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Original Article

Correlation between CX3CR1 249V/I polymorphism and premature coronary heart disease & blood lipid ratio

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Abstract: This study aimed to explore the correlation between CX3CR1 249V/I polymorphism of Fractalkine receptor and premature coronary heart disease (PCHD) & blood lipid ratio. Eligible patients were divided into PCHD group (n=447, age <50 years), late-onset coronary heart disease (LCHD) group (n=450, age >65 years) and healthy control group (n=447, age of 47-93 years old). The blood lipid level was detected in all. The total cholesterol/high density lipoprotein cholesterol (TC/HDLC) and the apolipoprotein B/apolipoprotein A1 (ApoB/ApoA1) were calculated. The CX3CR1 249V/I polymorphism distribution was analyzed by polymerase chain reaction-restriction fragment length polymorphism analysis (PCR-RFLP) method. The differences in CX3CR1 gene polymorphism and blood lipid ratio among the three groups were compared. The difference in distribution frequency of allele I249 among the three groups was statistically significant (P<0.0001); the TC/HDLC and the ApoB/ApoA1 ratios of PCHD group were significantly higher than those of LCHD group (P<0.0001) independent of 249V/I gene polymorphic variation. The allele I249 variation of CX3CR1 was correlated with the onset age of CHD. High blood lipid ratio was correlated with the onset age of CHD.

Keywords: CX3CR1, gene polymorphism, coronary heart disease (CHD), blood lipid ratio

Introduction

As an important factor in the occurrence and development of coronary heart disease (CHD), the inflammatory reaction has attracted many eyes recently [1, 2]. The chemokine Fractalkine and its receptor CX3CR1 mediate the emigration, adhesion and aggregation of leucocyte in the inflammatory process, mediate the inflammatory process, and participate in pathophysiological processes of atherosclerotic plaque formation, rupture and subsequent thrombus state, etc. [3-5]. Foreign studies indicate that CX3CR1 has two polymorphic sites, 249V/I and 280T/M. The variation of these two alleles has significant influence on CX3CR1 and the affinity of Fractalkine, which further influences the occurrence and development of atherosclerosis of coronary artery [6-8]. A study in China also reports that the allele I249 variation of CX3CR1 is related to the drop in CHD prevalence [9]. However, there is no report on the correlation between CX3CR1 polymorphic variation and the onset age of CHD. Moreover, a study of Chinese scholar shows that the blood lipid ratio is an important risk factor of myocardial infarction. The high blood lipid ratio is correlated with the instability and rupture of coronary plaque [10-13]. However, no domestic literature reports the relationship between the blood lipid ratio and the CHD onset age. This paper aimed at analyzing the distribution of CX3CR1 249V/I polymorphism in PCHD group, LCHD group and healthy control group, and further analyzing the difference in the blood lipid level and blood lipid ratio among the three groups.

Subjects and methods

Subjects

Patients hospitalized in Department of Cardiology in our hospital and some individuals having physical examination in our hospital were selected, all of which were Chinese Han. Patients conforming to any of the following items were classified into the case group: ① The blood vessel diameter stenosis ≥50% for at least one branch was confirmed by coronary

Table 1. Characteristics of the participants

Characteristics	PCHD group	LCHD group	control group
Age (year)	44.9 ± 4.1	72.8 ± 5.3	67.2 ± 6.1
Smoking	17.70%	24.90%	16.80%
SBP (mmHg)	121.63 ± 21.32	122.72 ± 20.90	115.32 ± 20.23
DBP (mmHg)	81.29 ± 12.54	79.34 ± 15.27	75.46 ± 16.38
BMI (kg/m^2)	23.89 ± 3.46	23.95 ± 2.57	22.94 ± 3.76
TC (mmol/L)	4.93 ± 1.09	5.11 ± 0.79	4.94 ± 1.06
LDLC (mmol/L)	3.19 ± 1.10	3.23 ± 0.54	3.22 ± 0.93
HDLC (mmol/L)	0.94 ± 0.27	1.20 ± 0.39	1.35 ± 0.45

Table 2. Genotype of 249V/I distributions in the three group participants

Genotype	PCHD group (n=447)	LCHD group (n=450)	Control group (n=447)
VV	215 (48%)[a,b]	140 (31%)[b]	113 (25%)[b]
VI	193 (43%)	265 (59%)	276 (62%)
II	39 (9%)	45 (10%)	58 (13%)
VI+II	228 (52%)[a,b]	310 (69%)[b]	334 (75%)[b]

angiography (CAG); ② Acute myocardial infarction; ③ Old myocardial infarction. Those with age <50 years were classified into the premature coronary heart disease (PCHD) group (n=447, aged 44.9 ± 4.1 years on average). Those with age >65 years were classified into the late-onset coronary heart disease (LCHD) group (n=450, aged 72.8 ± 5.3 years on average). Individuals conforming to any of the following items were classified into the healthy control group (n=447, aged 47-93 years, 67.2 ± 6.1 years on average): ① No vascular lesion confirmed by CAG; ② No clinical symptoms related to CHD; hospitalized patients due to other diseases, or healthy examinees with normal ECG, treadmill test and UCG. Since common traditional risk factors of CHD may have bias influence on test data, subjects conforming to any of the followings were excluded: ① Total cholesterol (TC) >7.2 mmol/L; ② Diabetes; ③ Systolic pressure >180 mmHg and (or) diastolic pressure >100 mmHg; ④ Having confirmed hepatic/renal insufficiency or thyroid disease.

