Homocysteine and Stroke Risk

Modifying Effect of Methylenetetrahydrofolate Reductase C677T Polymorphism and Folic Acid Intervention

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Background and Purpose—Elevated blood homocysteine concentration increases the risk of stroke, especially among hypertensive individuals. Homocysteine is largely affected by the methylenetetrahydrofolate reductase C677T polymorphism and folate status. Among hypertensive patients, we aimed to test the hypothesis that the association between homocysteine and stroke can be modified by the methylenetetrahydrofolate reductase C677T polymorphism and folic acid intervention.

Methods—We analyzed the data of 20 424 hypertensive adults enrolled in the China Stroke Primary Prevention Trial. The participants, first stratified by methylenetetrahydrofolate reductase genotype, were randomly assigned to receive double-blind treatments of 10-mg enalapril and 0.8-mg folic acid or 10-mg enalapril only. The participants were followed up for a median of 4.5 years.

Results—In the control group, baseline log-transformed homocysteine was associated with an increased risk of first stroke among participants with the CC/CT genotype (hazard ratio, 3.1; 1.1–9.2), but not among participants with the TT genotype (hazard ratio, 0.7; 0.2–2.1), indicating a significant gene–homocysteine interaction (P=0.008). In the folic acid intervention group, homocysteine showed no significant effect on stroke regardless of genotype. Consistently, folic acid intervention significantly reduced stroke risk in participants with CC/CT genotypes and high homocysteine levels (tertile 3; hazard ratio, 0.73; 0.55–0.97).

Conclusions—In Chinese hypertensive patients, the effect of homocysteine on the first stroke was significantly modified by the methylenetetrahydrofolate reductase C677T genotype and folic acid supplementation. Such information may help to more precisely predict stroke risk and develop folic acid interventions tailored to individual genetic background and nutritional status.

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Stroke is the leading cause of mortality and disability in China and the second leading cause of death in the world.[1,2] Hypertension has been identified as the most important risk factor for stroke.[3] Current data show that homocysteine is an important risk factor of stroke,[4] especially in hypertensive patients.[5,6] This study aimed to address several important questions related to the role of homocysteine in stroke and to fill in critical data gaps in the field.

First of all, can the homocysteine–stroke relationship be modified by individual genetic background? Homocysteine is a sulfur-containing amino acid produced by the demethylation of the essential amino acid methionine, and the methylenetetrahydrofolate reductase (MTHFR) C677T polymorphism is a major determinant of blood homocysteine levels.[7] Although the MTHFR C677T polymorphism has been studied extensively in relation to hyperhomocysteinemia in low folate

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settings,[8] most studies that have addressed the relationship between high levels of homocysteine and stroke risk did not take into account the potential modification effect of the MTHFR C677T genotype.[9] In fact, these previous studies have yielded inconsistent findings with regards to the association between the MTHFR TT genotype and stroke.[10–13] As a result, there is a particular need for studies that examine the joint effect of MTHFR genotype and homocysteine on stroke.

Second, according to a meta-analysis, the effect of the MTHFR 677C→T variant on homocysteine can be modified by the prevailing concentration of folate in the body.[7] Folic acid is the treatment of choice to lower homocysteine.[14] The primary findings of the CSPPT (China Stroke Primary Prevention Trial) showed that folic acid supplementation can reduce the first stroke by 21% in an overall hypertensive population.[15] However, questions remain on to what degree folic acid supplementation can reduce the adverse effect of homocysteine on the first stroke, specifically in subgroups defined by MTHFR genotype and baseline homocysteine.

Finally, there is a particular lack of relevant studies in Chinese populations, who have distinctive characteristics when compared with western populations, including a high prevalence of hypertension, elevated homocysteine, and the MTHFR C677T gene variant, and for whom stroke is the leading cause of death.[15] China is a vast country and one without mandatory folic acid fortification. Such characteristics offer an exceptional opportunity to address the aforementioned questions.

