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MTHFR C677T polymorphism, homocysteine, burden, and location of AMI and ACI

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Abstract. – OBJECTIVE: We aimed to investigate the relationship between homocysteine levels and *MTHFR C677T* polymorphisms and acute ischemic vascular events and focused on the differential effects of the *MTHFR C677T* polymorphisms on the burden and location of AMI and ACI.

PATIENTS AND METHODS: 102 acute cerebral infarction (ACI) and acute myocardial infarction (AMI) patients who were admitted to the First Hospital of Jilin University in northeast China as the patient group, 83 healthy people who were hospitalized during the same period served as a control group. *MTHFR C677T* genotypes were identified via Polymerase Chain Reaction (PCR)-Fluorescent Probe Method.

RESULTS: Patient group had higher serum homocysteine levels (p=0.013), lower serum folic acid (p<0.001), and Vit B12 levels (p=0.004) compared to the control group. Homocysteine levels in the patient group with the TT genotypes of the *MTHFR C677T* polymorphisms were higher than those with the CC and CT genotypes (p<0.05). Folic acid levels in the patients with TT genotypes were lower than those with the CC genotypes (p<0.05), but not in the control group (p>0.05). There were negative and significant associations between serum homocysteine levels and serum vitamin B12 levels in the control group (r=-0.234, p=0.033), but not between serum homocysteine levels and serum folic acid levels (r=-0.103, p=0.355). Conversely, there was a negative and significant association between serum homocysteine levels and serum folic acid levels in the patients' group (r=-0.257, p=0.01), but not between serum homocysteine levels and serum vitamin B12 levels (r=-0.185, p=0.64). No statistically significant differences in *MTHFR C677T* genotype and C/T alleles distribution were investigated between the patient and control group (p>0.05). The *MTHFR C677T* polymorphism did not differentially affect the burden and location of AMI and ACI.

CONCLUSIONS: Homocysteine played a common role in atherosclerosis-related acute ischemic vascular events. These correlations were modified by *MTHFR C677T* polymorphisms and influenced by folic acid levels. The *MTHFR C677T* polymorphisms were not directly related to acute ischemic vascular events, nor did they differentially affect the burden and location of AMI and ACI.

Key Words:
MTHFR, Gene polymorphism, Homocysteine, Acute cerebral infarction, Acute myocardial infarction.

Introduction

Atherosclerosis (AS) is a well-known risk factor and the leading cause of acute cerebral infarction (ACI) or acute myocardial infarction (AMI), which contributes to high mortality in the western and eastern population[1,2]. Elevated homocysteine levels promote atherosclerosis by inducing oxidant stress, impairing endothelial function, and increasing the risks of thrombosis[3]. Epidemiological results have proved that hyperhomocysteine (HHcy) levels are an important risk factor for AMI and stroke[4,5]. Serum homocysteine (Hcy) levels can reflect the severity of the AMI patients' conditions[6]. Lowering homocysteine levels may prevent atherosclerosis and vascular events[7].

Genetic susceptibility can influence Hcy metabolism. Methylenetetrahydrofolate reductase (*MTHFR*), an important enzyme in folic acid and Hcy metabolism, can catalyze the remethylation of Hcy to methionine[8]. Gene polymorphisms of the enzyme will induce higher homocysteine levels[9]. *MTHFR C677T* is the most widely mutated type of this enzyme, which will reduce the activity of *MTHFR* and increase Hcy levels[10]. However, there is little study on whether this gene polymorphism is directly related to atherosclerosis-associated acute vascular events, and no studies have focused on the differential effects of the *MTHFR C677T* polymorphisms on the burden and location of acute ischemic vascular events.

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In the present study, we aim to investigate the role of homocysteine levels and *MTHFR C677T* polymorphisms in acute ischemic vascular events. Furthermore, we research the contribution of Hcy levels and *MTHFR C677T* genotype to AMI and ACI. And subgroup analyses potential influencing factors affecting AMI and ACI.

