Original Article
Risk factors for H-type hypertension combined with acute cerebral infarction

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Abstract: Objective: To investigate whether the methylene tetrahydrofolate reductase (MTHFR) C677T mutation is an independent risk factor for H-type hypertension accompanied by acute cerebral infarction (ACI) and analyze the correlation between homocysteine (Hcy) concentration and H-type hypertension complicated with ACI. Methods: A total of 75 patients diagnosed with hypertension and ACI from December 2018 to December 2019 were enrolled as the case group, and 75 healthy subjects were included in the control group. The genetic polymorphism was detected by PCR-RFLP. The plasma Hcy concentration was detected by high-performance liquid chromatography (HPLC). Results: There was significant difference in the genotype frequencies of MTHFR C677T between the case group and the control group, and the difference between allele frequencies of C and T was significant (*P*<0.001). The case group showed higher plasma Hcy concentration and higher positive rate of Hcy in patients with heterozygous mutation (CT) and homozygous mutation (TT) than those of the control group (*P*<0.001). Spearman analysis showed that age, high plasma Hcy level, history of diabetes, coronary heart disease, TT genotype and systolic blood pressure were positively correlated with the incidence of cerebral infarction (*P*<0.001). Logistic regression showed that TT gene, stage 2 hypertension, age >70 years and plasma Hcy level ≥11 μmol/L were the independent risk factors for hypertension complicated with ACI. Conclusion: MTHFR C677T mutation is an independent risk factor for hypertension complicated with ACI, and plasma Hcy levels are positively correlated with the incidence of ACI in hypertension patients.

Keywords: Hypertension, MTHFR C677T, acute cerebral infarction, homocysteine, Hcy

Introduction

Acute cerebral infarction (ACI) is a type of cardiovascular and cerebrovascular disease with poor prognosis and high mortality rate, and has attracted extensive attention of researchers [1]. ACI occurred mostly in middle-aged and elderly patients. It will cause nerve injuries with high disability rate and mortality rate [2]. The main cause of ACI is chronic inflammation associated with atherosclerosis, resulting in platelets inflammation thrombosis, and even cerebral infarction [3]. ACI is widely found in patients with hypertension. The two diseases together will easily lead to the formation of coronary atherosclerosis and reduce vascular elasticity and blood flow, leading to microcirculation disorders and coronary sclerosis [4].

ACI and other cardiovascular and cerebrovascular diseases are common complications of H-type hypertension, with a high incidence, and the combination of the two can lead to aggravation of the disease, increase the difficulty of treatment, improve the disability and mortality, and have poor prognosis. Moreover, the formation of carotid atherosclerosis plaque is a key factor to promote the occurrence of cerebral ischemia in hypertension. Patients with hypertension complicated with ACI were only given simple antihypertensive and thrombolytic therapy, which is difficult to completely reverse the damage of their tissues and organs, leading to the occurrence of cardiovascular and cerebrovascular events from time to time. The reason is that most patients with hypertension have H-type hypertension (i.e., accompanied by high Hcy hyperlipidemia), which synergistically accel-

erates the sclerosis process and aggravates the coronary artery injury.

The plasma concentration of homocysteine (Hcy) is constant in healthy individuals, but secondary and primary diseases can lead to increased levels of Hcy, increasing the risk of cerebrovascular disease, coronary heart disease and peripheral blood disease, *etc*. [5]. Methylene tetrahydrofolate reductase (MTHFR), as an important enzyme in the metabolism of Hcy and folic acid, is primarily stored in liver cells. Its relative molecular weight is 74.5 kD.

Methyltetrahydrofolate is converted from 5,10-methylenetetrahydrofolate [6]. A study has shown that cerebral infarction is associated with MTHFR C677T, and the concurrency rate of TT gene mutations in China is higher than other countries [7]. Therefore, a simple and non-invasive detection of MTHFR should be developed to prevent cerebral infarction.

At present, the risk of cerebral infarction can also be evaluated through gene sequencing, which can effectively improve clinical prognosis. Hcy can be prevented based on vitamin B and folic acid supplements [8]. This study explored whether the MTHFR C677T mutation was an independent risk factor for H-type hypertension complicated with ACI, and plasma Hcy levels were measured to confirm its correlation with hypertension and ACI.