Using data from the CSPPT, we sought to examine the longitudinal association between baseline homocysteine and stroke in Chinese adults with hypertension but without history of stroke or myocardial infarction. We were particularly interested in testing the hypothesis that the association between homocysteine and stroke can be modified by the MTHFR C677T polymorphism and folic acid intervention. Findings from this investigation will help to fill in the critical identified data gaps and, more importantly, inform a more precise prediction and prevention of stroke using homocysteine, folate, and MTHFR C677T as biomarkers.

Materials and Methods

Study Population

All subjects were participants from the CSPPT. The CSPPT is a large community-based, randomized, multisite, double-blind, and actively-controlled trial designed to evaluate the efficacy of lowering both blood pressure (BP) and homocysteine compared with lowering BP alone in reducing the risk of stroke in hypertensive patients. The detailed inclusion and exclusion criteria, treatment assignment, and outcome measures of the trial have been described in previous publications[15–17] and shared on a related website (http://clinicaltrials.gov/ct2/show/NCT00794885).

Briefly, a total of 20 702 participants were enrolled from 32 communities in China and were randomized to the enalapril-folic acid group and 10 354 participants to the enalapril-only group. After excluding 278 participants without baseline homocysteine information, 20 424 participants were included in the final analyses.

Data Collection

Baseline Data Collection

Baseline demographic data, medical history, and medication use as well as seated BP measurements were collected and recorded by trained research staff during the study entry process according to standard operating procedures. Systolic BP and diastolic BP were calculated as the mean of three measurements. Each participant gave written informed consent before data collection.

Laboratory Tests

An ABI Prism 7900HT sequence detection system (Life Technologies) was used to determine MTHFR C677T polymorphism status. Serum folate and vitamin B12 levels were measured by a commercial laboratory using a chemiluminescent immunoassay (New Industrial), whereas baseline serum homocysteine, fasting lipids, and glucose levels were measured using automatic clinical analyzers (Beckman Coulter) at the core laboratory of the National Clinical Research Center for Kidney Disease, Nanfang Hospital, Guangzhou, China.

Follow-Up and Outcome Measurements

Participants were followed up every 3 months for a median of 4.5 years. The primary outcome of the CSPPT was a first stroke. Two independent neurologists separately identified all positive candidate cases based on medical records, imaging data, and event report forms. Suspected stroke cases were verified by the event adjudication committee. Stroke was identified and classified according to the *International Statistical Classification of Disease*, 10th edition.

Statistical Analysis

Data are presented as mean, median (SD or interquartile range), or proportion. Because the distribution of homocysteine levels was skewed toward high values, the variable was log-transformed before analysis. The Cox proportional hazards model was used to estimate the magnitude of effect of homocysteine for stroke. All P values for statistics were 2-tailed, and a $P<0.05$ was regarded as statistically significant. Empower (www.empowerstats.com; X&Y solutions Inc, Boston, MA) and R (http://www.R-project.org) were used for all statistical analyses.

Results

Baseline Characteristics

Baseline characteristics are shown in Table 1. Of the 20 424 participants included in the analysis, 15 626 (76.5%) were carriers of the MTHFR CC/CT genotype whereas 4798 (23.5%) carried the TT genotype. Homocysteine levels were higher among those with the TT genotype (≈15 μmol/L) than those with the CC/CT genotype (≈12 μmol/L). Likewise, folate and vitamin B12 levels were lower among those with the TT genotype than those with the CC/CT genotype. There were no obvious differences in other characteristics between the different MTHFR genotype groups and the 2 treatment groups.

Incidence of Stroke

During a median treatment duration of 4.5 years, first-ever stroke occurred in 621 participants: 502 were ischemic stroke and 117 were hemorrhagic stroke while 2 cases were with uncertain type of stroke. Of these stroke occurrences, 452 were among those with the MTHFR CC/CT genotype while 169 were among those with the TT genotype. The crude relative risk for rate of stroke in those with TT versus CC/CT genotype was 1.23 (95% confidence interval [CI], 1.02–1.47) and was 1.09 (0.91–1.31) in the multivariate-adjusted model.