Auxiliary examination

The blood lipid level of all selected cases was detected. TC, high density lipoprotein cholesterol (HDLC), apolipoprotein B (ApoB) and apolipoprotein A 1 (ApoA1) were determined. The TC/HDLC ratio and the ApoB/ApoA1 ratio were calculated.

Genome DNA extraction of whole blood

The genome DNA was extracted by blood cell Genomic Purification Kit (TaKaRa Company).

Analysis on CX3CR1 gene polymorphism

The CX3CR1 fragment containing 249V/I restriction enzyme cleavage site was amplified by PCR method. The forward primer was 5'-CCG AGGTCCTTCAGGAAATCT-3'. The reverse primer was 5'-TCAGCATCAGGTTCAGGAACTC-3'. PCR conditions: initialization at 94°C for 1 min; denaturation at 94°C for 30 s, annealing at 50°C for 40 s, and extension at 72°C for 55 s, a total of 34 cycles; and final elongation at 72°C for 5 min. The system was saved at 4°C. The sequencing was conducted for the PCR product. The overall length was 588 bp. The PCR product was confirmed as the target gene through BLAST in NCBI, and conformed to the project design. The digestion was conducted at the cleavage sites of 249V/I and 280T/M by the restriction enzyme PspI406I. The results indicated that among all 249V/I polymorphic sites, VV homozygote had two bands of 205 bp and 383 bp; II homozygote had only one band of 588 bp; VI heterozygote had three bands of 205 bp, 383 bp and 588 bp.

Statistical analysis

Measurement data were presented as $\bar{x}$ ± s. t-test was adopted for comparison between two groups. The analysis of variance was adopted for comparison among multiple groups. X^2 test was adopted for enumeration data. The significance level was 0.05.

Results

Comparison of clinical data among three groups

The difference in smoking proportion, blood pressure level, body mass index (BMI), TC and low density lipoprotein cholesterol (LDLC) level among three groups was not statistically significant (P>0.05). There was a large difference in

Table 3. Comparison of TC/HDLC and ApoB/ApoA1 among the three groups

Characteristics	PCHD group (n=447)	LCHD group (n=450)	control group (n=447)	*P* value
TC/HDLC	7.59 ± 2.02	6.38 ± 1.82	4.36 ± 1.25	<0.0001
ApoB/ApoAI	1.05 ± 0.35	1.01 ± 0.35	0.72± 0.18	<0.0001

age among three groups. The difference in the HDLC level among three groups was statistically significant (P<0.05). Healthy control group had the highest HDLC level, and LCHD group was secondary, followed by PCHD group (**Table 1**).

Genotype analysis on the polymorphic site 249V/I of CX3CR1 among three groups

The I249 allele frequency of the healthy control group and the LCHD group was significantly higher, while that of the PCHD group was the lowest. The differences in the genotype frequency of allele I249 and allele V249 among three groups were statistically significant (P<0.0001; **Table 2**). Multifactor logistic regression analysis revealed that CX3CR1 249V/I polymorphism was related to the onset age of CHD, and that the distribution frequency of VI+II genotype in the PCHD group was significantly lower than that in the LCHD group (OR=0.248, 95% CI: 0.107-0.565, P=0.003), which was independent of the rest risk factors of CHD.

Analysis on TC/HDLC and ApoB/ApoA1 of three groups

The PCHD group, the LCHD and the healthy control group had significantly higher, lower and the lowest blood lipid ratio, respectively. The difference among three groups was statistically significant (P<0.0001; **Table 3**). Eligible subjects of three groups were further divided as per VV and VI+II genotype, and their blood lipid ratios were compared. The blood lipid ratios of two genotypes in each group were very similar without statistical significance (P>0.05). However, the difference in the blood lipid ratio of each genotype among three groups was statistically significant, and the distribution trend was consistent (PCHD group > LCHD group > Healthy control group, P<0.0001; **Table 4**). The significant difference and the distribution trend of blood lipid ratio among three groups were not changed due to the polymorphic variation of the 249V/I genetic locus. This further illustrated that the correlation between CX3CR1 249V/I polymorphic variation and CHD onset age was not influenced by the blood lipid level.

