs174556 SNP of FADS1 and rs174617 SNP of FADS2 was not significantly associated with CHD ($P > 0.05$). Regression analysis in case group and control group indicated that hypertension, hyperlipidemia, age and FADS1 SNP were the main risk factors of CHD ($P < 0.05$). Conclusion: Gene polymorphism of rs174556 in FADS1 gene is likely to have association with CHD among Chinese Han population in North China. Since CHD is influenced by various factors like heredity and environment, larger sample size is needed for further investigation.

Keywords: Polyunsaturated fatty acids metabolic rate-limiting enzyme, FADS1 gene, FADS2 gene, single nucleotide polymorphisms, coronary heart disease

Introduction

Coronary Heart Disease (CHD) is a common cardiovascular disease with syndromes of angina pectoris and myocardial impairment mainly caused by coronary insufficiency, temporary or acute hypoxia and ischemia [1, 2]. The incidence of CHD in China has greatly increased, especially among middle aged and elderly people. CHD development is associated with various factors including heredity, environment and unhealthy living habits. In recent years a growing number of genetic studies have confirmed that genotyping associated with CHD mainly affects CHD development in thrombogenesis,

vascular contraction and expansion, lipid metabolism and inflammation. It was also reported that polyunsaturated fatty acids metabolic rate-limiting enzymes FADS1 and FADS2 had effects on the entire metabolic pathways of unsaturated fatty acid and body lipid level [3]. The morbidity of CHD among South Korean was reported to be closely related to FASD gene cluster polymorphisms.

In the study 240 patients diagnosed as CHD and 240 healthy controls from the Han population in Northern China were selected. Questionnaires were used to collect the basic information about the patients, and PCR was

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Table 1. Basic information in case group and control group

Group	Case Number	Hyperlipidemia	Hypertension	Serum Total Cholesterol cB/(mmol.L ⁻¹)	Triglyceride cB/(mmol.L ⁻¹)	FBG cB/(mmol.L ⁻¹)
Case	240	95	86	4.73±3.88	1.29±0.88	5.28±0.89
Control	240	116	134	4.51±1.35	1.48±1.45	6.02±1.43

Table 2. Frequency distribution of alleles and genotypes in case group and control group

Group	rs174556					rs174617 Locus				
	C	T	C	C/T	T/T	C	T	C/C	C/T	T/T
Case	320 (66.67)	160 (33.33)	114 (47.5)	92 (38.3)	34 (12.14)	348 (72.50)	132 (27.50)	135 (56.25)	78 (32.50)	27 (11.25)
Control	297 (61.88)	183 (38.13)	95 (39.58)	107 (44.58)	38 (15.83)	378 (78.75)	102 (21.25)	145 (60.42)	88 (33.67)	7 (2.92)

PS: Case group and control group.

applied to detect the rs174556 SNP of FADS1 and rs174617 SNP of FADS2. The association between single nucleotide polymorphisms (SNPs) of polyunsaturated fatty acids metabolic rate-limiting enzyme FADS1 and FADS2 genes and coronary heart disease (CHD) was analyzed.

Materials and methods

General information

From January 2010 to November 2011, 240 patients diagnosed as CHD in our hospital and healthy controls were selected. 240 cases included 126 males and 114 females. The patients were aged 59 to 69 years, with an average of 60.1±6.4 years. Meanwhile, 240 healthy controls included 121 males and 119 females. The healthy controls were aged 53 to 72 years, with an average of 62.8±6.9 years. General information of cases and controls including age, gender, educational level and clinical data on admission after routine physical checkup was comparable but not statistically significant ($P>0.05$). Written informed consent was obtained from all subjects involved in the study and researchers included were all qualified by hospital's training evaluation on research contents. The inclusion criteria were as follows [4-6]: 1) The included patients met diagnostic criteria for CHD of 1979 WHO/ISFC. 2) The patients with acute or chronic infection in a short time, or those with critical basic diseases of liver, kidney or lung were excluded. 3) There was no acute or chronic infection, anti-platelet drug use, bleeding or blood transfusion events within 3 months since admission. 4) The patients with circulatory failure on admission

were excluded. 5) There was no history of hypertension, allergy or allergic condition.

Methods

Five milliliter venous blood samples were obtained from subjects fasting for over 12 h early in the morning. E.Z.N.A.®SE Blood DNA Kit from Shanghai Solarbio Bioscience & Technology Co., LTD was used for blood DNA extraction. The rs174556 SNP of FADS1 and rs174617 SNP of FADS2 were obtained from NCBI dbSNP, with the primer sequence at rs174556 of F5'AAGCAGGGACCTCAAGAC-3'; R5'-AGCCCACCAAGAATGTAA-3', and that at rs174617 of F5'-GAACTGTGAGAGGCAACG-3'; R5'-CTGGGCAATAAAGCAAGA-3. SNPs were detected by polymerase chain reaction-restriction fragment length polymorphism: 1) PCR amplification: 94°C for 10 min, 94-95°C for 35-40s, 56-62°C for 1 min, and 72°C for 1 min, totally 40 cycles. The last amplification was completed under 72°C for 10 min. 5 ul PCR product was analyzed by 1% agarose gel electrophoresis with DL2000 Marker and detected by gel imaging analysis system. 2) Endonuclease digestion: restriction enzymes MboI and MspI were used for digestion. The C allele of the rs174556 SNP of FADS1 and rs174617 SNP of FADS2 created restriction sites and the 3 genotypes of C/C, C/T and T/T. The combined effect of two loci and frequency distribution of alleles and genotypes were analyzed.