Association Between Homocysteine and Stroke: Effect Modification by MTHFR C677T

In the enalapril-only group, the hazard ratio (HR) of log-transformed homocysteine (log-Hcy) for stroke was 3.1 (95% CI, 1.1–9.2) among participants with the CC/CT genotype and

Table 1. Clinical Characteristics of Participants by Methylenetetrahydrofolate Reductase Genotype and Treatment Group

Variables	All Populations (n=20 424)	CC+CT			TT		
		Enalapril (n=7811)	Enalapril-FA (n=7815)	*P* Value	Enalapril (n=2403)	Enalapril-FA (n=2395)	*P* Value
Male, n (%)	8352 (40.9)	3211 (41.1)	3170 (40.6)	0.49	975 (40.6)	996 (41.6)	0.48
Age, mean (SD), y	60.0 (7.5)	59.9 (7.6)	60.1 (7.5)	0.17	60.1 (7.5)	59.8 (7.5)	0.31
Body mass index, mean (SD)*	24.9 (3.7)	24.8 (3.7)	24.9 (3.7)	0.21	25.2 (3.7)	25.1 (3.6)	0.64
SBP, mean (SD), mm Hg	166.9 (20.4)	166.8 (20.3)	166.7 (20.3)	0.67	167.2 (20.9)	167.2 (20.7)	0.93
DBP, mean (SD), mm Hg	94.1 (11.9)	93.8 (12.0)	93.9 (11.9)	0.69	94.6 (12.1)	94.9 (11.4)	0.44
Smoking, n (%)				0.09			0.64
Never	14 077 (69.0)	5367 (68.7)	5397 (69.1)		1671 (69.5)	1642 (68.6)	
Former	1549 (7.6)	627 (8.0)	555 (7.1)		176 (7.3)	191 (8.0)	
Current	4789 (23.5)	1815 (23.2)	1856 (23.8)		556 (23.1)	562 (23.5)	
Alcohol drinking, n (%)				0.35			0.96
Never	14 093 (69.0)	5336 (68.3)	5409 (69.3)		1681 (70.0)	1667 (69.6)	
Former	1436 (7.0)	577 (7.4)	540 (6.9)		158 (6.6)	161 (6.7)	
Current	4883 (23.9)	1896 (24.3)	1857 (23.8)		563 (23.4)	567 (23.7)	
Laboratory results							
Fasting plasma glucose, mean (SD), mmol/L	5.8 (1.7)	5.8 (1.7)	5.7 (1.6)	0.50	6.0 (1.9)	5.9 (1.8)	0.27
TG, mean (SD), mmol/L	1.7 (1.2)	1.6 (0.9)	1.7 (1.4)	0.33	1.7 (0.9)	1.7 (1.0)	0.19
TC, mean (SD), mmol/L	5.5 (1.2)	5.5 (1.2)	5.5 (1.2)	0.21	5.6 (1.2)	5.6 (1.2)	0.33
HDL, mean (SD), mmol/L	1.3 (0.4)	1.3 (0.4)	1.3 (0.4)	0.71	1.3 (0.4)	1.3 (0.4)	0.13
Hcy, median (IQR), μmol/L†	12.5 (10.5–15.5)	12.0 (10.2–14.4)	12.1 (10.2–14.5)	0.92	15.3 (11.9–21.9)	15.1 (11.7–21.4)	0.39
Vitamin B12, median (IQR), pg/mL†	379.8 (314.9–476.7)	385.8 (319.4–484.6)	384.5 (318.2–482.2)	0.05	362.2 (304.6–454.1)	362.2 (304.4–450.7)	0.55
Folate, median (IQR), ng/mL†	8.1 (5.6–10.5)	8.5 (6.0–10.9)	8.5 (6.0–10.9)	0.68	6.5 (4.8–9.1)	6.5 (4.8–9.1)	0.38

Enalapril indicates enalapril-only group; Enalapril-FA, enalapril-folic acid group; DBP, diastolic blood pressure; Hcy, homocysteine; HDL, high-density lipoprotein; IQR, interquartile range; SBP, systolic blood pressure; TC, total cholesterol; and TG, triglycerides.

