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Original Article

Correlation between GDF 15 gene polymorphism and the collateral circulation in acute non-ST segment elevated myocardial infarction

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Abstract: Objective: To investigate the correlation between growth differentiation factor 15 (GDF 15) + 157 A/T polymorphism and the formation of collateral circulation in acute non-ST segment elevated myocardial infarction in Han population of Shandong province. Method: The medical records of 200 cases of patients undergoing selective coronary angiography were analyzed, and the arterial blood specimens of included patients were collected before coronary angiography. Based on the results of coronary angiography, patients were divided into acute myocardial infarction (AMI) group and normal control group; AMI group was divided into collateral group and non-collateral group by Rentrop's grading method; polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) and DNA sequencing methods were used to analyze the GDF 15 + 157 A/T polymorphism in the two groups. Results: There were statistically significant differences in GDF 15 + 157 A/T AA and AT distribution between AMI group and the control group (P = 0.002); and there was statistically significant difference in allele frequencies between the two groups (P = 0.006); for AMI group, there were statistically significant differences in GDFAA and AT genotype distribution between patients with and without collateral (P = 0.014), and there was statistically significant difference in allele frequencies between the two (P = 0.025). Conclusion: There was correlation between GDF 15 + 157 A/T polymorphism and the formation of collateral circulation in patients with non-ST-segment elevated myocardial infarction.

Keywords: Acute myocardial infarction, GDF 15, Gene polymorphism, SNPs, PCR-RFLP, Collateral circulation

Introduction

In acute non-ST-segment elevated myocardial infarction, coronary thrombosis and branch vessel blockage occurred, resulting in myocardial necrosis in the corresponding region dominated by the clogged blood vessels. Early and timely construction of collateral circulation plays an important role in regulating the infarct size. This re-establishment of collateral circulation can ensure the early myocardial activity in the corresponding region dominated by clogged blood vessels, protect the heart function, and improve the clinical outcome of patients [1-3]. The factors promoting the formation of collateral circulation in patients with acute myocardial infarction (AMI) have become a hot research spot in medical field.

Growth differentiation factor 15 (GDF 15) belongs to GDFs family, which is a member of the transforming growth factor β superfamily [4]; Previous study indicated that GDF 15-3148 loci polymorphism had a close relationship with collateral circulation in acute myocardial infarction [5, 6]. However, + 157 A/T loci polymorphism in GDF gene was not observed. This paper aims to explore the correlation between + 157 A/T polymorphism and the collateral circulation in patients with acute non-ST segment elevated myocardial infarction.

Materials and methods

Subjects

Inclusion criteria: From the beginning of January 2014, we selected 126 patients diagnosed AMI by coronary angiography in cardiac catheterization laboratory of Xiangya Hospital, Central South University. At the same time patients with chest pain but with normal coronary angiography were enrolled in the control group (n = 74).

Table 1. The characteristics of participants

Indices	AMI (n = 126)		Control group (n = 74)	*P* values
	Collateral circulation (n = 90)	NO-collateral circulation (n = 36)		
Age (Years)	58.3 ± 12.3	58.9 ± 11.4	59.3 ± 12.5	0.987
Sex (M/F)	61/29	25/11	48/26	0.265
Smoking (Y/N)	49/41	21/15	40/34	0.132
Hypertension	51/39	22/14	42/32	0.134
Diabetes	42/48	17/19	30/44	0.665
Hyperlipidemia	55/35	24/12	50/24	0.194
Family history of CHD	33/57	11/25	20/54	0.119

Table 2. Comparison of genotype frequency and allele frequency of GDF + 157 A/T locus between the control and AMI groups

Groups	n	Genotype		Allele	
		AA	AT	A	T
AMI group	126	102 (81.0)	24 (19.0)	228 (90.5)	24 (9.5)
Control group	74	45 (60.8)	29 (39.2)	119(80.4)	29 (19.6)
P values		0.002		0.006	
OR (95% CI)		2.738 (1.437-5.218)		2.315 (1.290-4.153)	

They all signed the informed consent and took arterial blood samples at the same time while performing coronary angiography.

