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Original Article

A clinical study of the relationship between microRNA143 gene polymorphism and hypertensive stroke

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Abstract: Hypertensive stroke threatens the health of patients. The role of microRNA143 in hypertensive stroke is unknown. This study was designed to investigate the relationship between microRNA143 gene polymorphism and hypertensive stroke to provide a theoretical basis for diagnosis and treatment. Clinical data of 600 hypertensive stroke patients were included in disease group. 600 healthy people were used as controls. The expression levels of microRNA143 in the plasma of two groups were analyzed. The polymorphism of microRNA143 gene and other clinical parameters in each group were also analyzed. The smoking history and hypertension history in the disease group were significantly higher than that of controls. Observation group of stroke in hypertensive patients with Fasting blood glucose level, low density lipoprotein (LDLC) level, high ensity lipoprotein (HDLC) levels, triglyceride (TG) level, total cholesterol level, and C-reactive protein levels of disease group were significantly higher than that of the controls ($P<0.05$). The frequency of A in SNP+513A/T of microRNA143 gene was significantly higher in disease group, compared to that of controls ($P<0.05$). The level of microRNA143 was positively correlated with LDLC, suggesting that LDLC may be a potential molecular marker of hypertensive stroke. MicroRNA143 gene polymorphism and lipid dyshomeostasis may play important role in hypertensive stroke. MicroRNA143 polymorphic loci might be used as the potential molecular marker of hypertensive stroke.

Keywords: Hypertensive stroke, microRNA143, gene polymorphism, risk factors

Introduction

Stroke, also known as cerebrovascular accident, is a nervous system disease caused by cerebral ischemia and brain tissue damage, which usually caused by vascular occlusion or cerebral vascular rupture [1]. The latest research suggests that stroke has become the number one reason of death from nervous system diseases in China, which has three major characteristics: high disability rate, high morbidity rate, and high mortality rate [2, 3]. At present, there is no effective treatment for stroke worldwide. The molecular mechanism of the pathogenesis of stroke is not yet clear. Therefore, prevention is the most useful tip for this disease [4, 5].

Stroke has many risk factors, including excessive drinking, lack of regular exercise, obesity, unhealthy diet, smoking, age, race, gender, vascular risk factors, hyperlipidemia, diabetes, and hypertension [6-8]. Stroke caused by high blood pressure is called hypertensive stroke. Hypertension is one of the risk factors that can be controlled [9, 10]. It is gradually recognized that some proteins paly important role in the pathogenesis of hypertensive stroke [11, 12]. Since cerebral ischemia or brain tissue damage caused by vascular occlusion or cerebral vascular rupture is the direct cause of hypertensive stroke, therefore, essential proteins and molecules that regulate angiogenesis and vascular injury and repair may be involved in the pathogenesis of hypertensive stroke [13-15]. For example, vascular endothelial growth factor and angiopoietin, which are involved in angiogenesis and impaired vascular repair, have been confirmed to be involved in hypertensive stroke [16-18].

Table 1. PCR

Template	1 μl
10×PCR Buffer	2.5 μl
DNTP Mixture (2 mM)	2 μl
Primer 1 (10 μM)	1 μl
Primer 2 (10 μM)	1 μl
Taq DNA Polymerase	1 μl
$MgCl_2$ (25 mM)	2.5 μl
H_2O	1 μl

Table 2. The PCR reaction

95°C	5 min
95°C	45 S
50°C	45 S
72°C	45 S
72°C	3 min
4°C	5 min

MicroRNA is a type of small RNA which has a lot of functions, including the regulation of cell proliferation and differentiation, cell death and signal transduction [15-17]. It has been shown that microRNA143 is associated with metabolic disorders in cancer. Serum microRNA143 [11] levels in rectal cancer patients or coronary heart disease patients are elevated. MicroRNA143 is also associated with cardiovascular disease. Hypertension is an important risk factor influencing the cardiovascular disease [12]. Therefore, microRNA143 may be involved in the occurrence and development of hypertensive stroke [13]. This study investigates the relationship between microRNA143 gene polymorphism and hypertensive stroke to provide a theoretical basis for the diagnosis and treatment of related diseases.

Materials and methods

Research objects

A total of 600 hypertensive stroke patients in Xinhui Affiliated Hospital of Southern Medical University from August 2013 to August 2015 were retrospectively included as disease group. 600 healthy people were included as controls. This study was approved by the ethics committee of Xinhui Affiliated Hospital of Southern Medical University. All the subjects of the study signed the informed consent.