*Calculated as weight in kilograms divided by height in meters squared.

†Wilcoxon signed rank test was used.

correspondingly 0.7 (95% CI, 0.2–2.1) among participants with the TT genotype, indicating a significant interaction between homocysteine and MTHFR genotype (*P*=0.008). We also conducted the analysis according to the 3 different MTHFR genotypes. The HRs of log-Hcy for stroke among those with the CC and CT genotypes, separately, were 2.1 (0.3–13.8) and 4.4 (1.2–16.6), respectively, which is similar to the HR for the CC and CT genotype combined (data not shown).

The analysis conducted according to tertiles of homocysteine yielded a similar pattern. In the enalapril-only group, the incidence of stroke was 2.3%, 2.6%, and 4.6%, respectively, among the lowest to highest tertiles of homocysteine for participants with the CC/CT genotype (Figure). Accordingly, the HRs of homocysteine tertiles 2 and 3 for stroke were 1.0 (0.7–1.4) and 1.4 (1.0–2.1), respectively (*P* for trend=0.033) in the CC/CT genotype. In contrast, the HRs of the corresponding tertiles in the TT genotype group were 0.7 (0.4–1.1) and 0.6 (0.4–1.2; Table 2).

Association Between Homocysteine and Stroke: Effect Modification by Folic Acid Intervention

For participants in the enalapril-folic acid group, the association between log-Hcy and stroke was no longer significant among those with the CC/CT genotype (HR, 0.7; 95% CI, 0.2–2.8). Again, there was no association with the TT genotype (HR, 0.6; 95% CI, 0.2–2.3). A similar pattern was observed when homocysteine was analyzed by tertiles (Table 2).

Efficacy of Folic Acid Supplementation in Subgroups Defined by MTHFR Genotype and Homocysteine

Folic acid supplementation reduced stroke risk by 15% (HR, 0.85; 0.70–1.02) among participants with the MTHFR CC/CT genotype and 30% (HR, 0.70; 0.51–0.95; *P* for interaction, 0.286) among those with the TT genotype. We further investigated the efficacy of folic acid supplementation in subgroups

defined by homocysteine tertiles and MTHFR genotypes. Among participants with the CC/CT genotype, folic acid supplementation significantly reduced stroke risk for those in the highest homocysteine tertile (HR, 0.73; 95% CI, 0.55–0.97). Interestingly, among those with the TT genotype, the reduction was most obvious for those in the lowest homocysteine tertile (HR, 0.44; 95% CI, 0.24–0.79; Table 3). The results were similar for ischemic stroke (Tables 2 and 3).

Discussion

To our knowledge, this is the first study to test the hypothesis that the association between homocysteine and stroke can be modified by MTHFR C677T polymorphism and folic acid intervention, using data from the CSPPT. This study has the following unique features. It was by far the largest randomized clinical trial on the primary prevention of stroke where all participants were from areas without folic acid fortification and did not take vitamins or folic acid supplements. The CSPPT included hypertensive participants without pre-existing stroke or myocardial infarction. With a low vascular disease burden and a low-frequency use of cardiac and vascular protective drugs, our results were less likely to be confounded by medications and vascular diseases. Another important feature was that >70% of the CSPPT hypertensive patients had elevated homocysteine, a major risk factor for stroke.[18] The CSPPT obtained individual data on MTHFR C677T genotype, folate, homocysteine levels, and other important factors. Taken together, this study provided an exceptional opportunity to address the study hypothesis.[19] Ultimately, the study gained important new insights regarding the interplay of homocysteine, MTHFR genotype, and folic acid intervention on first stroke.