Exclusion criteria: We excluded the patients with clinical manifestations of acute and chronic inflammatory disease, or with cancer, valvular disease; cardiomyopathy disease, severe kidney disease (serum creatinine was greater than 2.5 mg/dL), severe liver disease, blood diseases, neoplastic diseases and other heart disease.

Methods

Coronary angiography: Selective coronary angiography was performed according to Judkins method. More than 50% luminal diameter stenosis in any major coronary arteries (left main, left anterior descending artery, right coronary artery, circumflex artery, the main diagonal branch or obtuse marginal branch) was defined as significant coronary artery stenosis.

Collateral evaluation: Rentrop's classification system was used to evaluate the collateral circulation. Grade 0: no collateral vascular perfusion; grade 1: visible collateral vessels, but no contrast agent perfusion; grade 2: visible collateral vessels and partial epicardial artery perfusion; grade 3: visible collateral vessels, complete epicardial artery perfusion.

Grouping: The arterial blood specimens of included patients were collected before coronary angiography. According to the Rentrop's classification method, the AMI group was divided into two subgroups: Rentrop's grade 0 was included in non-collateral group; Rentrop's grades 1-3 were taken as collateral group.

DNA extraction: 3 mL fasting blood was collected with anticoagulant tubes containing $EDTANa_2$; after well mixing, genomic DNA extraction was performed with whole blood genomic DNA extraction kit (Beijing Fiesole Technology Co.).

Primers design and synthesis: Relevant literature [5] was reviewed for primer design; primers were synthesized by Shanghai Sangon Biological Technology Co., Ltd. Upstream: 5'GGCTGCTTGGGGGGTGGGAG3'; Downstream: 5'GCAAGTTTCTCGGGACCCTCAGAGTTGTAC3'.

Genotyping: We utilized PCR-RFLP technique to genotype the + 157 A/T locus. A total of 30 μL of the mixture was used for amplification and the PCR conditions for β-Fg-455G/A were: predenaturation at 95°C for 5 minutes; 35 cycles of denaturation at 95°C for 50 seconds, annealing at 58.2°C for 45 seconds and extension at 72°C for 60 seconds; and a final extension at 72°C for 7 minutes. Reaction was terminated by cooling to 4°C. Then, 6 μL of products were separated by 1.5% agarose gel electrophoresis (100 V) for 20 minutes and visualized with ethidium bromide staining.

PCR products were digested for 12 h; the reaction system was as follows: PCR product 8.75 μL, 10 × Buffer1 μL, endonuclease (BsrI enzyme) 0.25 μL; the total volume was 10 μL.

Table 3. Comparison of gene frequency and allele frequency of GDF 15 gene + 157 A/T locus between the collateral circulation group and the no-collateral circulation group

Groups	n	Genotype		Allele	
		AA	AT	A	T
Collateral circulation group	90	70 (77.8)	20 (22.2)	160 (88.9)	20 (11.1)
No-collateral circulation group	36	20 (55.6)	16 (45.4)	56 (77.8)	16 (22.2)
P values		0.014		0.025	
OR (95% CI)		2.800 (1.228-6.344)		2.285 (1.107-4.716)	

Table 4. Multivariable logistic regression result

Variables	B	SE	*P* value	OR (95.0% CI)
GDF + 157 A/T locus	0.231	0.087	0.028	1.562 (1.109-3.872)
Smoking	0.2221	0.315	0.521	1.321 (0.430-2.699)
Age	0.152	0.216	0.101	1.054 (1.021-1.087)
Sex	0.723	0.313	0.323	1.024 (0.963-3.217)
Hypertension	0.200	0.419	0.519	1.209 (0.776-3.127)
Diabetes	0.201	0.320	0.187	1.029 (0.910-3.374)
Hyperlipidemia	0.042	0.106	0.098	1.051 (1.112-2.087)
Family history of CHD	0.554	0.312	0.121	1.067 (0.967-3.217)

Statistical analysis

SPSS 19.0 statistical software was used for statistical analysis. Measurement data were presented as mean ± standard deviation (Mean ± SD) and compared by *t* test; whether genotype distribution meets Hardy Weinberg genetic equilibrium law, inter-group gene frequencies and alleles were compared using χ^2 test. $P < 0.05$ was considered statistically significant.