Patients and Methods

Patients

This study has been cleared by our Institution Ethics Review Board for human studies and the patients have signed informed consent. This study was a retrospective analysis that included 102 patients (because of acute cerebral infarction (ACI) or acute myocardial infarction (AMI) admitted to the First Hospital of Jilin University in northeast China) as a patient group, 83 healthy people who were hospitalized during the same period served as a control group. Patients were included if they met the diagnosis of ACI (within 7 days after symptom onset), patients with AMI must undergo coronary angiography (CAG) and percutaneous coronary intervention (PCI), and the onset is within one week. Patients were excluded from the study in case of: no complete clinical data; serious hepatic, renal, infectious, tumors, hematological, and immune system diseases; heart valve disease and atrial fibrillation; taking folic acid or B vitamins and other related supplements within the past 6 months; taking anticoagulant drugs, antibiotics, immunosuppressants or steroid hormones, antiepileptic drugs, methotrexate and other drugs that affect homocysteine metabolism within the past 6 months. All data were collected using EXCEL software.

Demographic Characteristics, Medical History, and Clinical Biochemical Test Index Collection

Demographic characteristics (sex and age), medical history and traditional risk factors for atherosclerosis (AS) [hypertension, diabetes mellitus history, as well as smoking and drinking history), and clinical biochemical test indexes (white blood cell count (WBC), neutrophils ratio (N ratio), lymphocytes ratio (L ratio), neutrophils/lymphocytes (N/L), alanine aminotransferase (ALT), aspartate aminotransferase (AST), serum uric acid (UA), total cholesterol (TC), triglycerides (TG), high-density lipoprotein (HDL), low-density lipoprotein (LDL), low-density lipoprotein/high-density lipoprotein (LDL/HDL), Glucose, homocysteine (Hcy), folic acid, and vitamin B12 (Vit B12) levels] were collected.

DNA Extractions and MTHFR C677T Genotyping Analysis

Fasting whole blood samples of all the participants were gathered on the second day of admission. The DNA extraction Kit (TianGen, Beijing, China) was used to extract DNA from whole blood samples according to the manufacturer's instructions. *MTHFR C677T* genotypic analysis was performed with an *MTHFR* genetic testing kit (YZYMED, Wuhan, China) *via* Polymerase Chain Reaction (PCR)-Fluorescent Probe Method.

The PCR program included a UNG treatment step at 37°C for 5 min, pre-denaturation for 5 minutes at 95°C, 40 cycles of 95°C for 15 s, and 60°C for 60 s. Finally, *MTHFR* genotypes are divided into three types: *CC*, *CT*, and *TT*.

Statistical Analysis

All statistical analyses were performed using SPSS 25.0 (IBM Corp., Armonk, NY, USA), and figures were designed using GraphPad Prism 8. Continuous variables with normal distribution were represented as the mean±standard deviation and compared by student's *t*-test. Median (*Q*1-*Q*3) were represented when the continuous variables with no-normal distribution and compared using Wilcoxon's rank-sum test. Categorical variables are expressed as frequency (percentages) and compared using the Chi-square test. Differences were examined as statistically significant at $p<0.05$.

Results

Demographic, Clinical, and Biochemical Test Index Characteristics

Overall, 102 patients with AS-related acute ischemic vascular disease [including AMI (N=74) and ACI (N=28)] and 83 control healthy people were enrolled in this study. The demographic, clinical, and biochemical test index characteristics were compared between the patient and control groups. Compared to the control group, acute ischemic vascular disease patients displayed higher rates of the male sex, drinking, and smoking history. The patient

group had higher WBC, N ratio, N/L, ALT, AST, LDL/HDL, and glucose levels, but lower L ratio and HDL levels compared to the control group. The patient group had higher LDL levels, but not statistically significant compared to the control group (Table I).

Serum Homocysteine, Folic Acid, Vit B12 Levels, and MTHFR Genotype in Control and Patient Group

As is shown in Figure 1A, the patient group had higher serum Hcy levels than the control group (p=0.013). To explore potential influence factors of the Hcy levels between the patient and control group, the serum folic acid, Vit B12 levels, and *MTHFR* genotype were also tested. We found the patients' serum folic acid (p<0.001) and Vit B12 levels (p=0.004) were lower than the control group (Figure 1B and 1C). Interestingly, no statistically significant differences in *MTHFR C677T* genotype and *C*/*T* alleles distribution were investigated between the patient and control group (Table II).