The inclusion and exclusion criteria of hypertensive stroke patients are in accordance with the standard [12]. In disease group, patients were diagnosed by both medical imaging and laboratory methods. Patients are older than 60 years old, without family history of cardiovascular or cerebrovascular disease. All is first time hypertensive stroke patient and has complete information.

Sample collection

Venous blood was collected from both patients and controls with citrate anticoagulantion. Blood samples were preserved in -80 degree for later use.

Genomic DNA extraction and PCR

The genomic DNA was extracted according to the manufacturer's instruction. Genomic DNA was used as template to perform PCR as follows (**Table 1**). The PCR reaction conditions are as follows (**Table 2**).

Comparison of clinical blood parameters

The blood parameters of hypertensive stroke patients and healthy volunteers were detected by the automatic blood testing machicine (Beijing DingGuo Biotechnology Co.). Indexes include fasting blood glucose levels, low density lipoprotein (LDL) triglyceride (TG) level, total cholesterol (total cholesterol, TC) levels, and C-reactive protein levels.

MicroRNA143 gene polymorphism

PCR products were sequenced. The polymorphism of microRNA143 gene was analyzed according to the sequence of microRNA143 in PubMed.

Allele frequency and genotype distribution of microRNA143 in hypertensive stroke patients and healthy volunteers were analyzed. The allele frequency of the microRNA143 was calculated as: number of microRNA143 gene/(number of microRNA143 gene+number of microRNA143 alleles).

Statistical analysis

SPSS 11 was used to analyze the data. Data are expressed as mean ± standard error. T test

Table 3. Summary of clinical data

	Control	Disease	T	*P*
Female/male (n)	207/393	225/375	0.41	0.62
Age (year)	64.2 ± 3.7	65.2 ± 3.5	2.88	0.07
BMI (Kg/m^2)	22.8 ± 1.5	23.2 ± 1.8	3.12	0.072
Smoking (%)	123 (20.5)	275 (45.8)	30.11	0.001
Drinking (%)	155 (25.8)	149 (24.9)	0.199	0.71
Hypertension (%)	137 (22.8)	415 (69.1)	85.12	0.0001
Diabetes (%)	37 (6.1)	53 (8.9)	1.31	0.26
Blood pressure (mmHg)	111 ± 3/78 ± 2	143 ± 6/90 ± 2	152.3	<0.0001
			57.10	0.0001

Table 4. Results of hematology analysis

	Control	Disease	T	*P*
C reactive protein (mg/L)	2.1 ± 1.2	9.5 ± 5.2	20.1	0.0009
TG (mmol/L)	1.4 ± 1.2	1.8 ± 1.2	2.8	0.02
TC (mmol/L)	4.5 ± 1.3	5.2 ± 1.3	5.1	0.001
HDLC (mmol/L)	1.5 ± 0.4	1.3 ± 0.6	2.81	0.001
LDLC (mmol/L)	2.2 ± 1.2	2.8 ± 1.1	5.11	0.001
Glucose (mmol/L)	5.4 ± 1.7	6.8 ± 2.2	7.21	0.001

was used to compare differences between groups. The risk factors of hypertensive stroke were analyzed by Logistic regression. P<0.05 was considered as statistically significant.

Results

Clinical data analysis

A total of 600 hypertensive stroke patients from August 2013 to August 2015 was included (**Table 3**) as the disease group. A total of 600 healthy people were included as controls. No significant differences was found in sex ratio, mean age, disease history, drinking history, diabetes history, or body mass index (BMI) between the two groups. The rates of smoking history and hypertension in the disease group were significantly higher than that of the control group.

Hematology analysis

As shown in **Table 4**, levels of fasting blood glucose, LDL, TG, TC, HDL, and C-reactive protein in hypertensive stroke patients were significantly higher than that of controls.

Allele frequency and genotype analysis of SNP+513A/T

As shown in **Table 5**, the A allele frequency of SNP+513A/T in disease group was significantly higher than that of the control group. Correlation analysis showed that microRNA143+1602AA gene type, high TG level, high cholesterol level, and smoking history are important risk factors of hypertensive stroke. MicroRNA143 polymorphic sites may be potential molecular marker of hypertensive stroke.

The analysis of risk factors

Logistic regression analysis showed that 513A/A genotype (**Table 6**), high TG level, smoking history, and high cholesterol were the major risk factors for hypertensive stroke.

The level of microRNA143 has a positive correlation with LDLC

The correlation analysis showed that the level of microRNA143 has a positive correlation with LDLC (**Figures 1** and **2**), suggesting that LDLC may be a potential molecular marker for hypertensive stroke.