Materials and methods

Subject enrollment

A total of 75 patients who were diagnosed as hypertension with ACI in our hospital from December 2018 to December 2019 were selected as the case group, including 42 males and 33 females. The included patients met *Consensus on the Diagnosis and Treatment of Hemorrhagic Transformation after Acute Cerebral Infarction w*ith liver and kidney dysfunction, cancer, thyroid and chronic gastrointestinal diseases (2009-edition) [9] and *Chinese Hypertension Prevention and Treatment Guidelines* [10]. Exclusion criteria: those w were excluded. Besides, 75 patients who underwent health examinations in our hospital during the same period were selected as the control group, including 36 males and 39 females. The included controls had no recent infections.

Both groups of subjects or their family members have signed informed consent. This study has been approved by the Ethics Committee of Hwa Mei Hospital, University of Chinese Academy of Sciences. The baseline data of two groups were comparable (*P*>0.05).

Specimen collection

Detection of MTHFR gene polymorphism: After fasting for 10 h, 2 ml of blood was drawn from the patients in the morning, added with EDTA for anticoagulation, and immediately stored in an incubator at 4°C, followed by centrifugation at 3500 r/min at 4°C for 1 h. Blood genomic DNA extraction kit was used to extract genomic DNA and stored at -20°C. MTHFR 667C/T detection kit (PCR-RFLP) was used for gene analysis. Plasma Hcy concentration was measured on Applied Biosystems' (ABI) GeneAmp™ PCR System 2700.

5 ml of venous blood was drawn from the patients, added with EDTA anticoagulation, and immediately placed in an incubator at 4°C. After centrifugation at 3000 r/min for 10 min, plasma samples were obtained and stored at -80°C. The plasma Hcy concentration was detected by HPLC-ED. MTHFR C677T polymorphism was detected using silica-coated magnetic particles with real-time polymerase chain reaction (PCR). Hcy level was detected by Hcy kit using 7600-020 biochemical analyzer. Saliva DNA was extracted using nano-magnetic bead solution which was purchased from Wuhan Wawasye Nanotechnology Development Co., Ltd. According to *Expert Consensus for the Diagnosis and Treatment of Type H Hypertension* (2016) [11], high Hcy level means Hcy ≥10 µmol/L.

MTHFR C677T gene sequencing: Quantitative fluorescence PCR was used to detect MTHFR C677T polymorphism. TaqManBHQ probes are as follows: 5-FAM-TGAAATCGG (G/C) CTCCC-GCAGACA-3; BHQ-Probe 2: 5-HEX-TGAAATC-GACTCCCGCAGACACC-3. Primers: 5-CCTCAAA-GAAAAGCTGCGTGAT-3 (forward), 5-CTGACCTG-AAGCACTTGAAGGA-3 (reverse); The reaction was set in a 10 µl volume. Conditions: Preconditioning at 50°C for 5 min, predenaturation at 94°C for 5 min, denaturation at 94°C for 25 s, annealing at 56°C for 25 s, extending at 72°C for 25 s, with a total of 35 cycles, and

Table 1. Baseline data [(mean ± SD)/n (%)]

Group	Case group (n=75)	Control group (n=75)	P
M/F	42/33	36/39	0.886
Age (year)	64.2±5.8	63.8±5.8	0.530
BMI (kg/m^2)	23.6±5.4	23.4±5.5	0.629
Smoking (n)	41 (54.66)	44 (58.66)	0.978
Drinking (n)	34 (45.00)	35 (46.66)	0.820
SBP (mmHg)	134.8±26.1	131.9±22.7	0.401
LDL-C (mM/L)	2.6±0.7	2.6±0.7	0.174
Glucose (mM/L)	5.5±1.0	5.6±1.0	0.081

Table 2. Distribution of MTHFRC 677T polymorphism [n (%)]

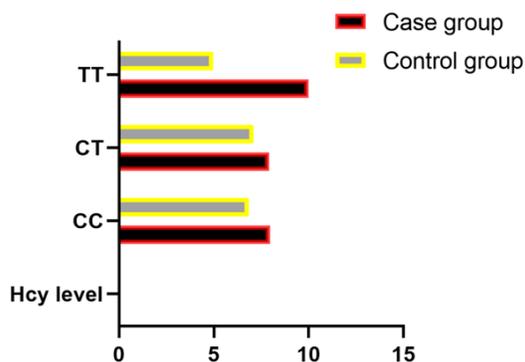| Genotype	Control group (n=75)	Case group (n=75)	OR	P
CC	33 (44.0)	13 (17.4)	1.00 (Control)	
CT	30 (40.0)	42 (56.0)	2.03 (1.03-4.02)	0.040*
TT	10 (13.4)	18 (24.0)	3.51 (1.05-3.02)	0.022*
CT+TT	39 (52.0)	59 (78.7)	2.22 (1.14-4.31)	0.017*

*P<0.05.