Correlation Analyses in Genotypes and Biochemical Indicators Between Patient and Control Group

To further explore possible reasons for the differences between the patient and control group, we compared the biochemical indicators with different genotypes in the two groups (Table III). The results show that Hcy levels (*vs. TT* genotype), WBC (*vs.* all genotypes), N ratio (*vs.* all genotypes), N/L (*vs.* all genotypes), ALT (*vs. CC* and *CT* genotypes), AST (*vs.* all genotypes), LDL/HDL (*vs. TT* genotype) were significantly higher than those in the control group. The folic acid level (*vs. CT* and *TT* genotypes), Vit B12 level (*vs. CC* and *CT* genotypes), and L ratio (*vs.* all genotypes) were significantly lower than those in the control group. Glucose levels of each genotype in patients were higher than those in the control group. Hcy level in the patients with the *TT* genotype was higher than those in the *CC* and *CT* genotypes, and the folic acid level in the patients with the *TT* genotype was lower than those in the *CC* genotype.

Table I. General clinical data between patients group and control group.

	Control (N = 83)	Patients (N = 102)	p
Sex			
Male Sex	34 (41.0%)	78 (76.5%)	< 0.001
Female Sex	49 (59.0%)	24 (23.5%)	
Age (years)	64.27 ± 7.63	62.97 ± 10.46	0.347
Smoking			
Yes	15 (18.1%)	41 (40.2%)	0.001
No	68 (81.9%)	61 (59.8%)	
Drinking			
Yes	4 (4.8%)	22 (21.6%)	0.001
No	79 (95.2%)	80 (78.4%)	
Hypertension			
Yes	42 (50.6%)	60 (58.8%)	0.299
No	41 (49.4%)	42 (41.2%)	
Diabetes Mellitus			
Yes	26 (31.3%)	37 (36.3%)	0.534
No	57 (68.7%)	65 (63.7%)	
WBC (*10^9/L)	6.36 (5.20, 7.11)	8.53 (7.39, 11.55)	< 0.001
N Ratio	0.63 (0.55, 0.66)	0.71 (0.63, 0.82)	< 0.001
L Ratio	0.28 (0.25, 0.33)	0.19 (0.13, 0.26)	< 0.001
N/L	2.21 (1.67, 2.68)	3.74 (2.38, 6.04)	< 0.001
ALT (U/L)	17.05 (13.03, 26.83)	25.60 (16.35, 43.65)	< 0.001
AST (U/L)	19.00 (15.80, 25.00)	49.30 (20.15, 149.80)	< 0.001
Uric acid (umol/L)	334.00 (276.50, 366.50)	334.50 (271.25, 398.00)	0.429
TC (mmol/L)	4.40 (3.78, 5.32)	4.44 (3.85, 5.36)	0.963
TG (mmol/L)	1.36 (1.11, 2.12)	1.67 (1.08, 2.58)	0.151
HDL (mmol/L)	1.06 (0.92, 1.26)	1.00 (0.86, 1.13)	0.047
LDL (mmol/L)	2.87 ± 0.90	3.17 ± 0.97	0.054
LDL/HDL	2.70 ± 0.89	3.17 ± 0.92	0.002
Glucose (mmol/L)	5.33 (4.89, 6.04)	6.48 (5.01, 8.62)	0.004