Homocysteine and Stroke Association: Effect Modification by MTHFR C677T Genotype

Previous studies had already demonstrated that high levels of homocysteine are closely associated with stroke.[4–6,20,21] Holmes meta-analysis showed that individuals with the MTHFR TT genotype had higher levels of homocysteine than those with the CC/CT genotype.[7] It was speculated that the effect of homocysteine on risk of stroke may differ by MTHFR genotype. The study by Mehlig et al[22] found that the association between elevated homocysteine levels and coronary heart disease was confined to carriers of the MTHFR 677C-allele, showing a reduced adverse association between homocysteine and coronary heart disease in MTHFR 677 T-allele carriers, particularly in T-homozygotes. Data from the Multicenter Chinese Stroke Study showed that hyperhomocysteinemia are more closely related to stroke recurrence among participants with the MTHFR TT genotype.[23] Although both studies suffered from inadequate sample size and lacked information on folate status, they still raised an interesting question about the gene–environment interaction between homocysteine, MTHFR C677T polymorphism, and vascular diseases. However, most studies that have addressed the relationship between high levels of homocysteine and stroke risk did not take into account the potential modification effect of the MTHFR C677T genotype and folate levels, in part, because these previous studies either did not measure or only measured a small fraction of the total study samples for the MTHFR genotype and homocysteine. In effect, because we did measure the homocysteine, folate and the MTHFR genotype, our study offers an exceptional opportunity to examine the joint effect of MTHFR genotype and homocysteine on the first stroke.

Our study is by far the largest of its kind, where all 20 424 participants had data on baseline homocysteine concentration and MTHFR genotype. In a previous publication, we reported an inverse relationship between baseline folate level and risk of stroke among participants in the enalapril-only group with the CC/CT genotype, whereas participants with the TT genotype had a persistently high risk of stroke across all folate quartiles.[15] However, the association between folate levels and cardiovascular disease (CVD) can be different from that of homocysteine levels and CVD. The study by Yang et al[24] found that a higher folate concentration was associated with a lower risk of all-cause mortality and CVD mortality, whereas

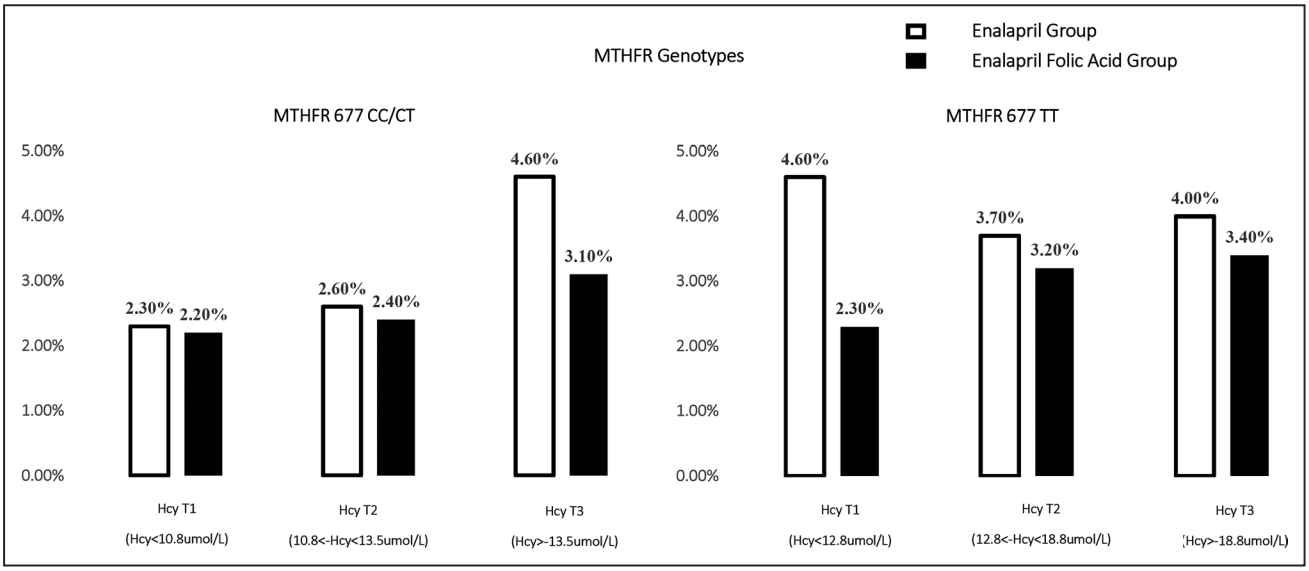

Figure. Rates of first stroke by treatment groups, methylene tetrahydrofolate reductase genotypes, and baseline homocysteine levels.