Figure 1. The relationship between the MTHFR genotype and Hcy level and ACI in the two groups. Hcy levels of patients with CC, CT and TT genotypes in the case group were significantly higher than those in the control group (P<0.05).

extending at 72°C for 5 min. Sterile distilled water was the negative control. A total of 97 samples were extracted and tested and the data were obtained within 45 min.

Statistical analysis

SPSS19.0 statistical software was used for data analysis. The measurement data were expressed as mean ± standard deviation (SD), and were compared by variance test, and the comparison between groups was adopted with t test. Count data were tested by X^2. The distribution frequency of gene was tested with Hardy-weinberg equilibrium. Spearman correlation analysis was used to determine the correlation between factors and ACI. Logistic regression analysis was used to analyze the influence of MTHFR genotype on Hcy level. P<0.05 means that the difference is statistically significant.

Results

Comparison of baseline data between the two groups

There was no significant difference between the case group and the control group in terms of gender, age, BMI, smoking history, alcohol consumption, SBP, LDL-C and glucose (P>0.05) (**Table 1**).

Distribution of MTHFR C677T polymorphism between the two groups

The genotype frequencies of T and TT were significantly increased in the case group (P= 0.001). The incidence of cerebral infarction caused by TT genotype was significantly higher than that of CC and CT (OR=1.29, 1.15-1.41) (**Table 2**).

Relationship between MTHFR polymorphism and plasma Hcy

The subjects were divided into three types on the basis of genotypes: TT, CT and CC. Among those with genetic mutations at C677T in MTHFR in the case group, the positive rates of plasma Hcy in heterozygous mutation (CT) and homozygous mutation (TT) were all significantly higher than those of the control group (P< 0.001) (**Figures 1**, **2**).

Risk factors for ACI in patients with hypertension

Multivariate analysis showed that age, hypertension grading, history of diabetes, coronary heart disease, systolic blood pressure, high plasma Hcy level and MTHFR C677T genotype were positively correlated with ACI (P<0.001). Triglyceride levels were negatively correlated
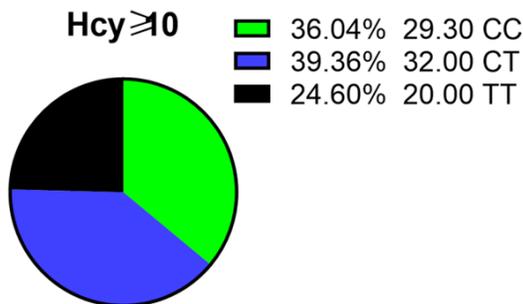
Hcy≥10

🟩	36.04%	29.30 CC
🟦	39.36%	32.00 CT
⬛	24.60%	20.00 TT

Figure 2. The relationship between the MTHFR genotype, Hcy level ≥10 and ACI. The positive rate of Hcy ≥10 of patients with CC and CT genotypes in the case group was significantly lower than that in the control group, but positive rate of the TT genotype was significantly higher than that of control group ($P<0.05$).

Table 3. Risk factors for cerebral infarction

Indices	r	P
History of diabetes	0.160	<0.001
History of hyperlipidemia	0.043	0.464
History of coronary heart disease	0.191	<0.001
Smoking history	0.100	0.094
Age	0.486	<0.001
Hypertension classification	0.466	<0.001
Hcy (μmo1/L)	0.174	0.001
SBP (mm Hg)	0.169	0.007
DBP (mm Hg)	-0.701	0.255
Heartbeats	-0.028	0.772
Fasting blood glucose (mmol/L)	-0.009	0.903
TC (mmol/L)	-1.800	0.031
TG (mmol/L)	-0.020	0.795
HDL-C (mmol/L)	-0.025	0.737
LDL-C (mmol/L)	-0.111	0.161
MTHFR TT genotype	0.229	<0.001

with the incidence of hypertension complicated with ACI ($P<0.05$) (**Table 3**).

The relationship between MTHFR polymorphism and plasma Hcy level and hypertension complicated with ACI

Logistic regression analysis included risk factors such as hypertension grading, diabetes, coronary heart disease, age, plasma Hcy and MTHFR C677T genotype. Age >70 years, MTHFR C677T genotype, plasma Hcy ≥11 μmol/L and grade 2 hypertension were all the independent risk factors for hypertension complicated by ACI (**Table 4**).

Discussion

ACI is a common cerebrovascular disease [12]. Studies have shown that the incidence of ACI is increasing yearly, especially among the young populations [13, 14]. Therefore, reducing complications as well as relieving clinical symptoms have become hot topics [15].