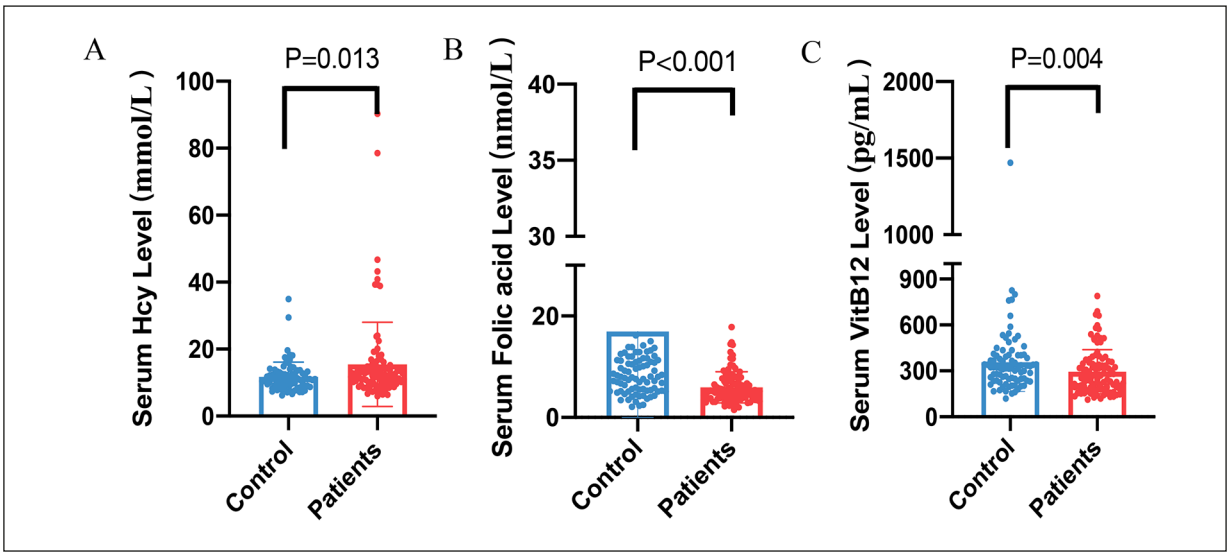

Figure 1. Serum Homocysteine, Folic acid, VitB 12 levels in Control and Patients group. **A**, Increased serum homocysteine levels was observed in patients compared to control groups(p = 0.013). **B**, Lower serum folic acid levels was observed in patients compared to control groups (p < 0.001). **C**, Lower serum Vit B12 levels was observed in patients compared to control groups (p = 0.004).

To further explore the reasons for the differences in Hcy levels between the patient and control group, we performed correlation analysis on serum homocysteine and folic acid levels, as well as serum homocysteine and Vit B12 levels (Figure 2). In the control group, there were negative and significant associations between serum homocysteine levels and serum vitamin B12 levels (r=-0.234, p=0.033) (Figure 2B), and no significant negative association between serum homocysteine levels and serum folic acid levels (r=-0.103, p=0.355) (Figure 2A). Conversely, there were negative and significant associations between serum homocysteine levels and serum folic acid levels (r=-0.257, p=0.01) (Figure 2C), and no significant negative association between serum homocysteine levels and serum vitamin B12 levels (r=-0.185, p=0.64) in the patient group (Figure 2D).

AMI Patients with Higher Stress Injury

Further analysis of potential causes affected AMI and ACI, we found patients with ACI were older, with higher rates of diabetes mellitus history. And the AMI patients presented with more severe stress injury, they showed higher WBC, N ratio, ALT, AST, and lower L ratio (Table IV). There were no statistically significant differences in serum Hcy level, folic acid level, Vit B12 level, *MTHFR C677T* genotype, and *C/T* alleles distribution were detected in AMI patients and ACI patients (**Supplementary Figure 1**, **Supplementary Table I**).

Table II. *MTHFR* genotype and allele distribution in control and patients groups.

Genotype/Allele	Control (N = 83)	Patients (N = 102)	X^2	p
MTHFR				
CC	18 (21.7%)	29 (28.4%)	-	-
CT	44 (53.0%)	41 (40.2%)	2.204	0.138
TT	21 (25.3%)	32 (31.4%)	0.018	0.892
CC	18 (21.7%)	29 (28.4%)	-	-
CT+TT	65 (78.3%)	73 (71.6%)	1.098	0.295
CC+CT	62 (74.7%)	70 (68.6%)	-	-
TT	21 (25.3%)	32 (31.4%)	0.825	0.364
C	80 (48.2%)	99 (48.5%)	-	-
T	86 (51.8%)	105 (51.5%)	0.004	0.949

Table III. Genotype and biochemical indicators correlation analysis between patients and control groups.