Table 2. Modifying Effect of the Methylene Tetrahydrofolate Reductase Genotype on the Association Between Homocysteine and First Stroke, Stratified by the Treatment Group

Outcomes	n	No. of Events (%)		Hazard Ratio (95% CI)*				*P* for Interaction*
		CC+CT	TT	CC+CT	*P* for trend	TT	*P* for trend	
Stroke								
Log-Hcy								
Enalapril	10214	250/7811 (3.2)	98/2403 (4.1)	3.1 (1.1–9.2)		0.7 (0.2–2.1)		0.008
Enalapril-FA	10210	202/7815 (2.6)	71/2395 (3.0)	0.7 (0.2–2.8)		0.6 (0.2–2.3)		0.456
Tertiles of Hcy, μmol/L								
Enalapril								
Tertile 1	3404	61/2617 (2.3)	36/787 (4.6)	Ref.	0.033	Ref.	0.150	0.016
Tertile 2	3427	69/2613 (2.6)	30/814 (3.7)	1.0 (0.7–1.4)		0.7 (0.4–1.1)		
Tertile 3	3383	120/2581 (4.6)	32/802 (4.0)	1.4 (1.0–2.1)		0.6 (0.4–1.2)		
Enalapril-FA								
Tertile 1	3398	58/2586 (2.2)	19/812 (2.3)	Ref.	0.924	Ref.	0.765	0.522
Tertile 2	3370	62/2586 (2.4)	25/784 (3.2)	0.9 (0.6–1.3)		1.1 (0.5–2.0)		
Tertile 3	3442	82/2643 (3.1)	27/799 (3.4)	1.0 (0.6–1.5)		0.9 (0.4–1.9)		
Ischemic stroke								
Log-Hcy								
Enalapril	10214	205/7811 (2.6)	82/2403 (3.4)	3.2 (1.0 10.4)		0.6 (0.2–2.2)		0.005
Enalapril-FA	10210	158/7815 (2.0)	57/2395 (2.4)	0.7 (0.2–3.2)		0.7 (0.2–2.9)		0.660
Tertiles of Hcy, μmol/L								
Enalapril								
Tertile 1	3404	45/2617 (1.7)	31/787 (3.9)	Ref.	0.010	Ref.	0.124	0.003
Tertile 2	3427	58/2613 (2.2)	25/814 (3.1)	1.2 (0.8–1.7)		0.6 (0.4–1.1)		
Tertile 3	3383	102/2581 (4.0)	26/802 (3.2)	1.7 (1.1–2.5)		0.6 (0.3–1.2)		
Enalapril-FA								
Tertile 1	3398	43/2585 (1.7)	16/812 (2.0)	Ref.	0.705	Ref.	0.832	0.769
Tertile 2	3370	49/2586 (1.9)	17/784 (2.2)	0.9 (0.6–1.4)		0.8 (0.4–1.6)		
Tertile 3	3442	66/2643 (2.5)	24/799 (3.0)	0.9 (0.6–1.4)		0.9 (0.4–2.0)		

CI indicates confidence interval; Enalapril, enalapril-only group; Enalapril-FA, enalapril-folic acid group; FA, folic acid; and Log-Hcy, log-transformed homocysteine.