Discussion

In recent years, the role of inflammatory reaction in the pathogenesis of CHD has drawn widespread attention. Therefore, in-depth exploration and study have been conducted on the roles that monocytes, T cells, natural killer cells and chemokine play in the inflammatory reaction of atherosclerosis [14-17]. Chemokine receptors mediate the process of inflammatory reaction through mediating the chemotaxis combination of lymphocytes, monocytes and endothelial cell surface of the vascular wall. The allele variation of certain chemokine receptors may lead to the change in the binding force between inflammatory cells and vascular endothelial cells, further influencing the course of atherosclerosis [18, 19].

Fractalkine is a membrane-bound chemokine. Most Fractalkine is expressed on the surface of activated endothelial cells. Fractalkine has the function of both chemotaxis and adhesion. Imai et al. find that CX3CR1 is a high-affinity receptor of Fractalkine. Cells expressing CX3CR1 can rapidly combine with membrane-bound Fractalkine or cells expressing Fractalkine with high affinity, and participate in the migration of leucocytes to the inflammation tissue. There is genetic variation of 5 single nucleotide polymorphic sites in the CX3CR1 transmembrane domain. The linkage disequilibrium is found for 2 genetic mutations-249V/I and 280T/M. The variation of polymorphic sites 249V/I and 280T/M obviously influences the affinity of CX3CR1 and Fractalkine, and further influences the migration, adhesion and aggregation of leukocytes in the inflammation. The variation of these two polymorphic sites is closely related to the occurrence and development of patients' atherosclerosis.

There are still controversies about the role of CX3CR1 gene polymorphism in the occurrence and development of CHD. In recent years, foreign scholars have discussed the correlation between CX3CR1 gene polymorphism and the susceptibility to atherosclerosis to varying degrees. Some believe that the varia-

Table 4. Comparison of TC/HDLC and ApoB/ApoA1 between the two genotypes in the three group

Characteristics	PCHD group		LCHD group		Control group		*P* value
	VV (n=215)	VI+II (n=228)	VV (n=140)	VI+II (n=310)	VV (n=113)	VI+II (n=334)	
TC/HDLC	7.64 ± 1.99	7.57 ± 2.25	6.67 ± 1.81	6.23 ± 1.76	4.28 ± 1.42	4.41 ± 1.13	<0.0001
ApoB/ApoAI	1.07 ± 0.24	1.09 ± 0.26	1.0 ± 0.27	0.96 ± 0.26	0.67 ± 0.30	0.69 ± 1.13	<0.0001

tion of CX3CR1 allele I249 is related to the drop in the occurrence risk of acute coronary syndrome [20-22], which indicates that allele I249 may have protective effect on cardiovascular disease of CHD patients. However, other scholars report that the variation of allele I249 of CX3CR1 is related to the increased risk of acute coronary events and stroke [21, 23].

In the present study, we detected the 249V/I polymorphism and the distribution of blood lipid ratio of the selected population. It is worth noting that since CHD is a chronic disease caused by multi-factors, common traditional risk factors of CHD are excluded upon selecting subjects of the present study. Therefore, the bias caused by the above-mentioned factors to research results is avoided. Moreover, the onset age of CHD was investigated in the present study. The included patients were divided into the PCHD group and the LCHD group according to the age. However, no age limit was set for the healthy control group. Those in the healthy control group did not have CHD and were randomly selected. The present study further explored the correlation between CX3CR1 gene polymorphism, blood lipid ratio and CHD onset age. The results revealed that the variation of the 249V/I polymorphic site of CX3CR1 gene was significantly correlated with the onset age of CHD. The distribution frequency of allele I249 in the CHD group was significantly lower than that in the healthy control group. The PCHD group was also significantly lower than the LCHD group. This indicated that the variation of allele I249 may have protective effect on cardiovascular diseases of CHD patients. The allele I249 variation was correlated with the advance of CHD onset age.

In addition, we compared the blood lipid ratio of three groups, and found that the PCHD group had significantly higher blood lipid ratio; the LCHD had lower blood lipid ratio; the healthy control group had the lowest blood lipid ratio. Many case-control studies at abroad have shown that the blood lipid ratio (especially ApoB/ApoA1) is the most important risk factor of myocardial infarction [10-13]. The results of our study showed that high blood lipid ratio was obviously correlated with CHD, which was especially evident in PCHD patients.

In summary, the results of the present study showed that the variation of allele I249 of CX3CR1 gene may not only reduce the onset risk of CHD, but also be correlated with the CHD onset age. High blood lipid ratio was correlated with the CHD onset age. However, the above-mentioned experimental results are not sufficient to draw the causal relationship between the polymorphic variation of chemokine receptor and the atherosclerosis. But the possibility of gene target therapy for atherosclerosis can be expected. In consideration of the restrictions of sample size, region, gender and race, and the diversity of CHD susceptbility genes, a multi-center study with a large sample size is needed to precisely display the causal relationship between CX3CR1 polymorphism and CHD susceptibility.

Disclosure of conflict of interest

None.

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