	Control (N = 83)			Patients (N = 102)		
	CC	***CT***	***TT***	***CC***	***CT***	***TT***
HCY (mmol/L)	10.37 (8.1, 12.7)	11.53 (9.45, 13.46)	10.42 (9.00, 12.75)	11.40 (8.91, 14.47)	12.07 (9.00, 13.80)	13.82 (10.37,23.95)*#
Folic acid (nmol/L)	7.80 (5.25, 12.00)	7.40 (4.95, 10.97)	9.00 (6.10, 11.55)	6.10 (4.25, 8.05)	5.10 (3.75, 7.20)*	4.50 (3.20,6.23)*$
VitB12 (pg/mL)	345.50 (234.75, 438.75)	315.50 (248.50, 382.50)	335.00 (230.00, 461.50)	227.00 (172.00, 351.50)*	248.00 (192.00, 331.50)*	268.00 (205.50, 479.75)
WBC (*10^9/L)	6.08 ± 1.23	6.15 ± 1.43	6.48 ± 1.89	10.09 ± 3.29*	8.89 ± 3.08*	9.16 ± 3.04*
N Ratio	0.63 ± 0.05	0.60 ± 0.08	0.61 ± 0.08	0.71 ± 0.17*	0.72 ± 0.10*	0.73 ± 0.12*
L Ratio	0.27 ± 0.05	0.30 ± 0.07	0.30 ± 0.08	0.20 ± 0.10*	0.20 ± 0.08*	0.20 ± 0.09*
N/L	2.33 (1.99, 2.68)	2.21 (1.59, 2.68)	2.18 (1.51,2.92)	3.55 (2.14, 8.18)*	3.81 (3.00, 5.68)*	3.68 (2.25,7.08)*
ALT (U/L)	15.10 (12.45, 24.80)	20.10 (14.10, 27.20)	14.30 (12.30, 22.40)	25.10 (16.55, 60.40)*	30.55 (17.43, 53.65)*	23.90 (13.90,39.80)
AST (U/L)	19.10 (14.30, 22.05)	21.30 (17.20, 26.70)	16.70 (14.40, 24.40)	63.80 (24.90, 193.40)*	48.35(20.93,162.00)*	37.30 (17.40,126.90)*
LDL/HDL	2.61 ± 0.76	2.90 ± 0.99	2.43 ± 0.60	3.23 ± 0.73	2.83 ± 0.63	3.50 ± 1.22*
Glucose (mmol/L)	5.44 (4.71 ,6.90)	5.36 (4.94,5.95)	5.05 (4.80, 8.17)	6.63 (5.08, 8.95)&	6.04 (4.96, 7.75)	7.10 (5.01,8.62)&

*$p < 0.05$ *vs*. each genotype in Control; &$p < 0.05$ *vs*. CT genotype in Control; $$p < 0.05$ *vs*. CC genotype in Patients; #$p < 0.05$ *vs*. CC and CT genotype in patients.