*Adjusted for age, sex, study center, smoking status, alcohol drinking, body mass index, glucose, total cholesterol, triglycerides, high-density lipids, folate levels, estimated glomerular filtration rate, baseline systolic blood pressure, baseline diastolic blood pressure, mean systolic blood pressure, and diastolic blood pressure over the treatment period.

homocysteine concentration was not significantly associated with the risk of all-cause mortality or CVD mortality. Our study extends previous study in this area and demonstrates that the association between homocysteine and stroke can be significantly modified by the MTHFR C677T genotype: in the control group, homocysteine was associated with increased risk of first stroke among participants with the CC/CT genotype, but not among participants with the TT genotype. Our data indicated a significant MTHFR gene–homocysteine interaction on first stroke (*P*=0.008).

Homocysteine and Stroke Association: Effect Modification by Folic Acid Intervention

As is well known, folic acid supplementation can lower homocysteine levels.[7,25] However, the conclusions of most previous randomized trials have been negative regarding the impact of folic acid supplementation on stroke risk.[26–30] Notably, these previous trials were conducted in populations living in areas with mandatory folic acid fortification. The CSPPT was the first randomized clinical trial to reveal that among hypertensive populations without folic acid fortification, folic acid supplementation can reduce stroke risk by 21%.[15] The present study further assessed the modifying effect of folic acid supplementation on the homocysteine–stroke association with regard to MTHFR genotype. We found that the HRs of log-Hcy for stroke among participants with the CC/CT genotype were reduced from 3.1 in the enalapril-only group to 0.7 in the enalapril-folic-acid group. These results provide evidence that folic acid intervention can significantly attenuate the homocysteine–stroke association for those with the CC/

Table 3. Efficacy of FA Supplementation in Preventing First Stroke in Subgroups Defined by Methylene Tetrahydrofolate Reductase Genotype and Homocysteine Tertiles

	Events (%)		Crude Hazard Ratio (95% CI)	Adjusted Hazard Ratio (95% CI)*	*P* Value*	*P* for Interaction*
	Enalapril Group	Enalapril-FA Group				
Homocysteine, μmol/L						
CC/CT						
Total	250/7811 (3.2)	202/7815 (2.6)	0.80 (0.67–0.97)	0.85 (0.70–1.02)	0.08	
Tertile 1 (<10.8)	61/2617 (2.3)	58/2586 (2.2)	0.96 (0.67–1.38)	0.99 (0.68–1.42)	0.94	0.41
Tertile 2 (10.8–13.5)	69/2613 (2.6)	62/2586 (2.4)	0.91 (0.64–1.28)	0.96 (0.68–1.36)	0.81	
Tertile 3 (>–13.5)	120/2581 (4.6)	82/2643 (3.1)	0.66 (0.50–0.88)	0.73 (0.55–0.97)	0.03	
TT						0.10
Total	98/2403 (4.1)	71/2395 (3.0)	0.72 (0.53–0.98)	0.70 (0.51–0.95)	0.02	
Tertile 1 (<12.8)	36/787 (4.6)	19/812 (2.3)	0.51 (0.29–0.89)	0.44 (0.24–0.79)	0.01	
Tertile 2 (12.8–18.8)	30/814 (3.7)	25/784 (3.2)	0.85 (0.50–1.45)	0.77 (0.45–1.33)	0.35	
Tertile 3 (>–18.8)	32/802 (4.0)	27/799 (3.4)	0.85 (0.51–1.42)	0.89 (0.53–1.50)	0.66	
Ischemic stroke						
CC/CT						0.35
Total	205/7811 (2.6)	158/7815 (2.0)	0.77 (0.62–0.95)	0.80 (0.65–0.99)	0.04	
Tertile 1 (<10.8)	45/2617 (1.7)	43/2585 (1.7)	0.97 (0.64–1.47)	1.03 (0.67–1.57)	0.91	
Tertile 2 (10.8–13.5)	58/2613 (2.2)	49/2586 (1.9)	0.85 (0.58–1.25)	0.88 (0.60–1.30)	0.52	
Tertile 3 (>–13.5)	102/2581 (4.0)	66/2643 (2.5)	0.63 (0.46–0.86)	0.69 (0.51–0.95)	0.02	
TT						
Total	82/2403 (3.4)	57/2395 (2.4)	0.69 (0.49–0.97)	0.69 (0.49–0.97)	0.04	
Tertile 1 (<12.8)	31/787 (3.9)	16/812 (2.0)	0.50 (0.27–0.91)	0.45 (0.24–0.85)	0.01	0.12
Tertile 2 (12.8–18.8)	25/814 (3.1)	17/784 (2.2)	0.70 (0.38–1.29)	0.65 (0.34–1.23)	0.18	
Tertile 3 (>–18.8)	26/802 (3.2)	24/799 (3.0)	0.93 (0.54–1.62)	0.99 (0.56–1.75)	0.98	

CI indicates confidence interval.

