Original Investigation

Efficacy of Folic Acid Therapy in Primary Prevention of Stroke Among Adults With Hypertension in China
The CSPPT Randomized Clinical Trial

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IMPORTANCE Uncertainty remains about the efficacy of folic acid therapy for the primary prevention of stroke because of limited and inconsistent data.

OBJECTIVE To test the primary hypothesis that therapy with enalapril and folic acid is more effective in reducing first stroke than enalapril alone among Chinese adults with hypertension.

DESIGN, SETTING, AND PARTICIPANTS The China Stroke Primary Prevention Trial, a randomized, double-blind clinical trial conducted from May 19, 2008, to August 24, 2013, in 32 communities in Jiangsu and Anhui provinces in China. A total of 20 702 adults with hypertension without history of stroke or myocardial infarction (MI) participated in the study.

INTERVENTIONS Eligible participants, stratified by *MTHFR* C677T genotypes (CC, CT, and TT), were randomly assigned to receive double-blind daily treatment with a single-pill combination containing enalapril, 10 mg, and folic acid, 0.8 mg (n = 10 348) or a tablet containing enalapril, 10 mg, alone (n = 10 354).

MAIN OUTCOMES AND MEASURES The primary outcome was first stroke. Secondary outcomes included first ischemic stroke; first hemorrhagic stroke; MI; a composite of cardiovascular events consisting of cardiovascular death, MI, and stroke; and all-cause death.

RESULTS During a median treatment duration of 4.5 years, compared with the enalapril alone group, the enalapril–folic acid group had a significant risk reduction in first stroke (2.7% of participants in the enalapril–folic acid group vs 3.4% in the enalapril alone group; hazard ratio [HR], 0.79; 95% CI, 0.68-0.93), first ischemic stroke (2.2% with enalapril–folic acid vs 2.8% with enalapril alone; HR, 0.76; 95% CI, 0.64-0.91), and composite cardiovascular events consisting of cardiovascular death, MI, and stroke (3.1% with enalapril–folic acid vs 3.9% with enalapril alone; HR, 0.80; 95% CI, 0.69-0.92). The risks of hemorrhagic stroke (HR, 0.93; 95% CI, 0.65-1.34), MI (HR, 1.04; 95% CI, 0.60-1.82), and all-cause deaths (HR, 0.94; 95% CI, 0.81-1.10) did not differ significantly between the 2 treatment groups. There were no significant differences between the 2 treatment groups in the frequencies of adverse events.

CONCLUSIONS AND RELEVANCE Among adults with hypertension in China without a history of stroke or MI, the combined use of enalapril and folic acid, compared with enalapril alone, significantly reduced the risk of first stroke. These findings are consistent with benefits from folate use among adults with hypertension and low baseline folate levels.

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Editorial

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Stroke is the leading cause of death in China and second leading cause of death in the world.[1] Primary prevention is particularly important because about 77% of strokes are first events.[2] Uncertainty remains regarding the efficacy of folic acid therapy for primary prevention of stroke because of limited and inconsistent data.[3]

Most relevant randomized trials were designed for secondary prevention and have not shown a beneficial effect of folic acid supplementation for prevention of cardiovascular disease,[4-12] although the results of some trials and meta-analyses do suggest a specific reduction in stroke risk.[6,8,13] This raises the possibility that folic acid supplementation might be more effective for stroke prevention than for other cardiovascular outcomes. However, none of the previous trials had stroke as the primary outcome. Furthermore, the ceiling effect in reducing stroke at around 0.8 mg/d of folic acid was evident in a previous meta-analysis of randomized trials.[14] Nevertheless, most relevant trials were conducted in regions with high dietary folate intake and/or grain fortification with folic acid and may not have been able to detect a beneficial effect.[14]

Methylenetetrahydrofolate reductase (MTHFR) is the main regulatory enzyme for folate metabolism. Polymorphism of the *MTHFR* gene C677T leads to a reduction in enzyme activity, resulting in decreased folate levels. A large meta-analysis of genetic studies and clinical trials[15] suggested that the effect of *MTHFR* C677T gene variants on stroke risk might be modified by folate status. Taken together, the efficacy of folic acid therapy in stroke prevention should be evaluated and interpreted in the context of primary vs secondary prevention and individual and combined effects of baseline folate levels and *MTHFR* gene C677T polymorphism.

The China Stroke Primary Prevention Trial (CSPPT) was designed to test the hypothesis that enalapril-folic acid therapy is more effective in reducing first stroke than enalapril alone among adults with hypertension in China.

Methods

Study Oversight

This study was approved by the ethics committee of the Institute of Biomedicine, Anhui Medical University, Hefei, China (FWA assurance number FWA00001263). All participants provided written informed consent. The trial protocol and statistical analysis plan are available in Supplement 1.