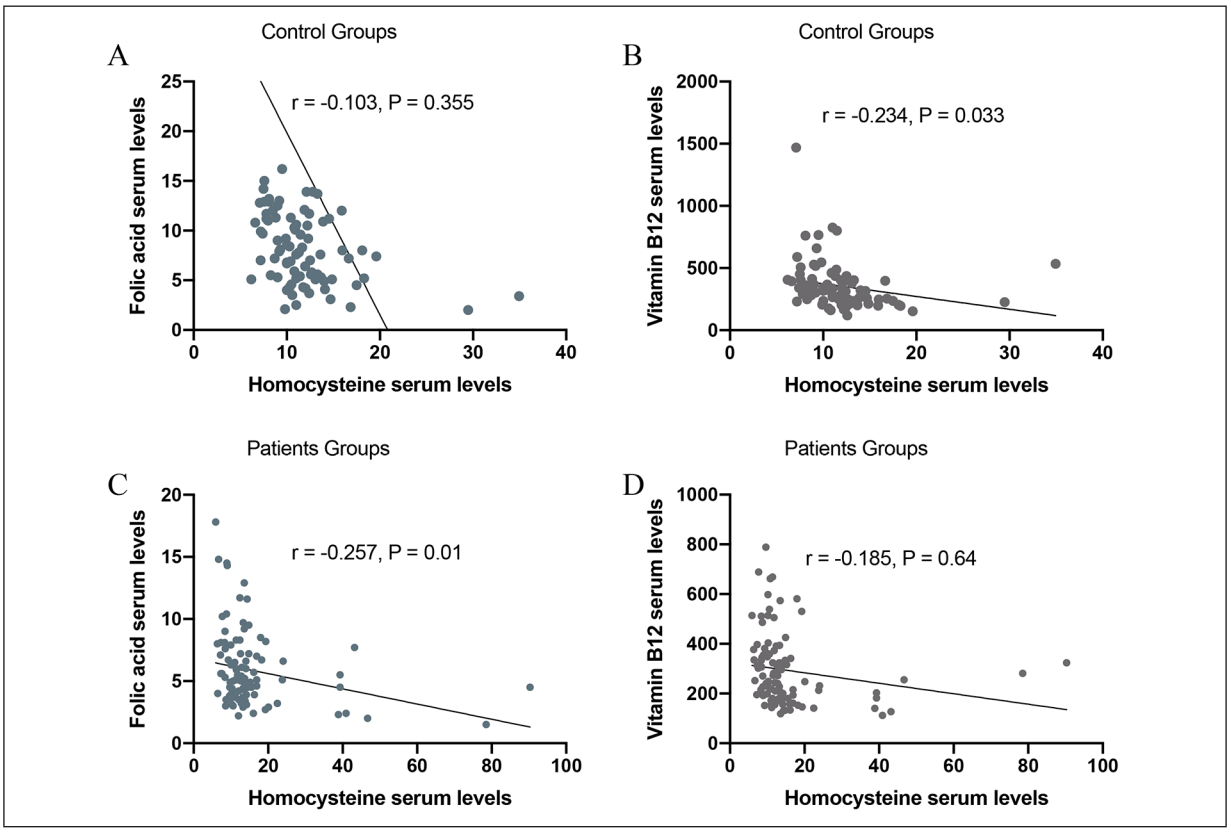

Figure 2. Correlation analysis on serum homocysteine with serum folic acid and Vit B12 levels in patients and control group. **A,** No significant negative association between serum homocysteine levels and serum folic acid levels (r = -0.103, $p = 0.355$) in control group. **B,** A negative and significant association between serum homocysteine levels and serum vitamin B12 levels (r = -0.234, $p = 0.033$) in control group. **C.** There were negative and significant association between serum homocysteine levels and serum folic acid levels (r = -0.257, $p = 0.01$) in patient group. **D,** No significant association between serum homocysteine levels and serum vitamin B12 levels (r = -0.185, $p = 0.64$) in patient group.

Discussion

In the present study, we analyzed the relationship among homocysteine levels, the *MTHFR C677T* genotypes, and atherosclerosis-related acute ischemic vascular events in 102 acute ischemic vascular events patients and 83 controlled healthy people. It has been found that patients with AMI and ACI had higher serum homocysteine levels, lower serum folate, and Vitamin B12 levels. The *TT* genotype of *MTHFR C677T* had higher levels of serum homocysteine than the *CC* and *CT* genotypes in the patient group. However, genotypes were not directly associated with acute ischemic vascular events, and the *MTHFR C677T* polymorphism did not differentially affect the burden and location of AMI and ACI.

No previous study has reported the effect of serum homocysteine and *MTHFR C677T* gene polymorphisms on acute ischemic vascular events and the burden of site distribution. Our study showed patients with AMI and ACI have higher serum homocysteine levels than the control people. In our subgroup analysis, there were no statistically significant differences in serum Hcy levels between AMI patients and ACI patients. The *MTHFR C677T* genotype had no direct contribution to the patients. Higher homocysteine levels are an important risk factor for atherosclerosis and induction of acute vascular events. Elevated homocysteine levels cause prothrombotic and proinflammatory effects[11,12], induce oxidative stress, and lead to endothelial dysfunction and vascular damage[13]. Studies[14-17] have found a close relationship between higher homocysteine levels and AMI or ACI.

Our study found that the *MTHFR 677 TT* genotype was significantly related to higher homocysteine levels in AMI and ACI patients. It has been known that *MTHFR C677T* gene polymorphisms

Table IV. General clinical data between AMI patients and ACI patients.

	AMI (N = 74)	ACI (N = 28)	*p*
Sex			
Male Sex	59 (79.7%)	19 (67.9%)	0.207
Female Sex	15 (20.3%)	9 (32.1%)	
Age (years)	61.56 ± 9.44	66.21 ± 12.49	0.028
Smoking			
Yes	32 (43.2%)	9 (32.1%)	0.308
No	42 (56.8%)	19 (67.9%)	
Drinking			
Yes	4 (14.3%)	18 (24.3%)	0.271
No	24 (85.7%)	56 (75.7%)	
Hypertension			
Yes	41 (55.4%)	19 (67.9%)	0.254
No	33 (44.6%)	9 (32.1%)	
Diabetes Mellitus			
Yes	22 (29.7%)	15 (53.6%)	0.025
No	52 (70.3%)	13 (46.4%)	
WBC (*109/L)	10.07 ± 3.20	7.08 ± 1.77	< 0.001
N Ratio	0.76 ± 0.11	0.61 ± 0.12	< 0.001
L Ratio	0.17 ± 0.08	0.28 ± 0.07	< 0.001
N/L	4.64 (3.33, 7.55)	2.28 (1.69,3.01)	< 0.001
ALT (U/L)	34.05 (21.33, 51.68)	14.70 (10.75, 24.35)	< 0.001
AST (U/L)	108.20 (41.80, 205.33)	17.05 (14.63, 20.93)	< 0.001
Uric acid (umol/L)	348.50 (270.50, 400.25)	321.50 (278.00, 393.25)	0.977
TC (mmol/L)	4.68 ± 1.15	4.31 ± 1.02	0.119
TG (mmol/L)	1.61 (1.07, 2.51)	1.74 (1.05, 2.75)	0.605
HDL (mmol/L)	1.03 ± 0.25	0.97 ± 0.19	0.136
LDL (mmol/L)	3.23 ± 0.96	2.91 ± 0.92	0.125
LDL/HDL	3.21 ± 0.88	3.06 ± 0.92	0.667
Glucose (mmol/L)	6.56 (5.10, 8.62)	5.40(4.73, 8.48)	0.241