In recent years, with the continuous development of medical technology, the application of gene detection in the prevention of cerebral infarction is more and more popular. Genetic polymorphisms in the human genome demonstrated that genes of different populations or individuals are different, which reflects the results of the interaction between different genetic compositions and the environment during human evolution, leading to different susceptibility. At present, the genetics of cerebral infarction disorder is gaining research interest. Many experts also explored the genetic polymorphism with regard to cerebral infarction and the possible causes of cerebral infarction [15].

Hcy and MTHFR are key enzymes of folate metabolism. When Hcy is methylated, 5-methyltetrahydrofolate is converted to methyl, which is then transformed into key enzymes by mediating MTHFR [16]. A study has shown that reduced MTHFR activity can lead to a reduction in the conversion of Hcy to methionine, ultimately increasing Hcy levels [17]. Clinical diagnosis of cardiovascular and cerebrovascular diseases is mostly based on Hcy concentration, which is the main influencing factor of cerebral infarction, but there is no data on the specific mechanism of the disease. Some studies believed that the formation of thrombus and atherosclerosis are related to the damage to vascular endothelial cells and local vascular smooth muscle caused by Hcy levels [18, 19]. Hcy causes damage to the lining of blood vessels, increasing the possibility of clot formation and cerebrovascular disease [20]. A study has shown that when the level of Hcy increases, the incidence of cerebral infarction is elevated, and when the level of Hcy decreases, the incidence of cerebral infarction and ischemic cerebrovascular disease decreases significantly [21]. Therefore, it is of great significance to jointly detect the polymorphism of Hcy and MTHFR C677T genes.

Table 4. Logistic regression analysis

Factors	β	SE	Wald	OR (95% CI)	P
Age >70 years	2.192	0.348	34.546	8.964	<0.001
Hypertension ≥2 grade	2.285	0.483	22.274	9.839	<0.001
Hcy ≥11 μmol/L	0.690	0.323	4.539	1.993	0.032
MTHFR C677T genotype	1.193	0.443	7.226	3.301	0.006

MTHFR C677T polymorphisms include 677CC type (wild type), 677CT type (heterozygous mutant type) and 677TT (homozygous mutant type). The C677T mutation makes it intolerant to heat and reduces its activity. Compared with CC type, enzyme activity of CT and TT types is 65% and 30%, respectively. Hcy methylation is blocked again. Therefore, Hcy cannot be methylated to methionine and cause accumulation, resulting in a decrease in folic acid levels and hyperhomocysteinemia [22, 23].

This study showed that the increased Hcy level was significantly associated with the MTHFR genotype, and the Hcy level of CT and CC gene carriers was significantly higher than that of TT gene carriers. This conclusion was consistent with the results of Tian et al. [24], suggesting that the carriers of TT genotype of MTHFR C677T polymorphism had a higher incidence of Hcyemia than other populations. The present study has shown that by lowering the plasma level of Hcy, the risk of hypertension complicated by ACI can be reduced. At present, folic acid is commonly used to regulate Hcy level. Long-term use of folic acid can effectively prevent the occurrence of cerebral infarction.

Studies revealed that people who took folic acid for a long time had a significantly lower incidence of cerebral infarction than those who did not take folic acid, and it was found that vitamin B12 combined with folic acid had better efficacy in preventing cerebral infarction [25]. Folic acid can also reduce the occurrence of cardiovascular mortality. This study has shown that Hcy is a major risk factor for hypertension and ACI. The results of this study showed that the genotype frequency of TT in the case group was significantly higher than that in the control group, and higher than the other two genotypes. MTHFR C677T gene polymorphism is closely related to Hcy level, and both MTHFR gene polymorphism and serum high Hcy level may be risk factors for ischemic stroke. Hypertension is the leading controllable risk factor for stroke. The control of hypertension can curb the increasing trend of cardiovascular and cerebrovascular diseases and deaths. For hypertensive patients with elevated serum Hcy, more attention should be paid to supplementing folic acid while lowering blood pressure to reduce the incidence of stroke in patients with H-type hypertension. Therefore, people with TT genotype, especially those with H-type hypertension, need to detect this gene in time, so as to achieve early prevention and early intervention.

In summary, genetic defects in folic acid metabolism can be used to predict the risk of H-type hypertension and ACI by monitoring the polymorphism of MTHFR C677T gene, and age >70 years, MTHFR C677T genotype, plasma Hcy ≥11 μmol/L, grade 2 hypertension are the independent risk factors for hypertension and ACI. B6 and B12 can be prescribed for reducing Hcy concentration.

Disclosure of conflict of interest

None.

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