The principal investigator, under the oversight of an academic steering committee and executive committee, was responsible for the study design and conduct. All outcome events, including primary and secondary outcomes, were reviewed and adjudicated by an independent end-point adjudication committee whose members were unaware of study group assignments. An independent data and safety monitoring board (DSMB) performed interim monitoring analyses for safety and efficacy with the support of the statistical group. After the study was completed and the database was locked, a writing group prepared the manuscript, which was subsequently revised by all of the authors.

Participants

Eligible participants were men and women aged 45 to 75 years old who had hypertension, defined as seated resting systolic blood pressure of 140 mm Hg or higher or diastolic blood pressure of 90 mm Hg or higher at both the screening and recruitment visits or were taking an antihypertensive medication. The major exclusion criteria included history of physician-diagnosed stroke, myocardial infarction (MI), heart failure, coronary revascularization, or congenital heart disease (Supplement 1).

Trial Design

The CSPPT was a multicommunity, randomized, double-blind clinical trial conducted from May 19, 2008, to August 24, 2013, in 32 communities in the Jiangsu and Anhui provinces of China, with a study coordination center in each province. The trial consisted of 3 stages: screening and recruitment, a 3-week run-in treatment period, and a 5-year randomized treatment period.

Screening and Recruitment

During the screening stage, each participant completed a physical examination and questionnaires on lifestyle and history of disease and medication use. Genotyping for *MTHFR* C677T polymorphisms was also performed.

Run-in Treatment

All eligible participants, as determined using the above inclusion and exclusion criteria, were asked to take an oral daily dose of 10 mg of enalapril for a total of 3 weeks. Participants who demonstrated good adherence to the treatment and were tolerant of enalapril were entered into the next stage.

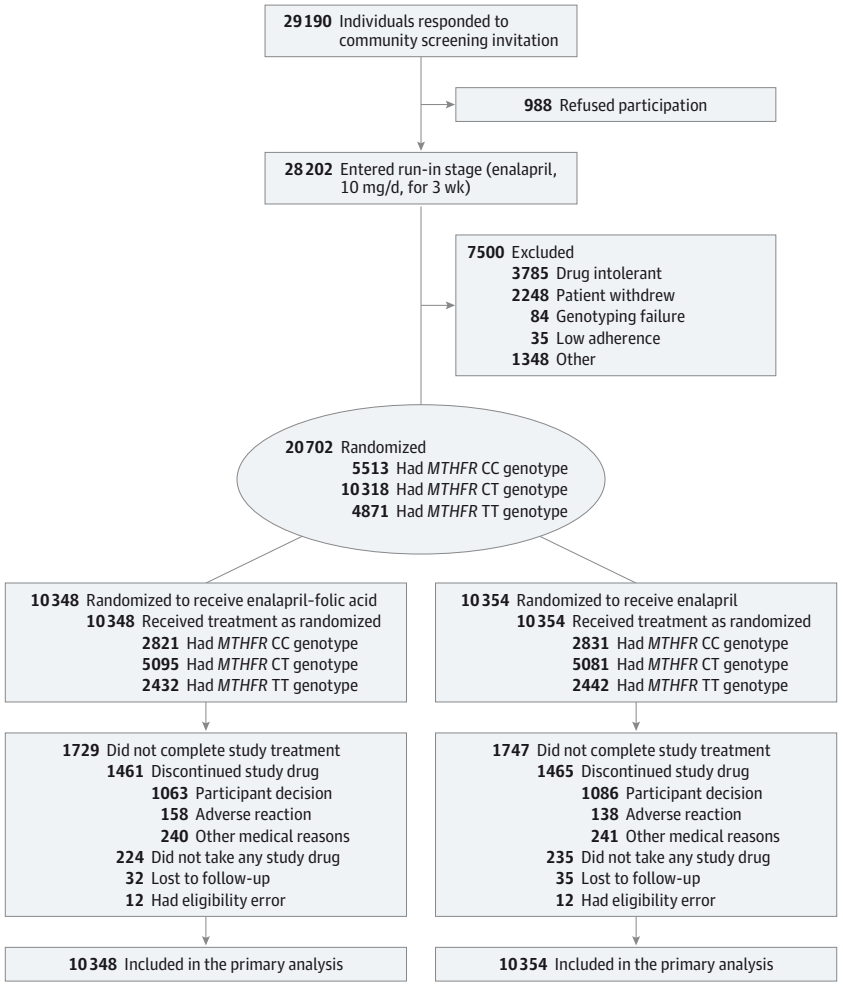
Randomization and Treatment

Eligible participants, stratified by *MTHFR* C677T genotypes (CC, CT, or TT), were randomly assigned, in a 1:1 ratio, to receive 1 of 2 treatments: a daily oral dose of 1 tablet containing 10 mg of enalapril and 0.8 mg of folic acid (single-pill combination; the enalapril-folic acid group) or a daily oral dose of 1 tablet containing 10 mg of enalapril only (the enalapril group) (**Figure 1**). Both types of tablets were concealed in a single-capsule formulation and were identical in appearance, size, color, and taste. Randomization was performed centrally by means of 4 randomization tables: 1 was a randomization of drug code and treatment allocation, and the other 3 were *MTHFR* C677T genotype–specific randomized sequences with a fixed-block size of 4. All study investigators and participants were blinded to the randomization procedure and the treatment assignments. During the trial period, concomitant use of other antihypertensive drugs (mainly calcium channel blockers or diuretics), but not B vitamins, was allowed.

