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Correlation of MMP−2 gene polymorphism with disease relapse as well as inflammatory mediators and oxidative stress molecules in patients with ischemic stroke

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ABSTRACT

Objective: To study the correlation of MMP-2 gene polymorphism with disease relapse as well as inflammatory mediators and oxidative stress molecules in patients with ischemic stroke. **Methods:** Patients with primary and recurrent ischemic stroke who were treated in Shaanxi Provincial People's Hospital between January 2015 and December 2016 were selected as the primary group and recurrent group respectively; healthy volunteers who received physical examination during the same period were selected as control group. Serum MMP-2 gene polymorphism and inflammatory mediator levels as well as peripheral blood oxidative stress molecule expression were determined. **Results:** Serum MMP2 gene 735 loci CC genotype constituent ratio of recurrent group was significantly higher than that of control group and primary group while CT genotype and TT genotype constituent ratio were significantly lower than those of control group and primary group; serum HMBG1, FKN, TNF-α, IL-1β and IL-17 levels as well as peripheral blood Keap1 mRNA expression in stoke patients with MMP2 gene CC genotype were significantly higher than those in stoke patients with MMP2 gene CT genotype and MMP2 gene TT genotype while peripheral blood Nrf2, ARE, NQO1 and HO1 mRNA expression were significantly lower than those in stoke patients with MMP2 gene CT genotype and MMP2 gene TT genotype. **Conclusions:** MMP-2 gene CC genotype in patients with ischemic stroke can aggravate inflammatory response and oxidative stress response, and is closely related to disease relapse.

1. Introduction

Ischemic stroke is the most common clinical type of stroke, which is the intracranial arterial thromboembolism and cerebral ischemia hypoxia developed on the basis of atherosclerosis. For patients with primary ischemic stroke, intervention, thrombolysis and other reperfusion treatments can effectively restore cerebral blood perfusion, reduce brain tissue damage and improve nerve function[1]. After the disease is in the sequela period, antiplatelet, neurotrophy and other drug treatments are also needed, which can not only promote the reconstruction of the nerve function, but also prevent recurrence of ischemic stroke[2,3]. Nevertheless, patients with ischemic stroke still have a higher risk of recurrence, and early assessing the risk of recurrence and implementing intervention can improve outcomes. Matrix metalprotease 2 (MMP2) is the MMPs family member closely associated with the properties of atheromatous plaques, which is involved in plaque stability reduction, thrombosis and other processes. Recent studies have shown that the MMP2 gene promoter upstream 735 loci polymorphism is closely related to the gene expression[4]. In the following study, the correlation of MMP-2 gene polymorphism with disease relapse as well as inflammatory mediators and oxidative stress molecules in patients with ischemic stroke was analyzed.

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2. Research subject information and methods

2.1 General information of research subjects

Patients with ischemic stroke, including 35 primary patients and 25 recurrent patients, who were treated in Shaanxi Provincial People's Hospital between January 2015 and December 2016 were selected as the primary group and recurrent group of the study respectively. All the patients were in line with the diagnostic criteriafor ischemic stroke, and the patients with cerebral arteriovenous malformation, those associated with respiratory tract infection or other infectious diseases, and those with severe liver and kidney function damage were excluded. Primary group included 19 men and 16 women that were 46-63 years old; recurrent group included 14 men and 11 women that were 44-65 years old. 50 healthy volunteers who received physical examination during the same period were selected as control group, including 35 men and 25 women that were 41-65 years old. There was no significant difference in the general data of the three groups (P>0.05).

2.2 MMP2 gene polymorphism detection methods

3 mL cubital venous blood was collected, blood genomic DNA extraction kit was used to isolate genomic DNA in peripheral blood samples, the primers for MMP2 gene 735 loci were designed, PCR reaction was conducted, PCR products were collected for agarose gel electrophoresis, and MMP2 gene 735 loci polymorphism was judged after development. The product size of the CC genotype was 164 bp, the product sizes of the CT genotype were 164 bp, 143 bp and 68 bp, and the product sizes of the TT genotype were 143 bp and 68 bp.

2.3 Serum inflammatory mediator level detection methods

5 mL cubital venous blood was collected and centrifuged at 3 000 r/min for 20 min to get serum samples, and enzyme-linked immunosorbent assay kits were used to determine the contents of HMBG1, FKN, TNF-α, IL-1β and IL-17.

2.4 Peripheral blood oxidative stress molecule detection methods

3 mL cubital venous blood was collected, added in Ficoll separating medium and centrifuged, the mononuclear cells suspended in the middle were absorbed, the total RNA was extracted from cells for fluorescence quantitative PCR amplification, the amplified genes included Keap1, Nrf2, ARE, NQO1, HO1 and GAPDH, and the amplification curve was referred to calculate the Keap1, Nrf2, ARE, NQO1 and HO1 mRNA expression.