ACI: Acute cerebral infarction; AMI: Acute myocardial infarction.

are the most common mutation cause of hyperhomocysteine[9]. *MTHFR* gene mutation may play an important role in vascular injury-related diseases[18]. Some studies[19-21] have shown that *MTHFR TT* genotype patients have more severe coronary lesions, and the *TT* genotype has a strong relationship with the risk of ischemic stroke, while other research[22,23] failed to demonstrate this direct correlation. We also did not find a direct correlation between *MTHFR C677T* genotypes and acute ischemic vascular events in our study. These deviations may be attributed to small study populations, regional differences, and inconsistent assessment methods. However, we could not deny the contribution of *MTHFR C677T* genetic mutations to homocysteine levels. Hereditary factors may play an important push role in promoting homocysteine-induced vascular disease.

Our present study also found the AMI and ACI patients had lower serum folate and Vit B12 levels than the control group, and the folic acid levels in the patients with the *TT* genotype were lower than those with the *CC* genotype. Folic acid and vitamin B12 are two important regulators of homocysteine metabolism[24]. Studies[25] show that lower folate level is linked to an increased risk of stroke, folate supplementation may reduce serum homocysteine levels and prevent stroke. The CSPPT study declared that low levels of folate and Vit B12 were a significant association with first ischemic stroke in China hypertensive patients, but daily 0.8 mg folic acid supplementation would reduce first ischemic stroke in patients with low levels of folate and Vit B12 with *MTHFR 677 CC* genotype and patients with higher levels of folate and Vit B12 with *MTHFR 677 TT* genotype[26]. This was mainly due to folate treatment significantly reduced homocysteine levels. Interestingly, a study found the influence of the *MTHFR C677T* mutation on homocysteine levels was larger in low folic acid regions, folic acid treatment had no significant effect on decreasing stroke risk in countries where folic acid fortification food is mandatory[26,27]. Although our study found a negative and significant association between serum homocysteine levels and serum

folic acid levels in the patients, but not the control group. The abnormal homocysteine levels induced by genotype difference could be corrected by folic acid supplementation. However, the exact amount of supplementation is still under discussion.

In our study, we also found higher WBC, N ratio, ALT, AST, LDL/HDL, and lower L ratio in patients with acute ischemic vascular events, especially in AMI patients. Acute inflammatory response after ischemic is related to poor outcomes[28,29]. In addition, LDL/HDL was a more sensitive predictor of recurrent ischemic events than total cholesterol, LDL, or HDL[30]. However, the predictive value of inflammatory markers and LDL/HDL for recurrent AMI and ACI still requires further study.

Although *MTHFR* genotype and serum Hcy levels did not affect the site and burden of acute vascular events. Whether the influencing factors of cardiac, cerebral, and lower extremity ischemic vascular events are different and exploring these will be of great value in preventing and improving the prognosis of the disease.

Limitations

However, there are still many limitations. First, the small sample size of the study may lead to biassing results. Second, the intervention of folic acid supplementation was not carried out, and the choice of folic acid dose in patients with different genotypes remains to be investigated.

Conclusions

In summary, we elaborated on a significant relationship between homocysteine levels and both AMI and ACI in China's northeast patients. Homocysteine played a common role in atherosclerosis-related acute ischemic vascular events. This correlation was modified by *MTHFR C677T* polymorphisms and influenced by folic acid levels. Whether reducing homocysteine by supplementing folic acid could improve clinical outcomes in these patients still needs further study.

Conflict of Interest

The Authors declare that they have no conflict of interests.

Funding

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Informed Consent

Written consent forms were signed by all the participating patients.

Ethics Approval

This study has been cleared by the First Hospital of Jilin University Institution Ethics Review Board for human studies (#2020-413, September 18th, 2020).

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