Results

General information

No statistically significant differences had been found in age, sex, smoking history, hyperlipidemia, hypertension, diabetes, and family history between acute myocardial infarction and control groups ($P > 0.05$); there was no statistically significant difference in baseline data between collateral group and non-collateral group ($P > 0.05$), shown in **Table 1**.

Hardy Weinberg genetic equilibrium

The genotype distribution of GDF + 157 A/T locus in both groups were in line with the law of Hardy Weinberg genetic equilibrium ($P > 0.05$), with a group representative.

GDF 15 gene + 157 A/T locus genotype frequencies and allele frequencies

As shown in **Table 2**, between the two groups there was statistically significant difference in + 157 A/T locus genotype frequency ($P = 0.002$); and there was statistically significant difference in allele frequencies ($P = 0.006$).

GDF 15 gene + 157 A/T locus genotype frequencies and allele frequencies in non-collateral circulation group and collateral circulation group

As shown in **Table 3**, there was statistically significant difference in + 157 A/T genotype frequencies between the two groups ($P = 0.014$); and there was statistically significant difference in allele frequencies ($P = 0.025$). For the genotype distribution, + 157 A/T AT genotype had a trend to significantly promote the formation of collateral circulation in patients with AMI.

Multivariable logistic regression analyses for the relation between GDF 15 gene + 157 A-/-T polymorphism

As shown in **Table 4**, we found GDF 15 gene + 157 A-/-T polymorphism was independently related to the formation of collateral circulation in patients with non-ST-segment elevated myocardial infarction, after adjustments of other traditional risk factors, such as smoking, hypertension, diabetes, hyperlipidemia and family history of CHD.

Discussion

Collateral circulation is the non-capillary anatomical connection between different parts of the same blood vessel and between different coronary arteries [7]. When the existing cardiac coronary is unable to provide adequate blood

flow, collateral circulation is a potentially important source of supply for vessels [8]. Growth differentiation factor 15 is mainly involved in regulating many cell functions and biological processes, such as multiple organ growth, differentiation and tissue repair [9]. Recent studies have found that GDF 15 not only has the above biological function, but also is involved in the development and progression of cardiovascular disease [10-11]. In 2002, Brown et al [12] reported that serum protein level of GDF 15 was an independent risk factor for women suffering from atherosclerosis and other cardiovascular events, and firstly linked GDF 15 with cardiovascular disease. To clarify the correlation between GDF 15 gene polymorphism and the formation of collateral circulation in patients with non-ST segment elevated myocardial infarction (NSTEMI), patients with NSTEMI and individuals with normal coronary angiography were taken as the subjects. Two genotypes of + 157 A/T locus, AA and AT, were found both in the acute myocardial infarction group and the control group; the statistics showed that there were statistically significant differences in the two genotypes between AMI group and control group ($P < 0.05$); and AT genotype may be a risk factor of acute myocardial infarction; the risk of AMI in people with AT genotype could be increased by 2.73 times. At the same time, the possibility of the existence of collateral circulation in patients with AMI carrying AT genotype could be increased by 2.8 times. However, in this study, there were no statistically significant differences in + 157 A/T allele frequency; this may be due to that the sample size of this study was small. Therefore, the sample size should be increased for further study.

GDF 15 + 157 A/T polymorphism had a certain correlation with the formation of collateral circulation in non-ST-segment elevated acute myocardial infarction. AT genotype may be associated with the prevalence of acute myocardial infarction, and the possibility of collateral circulation in patients with AMI carrying AT genotype was large, so it can be used as a biological indicator for the prediction of myocardial infarction. After adjusting of other traditional risk factors, GDF 15 gene + 157 A-/-T polymorphism was independently related to the formation of collateral circulation in patients with non-ST-segment elevated myocardial infarction. But the sample size was small, with some limitations, so it may not truly reflect the correlation between the polymorphism and the formation of collateral circulation in acute non-ST segment elevated myocardial infarction, which still needs to be further verified by expanding the sample size.

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Disclosure of conflict of interest

None.

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