2.5 Statistical methods

SPSS 20.0 software was used to input and analyze data, measurement data comparison among three groups was by variance analysis, count data analysis was by chi-square test and test results P<0.05 was the standard of statistical significance in differences.

3. Results

3.1 MMP2 gene polymorphism of three groups of subjects

Serum MMP2 gene 735 loci CC genotype constituent ratio of recurrent group was significantly higher than that of control group and primary group while CT genotype and TT genotype constituent ratio were significantly lower than those of control group and primary group (P<0.05); serum MMP2 gene 735 loci CC genotype, CT genotype and TT genotype constituent ratio were not significantly different among primary group and control group (P>0.05).

Table 1.

Constituent ratio of different MMP2 gene 735 loci genotypes of three groups of subjects [n(%)].

Groups	n	CC genotype	CT genotype	TT genotype
Control group	50	28(56%)	13(26%)	9(18%)
Primary group	35	19(54.29%)	9(25.71%)	7(20%)
Recurrent group	25	20(80%)*&	2(8%)*&	3(12%)*&

*: compared with control group, P<0.05; &: compared with primary group, P<0.05.

3.2 Serum inflammatory mediators in stroke patients with different MMP2 gene polymorphism

Analysis of serum inflammatory mediators HMBG1 (pg/mL), FKN (pg/mL), TNF-α (ng/mL), IL-1β (ng/mL) and IL-17 (ng/mL) in stroke patients with different MMP2 gene polymorphism was as follows: serum HMBG1, FKN, TNF-α, IL-1β and IL-17 levels in stoke patients with MMP2 gene CC genotype were significantly higher than those in stoke patients with MMP2 gene CT genotype and MMP2 gene TT genotype (P<0.05); serum HMBG1, FKN, TNF-α, IL-1β and IL-17 levels were not significantly different between stoke patients with MMP2 gene CT genotype and MMP2 gene TT genotype (P>0.05).

Table 2.

Comparison of serum inflammatory mediators among stroke patients with different MMP2 gene polymorphism.

MMP2 polymorphism	n	HMBG1	FKN	TNF-α	IL-1β	IL-17
CC genotype	39	75.59±9.48[ab]	93.48±11.24[ab]	36.51±6.52[ab]	13.29±1.85[ab]	18.59±2.32[ab]
CT genotype	11	41.38±6.48	36.69±5.21	22.13±3.25	7.68±0.93	8.59±1.03
TT genotype	10	42.19±5.85	37.12±5.28	21.98±3.52	7.75±0.98	8.84±1.08

[a]: compared with CT genotype, P<0.05; [b]: compared with TT genotype, P<0.05.

Table 3.

Comparison of peripheral blood oxidative stress molecules among stroke patients with different MMP2 gene polymorphism.

MMP2 polymorphism	*n*	Keap1	Nrf2	ARE	NQO1	HO1
CC genotype	39	2.14±0.34[ab]	0.38±0.05[ab]	0.32±0.06[ab]	0.48±0.08[ab]	0.43±0.06[ab]
CT genotype	11	1.15±0.19	1.03±0.19	1.07±0.15	1.15±0.17	1.04±0.17
TT genotype	10	1.08±0.15	0.98±0.12	1.12±0.14	1.08±0.12	1.07±0.12

[a]: compared with CT genotype, $P<0.05$; [b]: compared with TT genotype, $P<0.05$.

3.3 Peripheral blood oxidative stress molecule expression in stroke patients with different MMP2 gene polymorphism

Analysis of peripheral blood oxidative stress molecules Keap1, Nrf2, ARE, NQO1 and HO1 expression among stroke patients with different MMP2 gene polymorphism was as follows: peripheral blood Keap1 mRNA expression in stoke patients with MMP2 gene CC genotype was significantly higher than that in stoke patients with MMP2 gene CT genotype and MMP2 gene TT genotype while Nrf2, ARE, NQO1 and HO1 mRNA expression were significantly lower than those in stoke patients with MMP2 gene CT genotype and MMP2 gene TT genotype ($P<0.05$); peripheral blood Keap1, Nrf2, ARE, NQO1 and HO1 expression were not significantly different between stoke patients with MMP2 gene CT genotype and MMP2 gene TT genotype ($P>0.05$).