Follow-up

Participants were scheduled for follow-up every 3 months. At each follow-up visit, vital signs, study drug adherence, con-

Figure 1. Flow of Participants in the China Stroke Primary Prevention Trial



MTHFR indicates methylenetetrahydrofolate reductase.

comitant medication use, adverse events, and possible endpoint events were documented by trained research staff and physicians.

Laboratory Assays

MTHFR C677T (rs1801133) polymorphisms were detected on an ABI Prism 7900HT sequence detection system (Life Technologies) using the TaqMan assay. The concordance rate for duplicates was 99.4%. Serum folate and vitamin B_{12} at both the baseline and the exit visits were measured by a commercial laboratory using a chemiluminescent immunoassay (New Industrial). Serum homocysteine, fasting lipids, and glucose levels at both the baseline and the exit visit 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.

Outcome Assessment

More details on definition and event adjudication can be found in Supplement 1. Briefly, the primary outcome was a first nonfatal or fatal stroke (ischemic or hemorrhagic), excluding subarachnoid hemorrhage and silent stroke. Source data for all suspected stroke cases including medical records and imaging data as well as event report forms were submitted to the event adjudication committee for further verification. Secondary outcomes included a composite of cardiovascular events consisting of cardiovascular death, MI, and stroke; first ischemic stroke (fatal and nonfatal); first hemorrhagic stroke (fatal and nonfatal); MI; and all-cause death. Myocardial infarctions needed to meet the criteria for ischemic symptoms or corresponding electrocardiographic changes plus evidence of myocardial damage. Cardiovascular death included sudden cardiac death; death due to MI, heart failure, stroke, or cardiovascular invasive procedures; death due to cardiovascular hemorrhage; and death due to other known vascular causes. All-cause death included death due to any reason. Evidence for death included death certificates from hospitals or reports of home visit by investigators.

Safety outcomes included all adverse events reported, any drug-related adverse events, any serious adverse events, adverse events leading to drug withdrawal, and abnormal laboratory test results with clinical significance.

In the exploratory analyses, we further investigated the modifying effect of baseline serum folate level (in quartiles)

and possible interaction with *MTHFR* C677T genotype on the effect of folic acid therapy on the primary outcome.

Statistical Analysis

Based on a large epidemiological study, the annual incidence rate of stroke among Chinese adults aged 45 to 75 years with hypertension was approximately 1.0%.[16] In consideration of better blood pressure management among participants in the trial, we assumed a stroke annual incidence rate of 0.7% in the enalapril group. Our meta-analysis[13] of the 8 previous reports of randomized trials estimated a stroke hazard ratio (HR) of 0.82 for folic acid supplementation. In subgroups of participants from regions without folic acid fortification of grain, the effect size was larger at an HR of 0.75. The CSPPT participants were from regions without folic acid fortification. However, to be conservative, we assumed an effect of an HR of 0.80 during the 5-year follow-up, with a type I error rate of 5% and 80% power; thus, a sample size of 20 337 would be required. This trial, with a sample size of 20 702, was adequately powered to address the primary study hypothesis.

The interim efficacy analyses focused on the primary outcome only, and the O'Brien-Fleming alpha spending function was used for defining boundaries of statistical significance.[17] Results from the interim analyses were accessible only to DSMB members. The DSMB could have recommended terminating the trial in one of the following scenarios: significant efficacy difference between the 2 treatment groups; much greater risk-benefit ratio in the enalapril-folic acid group; or a low likelihood of success of the trial within a reasonable period (eg, low treatment adherence, low incidence of outcome events).

The intention-to-treat (ITT) set included all participants randomized to treatment. The ITT set was used for the primary efficacy analysis. The per-protocol set consisted of all participants with no major deviation from the protocol and with an overall treatment adherence rate of 70% or higher at the end of the study. The per-protocol set was mainly used for the sensitivity analysis of the primary outcome. The safety set consisted of ITT participants excluding those who did not take any study medication or who had no record of follow-up after randomization.