Discussion

Hypertensive stroke threatens patients' health. The stroke caused by hypertension is called hypertensive stroke. Hypertension is one of the most important risk factors that can be controlled [1-3]. Previous studies [14] showed that microRNA143 may be a risk factor for hypertensive stroke. This study investigated the relationship between microRNA143 gene polymorphism and hypertensive stroke in order to provide a theoretical basis for the diagnosis and treatment of hypertensive stroke.

Results showed that smoking history rate, hypertension rate, and levels of fasting blood glucose, LDLC, TG, TC, and C-reactive protein were significantly higher in disease group, compared with that of control group. Interestingly, a previous study showed that LDLC was the common risk factor for ischemic stroke in men and

Table 5. Analysis of SNP+513A/T allele frequency and genotype

Group	Gene TT	Gene AT	Gene AA	T Allele gene frequency (%)	A Allele gene frequency (%)	T	P	Chi square	P
Disease	54	276	270	32	68	6.7	0.007	14.03	0.013
Control	126	282	192	44.5	55.5				

Table 6. Analysis risk factors of smoking, HDL, High triglyceride and microRNA polymorphism

	Coefficient	Standard error	X^2	P	95% CI
Smoking	2.2	0.89	6.12	0.0043	1.5-13
HDL	0.83	0.57	2.1	0.012	0.77-7.3
High triglyceride	0.77	0.33	0.38	0.01	1.0-3.3
+513A/A gene	0.85	0.28	12.8	0.001	1.3-3.9

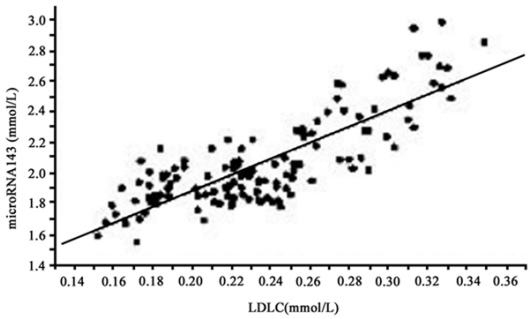

Figure 1. The level of microRNA143 has a positive correlation with LDLC (low density lipoprotein cholesterol).

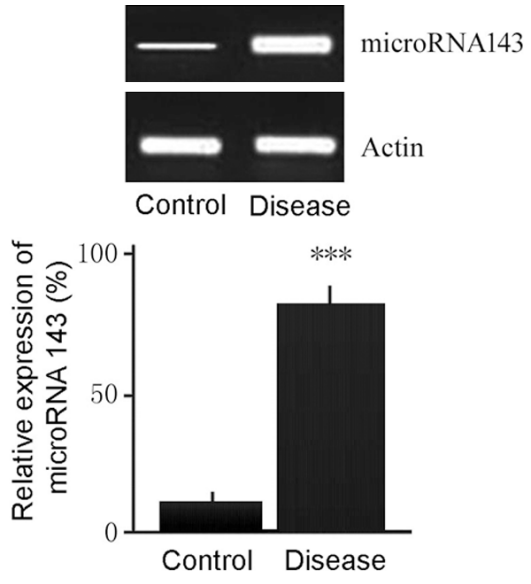

Figure 2. Relative expression of microRNA143 in control and disease group.

women, whereas Non-HDLc, TC and TC: HDLC ratio levels were associated with ischemic stroke as risk factors only in women [18]. However, the difference of LDLC, TC level between men and women was not evaluated in the present study. Results also showed that HDLC levels in patients were significantly higher than that of controls. A allele frequency of SNP+513A/T in disease group was found significantly higher than that of the control group. Disease group also showed higher microRNA143 levels, suggesting microRNA143 might be involved in the pathogenesis of hypertensive stroke. However, the exact mechanism by how microRNA143 participates in the pathogenesis or development of stroke remains unclear and requires further investigation. The Correlation analysis showed that microRNA143+1602AA gene type, high TG level, high cholesterol, and smoking history are im-portant risk factors of hypertensive stroke. MicroRNA143 gene polymorphism may be used as potential markers for hypertensive stroke. These results also suggest that microRNA143 gene polymorphism and lipid dyshomeostasis may be important risk factors of hypertensive stroke [19, 20].

It is worth to mention that it would be better to validate these results by large-cohort clinical studies. As the present study does not specify the role of microRNA143 in the occurrence and development of hypertensive stroke, animal model would be another choice in the future to investigate the role and mechanism of microRNA143 level in the progression of hypertensive stroke.

In summary, this study showed that microRNA143 gene polymorphism and lipid dyshomeostasis may be important risk factors of hypertensive. MicroRNA143 gene polymorphism may be used as potential molecular marker of hypertensive stroke.

Disclosure of conflict of interest

None.

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