Statistical analysis

Conjoint analysis of the rs174556 SNP of FADS1 and rs174617 SNP of FADS2 was carried out using UNPHASED 3.012 Software.

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Table 3. Conjoint analysis of gene SNPs in case group and control group

Group	Case	Control	χ^2	P
rs174556 (C/C)-rs174617 (T/T)	78	91	2.93	0.08
rs174556 (C/C)- rs174617 (C/T)	18	23	1.04	0.33
rs174556 (C/C)- rs174617 (C/C)	54	46	0.36	0.56
rs174556 (C/T)-rs174617 (T/T)	53	42	0.95	0.31
rs174556 (T/T)-rs174617 (C/T)	17	13	0.41	0.52

Table 4. Regression analysis on the risk factors for CHD in case group and control group

Variable	β Value	P Value	OR Value
Gender	-0.175	0.689	0.836
Age	-1.568	0.032	0.567
Hypertension	2.072	0.000	7.957
Hyperlipidemia	-1.418	0.058	0.243
FADS1 SNP	2.432	0.001	11.452

Qualitative data were analyzed by χ^2 test. Statistical analysis was performed using SPSS 17.0 software and $P < 0.05$ indicated significant difference.

Results

Basic information in case and control groups

Basic information, which included hyperlipidemia, hypertension, serum total cholesterol, triglyceride and fasting blood-glucose (FBG), showed significant differences between case group and control group ($P < 0.05$, **Table 1**).

Frequency distribution of alleles and genotypes in case group and control group

Results of χ^2 goodness of fit test showed that the frequency distribution of alleles and genotypes of the rs174556 SNP of FADS1 and rs174617 SNP of FADS2 conformed to Hardy-Weinberg equilibrium ($P > 0.05$). The genotype frequency distribution of rs174556 in case group and control group was significantly different ($P < 0.05$), while that of rs174617 in the two groups was not significantly different ($P > 0.05$, **Table 2**).

Conjoint analysis of gene SNPs in case and control groups

Linkage disequilibrium (LD) of the rs174556 SNP of FADS1 and rs174617 SNP of FADS2 was detected with genetic software, and the result

was $D' = 0.57$, $r^2 = 0.53$, indicating that the two loci were located in different regions and exhibited gene interaction. The combined effect analysis of gene SNPs in case group and control group showed that the combined effect of the rs174556 SNP of FADS1 and rs174617 SNP of FADS2 was not significantly associated with CHD ($P > 0.05$, **Table 3**).

Regression analysis on the risk factors for CHD in case group and control group

Regression analysis in case group and control group indicated that hypertension, hyperlipidemia, age and FADS1 SNP were the main risk factors of CHD ($P < 0.05$, **Table 4**).

Discussion

CHD is a systemic chronic angiocardopathy caused by local coronary atherosclerosis and related myocardial ischemic-anoxic injury. CHD grade analysis is carried out according to the coronary artery obstruction degree. Genome-wide association study on CHD patients suggested that decrease of body lipid like cholesterol was closely associated with minor allele carriers of SNPs on FADS gene cluster. Polymorphisms of polyunsaturated fatty acids metabolic rate-limiting enzyme FADS1 and FADS2 genes on chromosome 11 were closely related to body arachidonic acid level. It was reported that fat level increase in CHD patients was bound up with the incidence of myocardial infarction [7]. In the study, the rs174556 locus of FADS1 was found to be closely related to CHD development. Furthermore, individuals with T minor allele had higher risks of developing CHD owing to FADS1 activity's direct influence on unsaturated fatty acid synthesis. Studies have reported that CHD patients with T minor allele on rs174556 of FADS1 have higher levels of linoleic acid and α -linolenic acid, but have lower arachidonic acid level [8]. Meanwhile, foreign researchers have studied on the association between polymorphisms of 3 alleles including rs174556 of FADS1 gene cluster and plasma fatty acid and erythrocyte membrane fatty acid levels in 571 subjects [9]. Results indicated that the rs174556 and rs174561 of FADS1 gene clusters were closely related to arachidonic acid level after multivariate exclusion. Moreover, rs174556 of FADS1 gene cluster was strongly related to arachidon-

ic acid level by influencing inflammatory medium and then CHD development, which indirectly confirmed our results in the study and may account for their association.

However, further studies are required to detect plasma fatty acid levels of the subjects and analyze effects of FADS1 gene cluster polymorphisms among Han population in Northern China on the arachidonic acid level and its changes. In addition, it was reported that body γ -linolenic acid, timnodonic acid and other polyunsaturated fatty acids decreased owing to the reduced activity of fatty acid desaturase in synthetic process, which may cause CHD development [10, 11]. Lattka E et al. have reported that risk allele frequency of FADS gene cluster of CHD patients is significantly higher than that of healthy controls, consistent with our results [12].

In conclusion, gene polymorphism of rs174556 in FADS1 gene is likely to have association with CHD among Chinese Han population in North China. Since CHD is influenced by various factors like heredity and environment, larger sample size is needed for further investigation.

Disclosure of conflict of interest

None.

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