4. Discussion

MMP2 in the circulating blood is the molecule that plays an important role in the development and change of ischemic stroke, which can degrade the collagen, laminin, elastin and other compositions in the atheromatous plaque fibrous cap, thus result in plaque rupture and thrombosis and lead to the occurrence of ischemic stroke[5,6]. MMP2 gene expression levels directly determine the content of MMP2 in blood circulation, the gene locates in chromosome 16q13-21, and there is polymorphism of allele C replacement by T in promoter upstream 735 bp. Promoter is directly involved in the regulation of gene expression, upstream 735 bp allele C transition to T will affect the Sp1 loci binding activity, thereby inhibiting gene transcription and reducing gene expression[7,8]. In order to define the relationship between MMP2 gene promoter upstream 735 bp loci polymorphism and ischemic stroke recurrence, the constituent ratio of different MMP2 gene 735 loci genotypes in recurrent ischemic stroke was analyzed in the study, and the results showed that serum MMP2 gene 735 loci CC genotype constituent ratio of recurrent group was significantly higher than that of control group and primary group while CT genotype and TT genotype constituent ratio were significantly lower than those of control group and primary group. That means that MMP2 gene promoter upstream 735 bp loci allele T change to C is associated with recurrence of ischemic stroke, which may be related to the increased gene transcription activities after allele T change to C.

Inflammation is important pathological change in the process of atherosclerosis, and inflammatory cell infiltration in vascular endothelium can cause atheromatous plaque formation and lead to plaque nature change and rupture. Studies have shown that HMBG1, FKN, TNF-α, IL-1β, IL-17 and other inflammatory mediators are closely related to the occurrence and recurrence of ischemic stroke[9]. HMGB1 promotes macrophage activation and aggregation, and the macrophages in atheromatous plaque devour lipid and form foam cells, thus accelerating the formation of atheromatous plaque; FKN is a chemokine of the CX3C family, which has chemotaxis on lymphocyte and mononuclear macrophage adhesion to the vascular walls and atheromatous plaques[10]; TNF-α and IL-1β are the pro-inflammatory factors secreted by mononuclear macrophages, which have promoting effect on cascade activation of the inflammatory response, and can also increase the release of oxygen free radicals, cause endothelial injury, and accelerate the plaque rupture [11]; IL-17 is the cytokine secreted by the Th17 cells, which has promoting effect on the activation of various inflammatory cells and the release of inflammatory mediators[12]. In order to define the effect of MMP2 gene polymorphism on the generation of above inflammatory mediators in patients with ischemic stroke, the serum levels of inflammatory mediators in patients with different MMP2 gene 735 loci genotypes were analyzed in the study, and the results showed that serum HMBG1, FKN, TNF-α, IL-1β and IL-17 levels in stoke patients with MMP2 gene CC genotype were significantly higher than those in stoke patients with CT genotype and TT genotype. That means that MMP2 gene promoter upstream 735 bp loci allele C change to T can increase the synthesis and secretion of a variety of inflammatory mediators, aggravate the inflammatory response and participate in the recurrence of ischemic stroke.

Oxidative stress is an important pathological link that causes neural function damage in the progression of ischemic stroke, Keap1-Nrf2/ARE pathway is an important signaling pathway that regulates oxidative stress reaction, and it can affect the expression of downstream HO1, NQO1 and other antioxidant enzymes to participate in the regulation of oxidative stress reaction[13]. In physiological conditions, Keap1 is combined with Nrf2 and the Nrf2/ARE pathway is in inhibited state; when the oxidative stress

response is activated, the Keap1 and Nrf2 are dissociated, and the Nrf2 is in free state, transfers into the nucleus and is combined with ARE to start the expression of genes such as HO1 and NQO1. In the course of the ischemic stroke, the Nrf2/ARE pathway will be activated as compensation, and mitigate the oxidative stress damage to the nervous system by the generation of the downstream HO1 and NQO1[14]; when compensated activation of Nrf2/ARE is insufficient, it will aggravate the oxidative stress and increase the risk of stroke recurrence[15]. In order to define the effect of MMP2 gene polymorphism on the oxidative stress molecule generation in patients with ischemic stroke, peripheral blood oxidative stress molecule expression in patients with different MMP2 gene 735 loci genotypes were analyzed in the study, and the result showed that peripheral blood Keap1 mRNA expression in stoke patients with MMP2 gene CC genotype was significantly higher than that in stoke patients with CT genotype and TT genotype while Nrf2, ARE, NQO1 and HO1 mRNA expression were significantly lower than those in stoke patients with CT genotype and MMP2 gene TT genotype. This means that MMP2 gene promoter upstream 735 bp loci allele T change to C can inhibit the activation of Nrf2/ARE anti-oxidative stress pathway, thus increase the oxidative stress reaction damage to nerve function, and increase the risk of ischemic stroke recurrence.

MMP-2 gene promoter upstream 735 bp loci allele T change to C and the emergence of CC genotype C in patients with ischemic stroke are associated with recurrence of ischemic stroke, and the emergence of the CC genotype can aggravate inflammation and oxidative stress.

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