*Adjusted for age, sex, study center, smoking status, alcohol drinking, body mass index, glucose, total cholesterol, triglycerides, high-density lipids, folate levels, estimated glomerular filtration rate, baseline systolic blood pressure, baseline diastolic blood pressure, mean systolic blood pressure, and diastolic blood pressure over the treatment period.

CT genotype. Furthermore, our study revealed that the efficacy of folic acid intervention differed by subgroups defined by MTHFR C677T genotype and homocysteine. Within the MTHFR CC/CT genotype, those in the highest homocysteine tertile benefited more. Within the MTHFR TT genotype group, those with lower homocysteine benefited more. Although we cannot exclude the possibility of chance, this opposite trend could be because of following reasons.

Compared with participants with CC/CT genotype, those with TT genotype may have both folate insufficiency and decreased MTHFR enzyme activity (a key enzyme in homocysteine metabolism). On average, the MTHFR enzyme loses 70% of its activity in homozygous 677TT carriers. Jacques et al[31] suggested that participants homozygous for the T677 allele might have higher folate requirements to regulate homocysteine, especially if they also have low folate status. Therefore, we speculate that the current dosage (0.8-mg folic acid) might not be adequate to overcome the coexistence of low folate status and the enzyme dysfunction among TT participants with higher homocysteine levels.

Furthermore, 677TT carriers could suffer from long-standing injury, it might need a longer-term and higher dosage of folic acid to overcome the biologically dysfunction. Based on these results, the CSPPT group is planning to conduct another randomized controlled trial to evaluate the effect of a higher dose of folic acid on reducing the stroke risk among participants with TT genotype to identify optimal dosage of folic acid according MTHFR genotypes.

Although the reasons for the modifying effect of the MTHFR polymorphisms seen in the present study are unclear, our study findings have important clinical and public health implications.

The characteristics of hypertensive patients in China differ from those of Western populations. More than 50% of hypertensive patients in China have hyperhomocysteinemia,[32] and among these ≈24% carry the MTHFR TT genotype, which is much higher than that of European populations.[33] The precision evaluation of the association between homocysteine levels and the MTHFR C677T polymorphism for stroke risk is not only feasible but also important among

this population. The 2010 Chinese Guidelines for the Management of Hypertension already recommend that hypertensive patients have their homocysteine levels tested and point out that hyperhomocysteinemia is a risk factor for CVD.[34] The results of our study further underscore the importance of homocysteine, folate, and MTHFR as biomarkers in assessing stroke risk and highlight the opportunity to more precisely predict stroke risk and develop folic acid interventions that can be tailored to an individual's genetic background and nutritional status.[35] This study is an important step toward precision medicine that aims for the development of personalized therapy and has the potential to improve clinical outcomes.[36]

Limitations

Our study had some limitations. First, the population comprised hypertensive participants, limiting the generalizability of our results. Second, as a post hoc analysis, this study may have been underpowered for certain subgroup analyses, and may suffer from residual confounding. It is possible that these finding might be because of chance.

Summary

Among Chinese hypertensive patients, the effect of homocysteine on first stroke was significantly modified by the MTHFR C677T genotype and folic acid supplementation. Furthermore, the efficacy of folic acid supplementation on stroke differed by subgroups defined by MTHFR C677T genotype and homocysteine subgroups. Such information can be used to more precisely predict stroke risk and develop folic acid intervention strategies tailored to an individual's genetic background and nutritional status.

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