If information on the number of pills taken by a certain visit was missing but such data at the last visit were available, the missing data were filled by the method of last observation carried forward. Otherwise, the number entered was 0. For all other variables, missing data were treated as missing in all efficacy and safety analyses. Because of the relatively small amount of missing data, we did not expect that the missing data would substantially change the major results for the primary outcome.

For testing the primary hypothesis, the efficacy analyses for the primary outcome were conducted according to the ITT principle. The efficacy index for an outcome was the time from randomization to the first event of the outcome of interest. The cumulative event rates of an outcome in the enalapril-folic acid and the enalapril groups, respectively, were estimated using the Kaplan-Meier method. The crude and adjusted HRs and their 95% confidence intervals were estimated by the Cox proportional hazards regression model. Given previous interim efficacy analyses performed for the primary outcome (see the statistical analysis plan in Supplement 1), this final analysis, according to the spending function, used an unadjusted 2-tailed $P<.048$ as the significance cutoff for the efficacy analysis of the primary outcome. Two sensitivity analyses for the primary outcome were also performed. The first sensitivity analysis was to estimate HRs using the per-protocol set. In the second sensitivity analysis, a composite outcome consisting of the primary outcome and all-cause death was used. The main purpose of this analysis was to address potential differential competing risks from other causes of death between the 2 treatment groups. A similar approach was applied to all of the efficacy analyses of the secondary outcomes, but an unadjusted 2-tailed $P<.05$ was used.

In the exploratory analyses, we first investigated the modifying effect of serum folate level (in quartiles) and possible interaction with *MTHFR* C677T genotype on the efficacy of folic acid therapy. Other variables for subgroup analyses included sex, baseline age, serum homocysteine level (in quartiles), serum vitamin B_{12} level (in quartiles), and smoking. R software, version 2.15.1 (http://www.R-project.org/) was used for all statistical analyses.

Results

Study Participants and Baseline Characteristics

As shown in Figure 1, of the 29 190 candidates screened, a total of 20 702 participants with an average age of 60.0 years (SD, 7.5 years) were enrolled and randomized between May 2008 and August 2009. A total of 10 348 and 10 354 participants were assigned to the enalapril-folic acid and enalapril groups, respectively.

The percentages of self-reported hyperlipidemia and diabetes were low at 2.7% and 3.1% to 3.2%, respectively, as was the use of lipid-lowering drugs (0.8%), glucose-lowering drugs (≤1.6%), and antiplatelet drugs (≤3.1%) in both groups. The rate of vitamin B_{12} deficiency (<200 pg/mL) was low (1.5%) in both groups (**Table 1**).

The frequency of *MTHFR* C677T polymorphisms was 27.3% (n = 5652) for CC, 49.2% (n = 10 176) for CT, and 23.5% (n = 4874) for TT genotypes, which were all in Hardy-Weinberg equilibrium within province strata. When further stratified by *MTHFR* genotypes (eTable 1 in Supplement 2), there was no significant difference in baseline characteristics between the enalapril-folic acid and enalapril groups within each genotype stratum (all $P>.05$).

Treatment Adherence

The majority of participants (n = 7159 [69.2%] in the enalapril-folic acid group and n = 7152 [69.1%] in the enalapril group) took at least 70% of their study medication throughout the trial and had no major protocol violations. The rates of discontinuation of study treatment were 14.2% in the enalapril-folic acid group and 14.1% in the enalapril group. The majority of the participants in both groups who discontinued the intervention still continued follow-up for outcome events. A total of 32 (0.3%) participants in the enalapril-folic acid group and 35 partici-

Table 1. Baseline Characteristics of the Study Participants

Characteristics	Enalapril-Folic Acid Group (n=10 348)	Enalapril Group (n=10 354)
Male, No. (%)	4245 (41.0)	4252 (41.1)
Age, mean (SD), y	60.0 (7.5)	60.0 (7.6)
Body mass index, mean (SD)[a]	25.0 (3.7)	24.9 (3.7)
MTHFR C677T polymorphisms, No. (%)		
CC	2821 (27.3)	2831 (27.3)
CT	5095 (49.2)	5081 (49.1)
TT	2432 (23.5)	2442 (23.6)
Cardiovascular risk factors, No. (%)		
Smoking		
Never	7119 (68.8)	7135 (68.9)
Former	761 (7.4)	809 (7.8)
Current	2461 (23.8)	2408 (23.3)
Alcohol drinking		
Never	7158 (69.2)	7113 (68.7)
Former	715 (6.9)	744 (7.2)
Current	2466 (23.9)	2494 (24.1)
Self-reported hyperlipidemia	284 (2.7)	278 (2.7)
Self-reported diabetes	317 (3.1)	335 (3.2)
Laboratory results		
Total cholesterol, mean (SD), mg/dL	213.6 (46.0)	213.2 (45.8)
Triglycerides, mean (SD), mg/dL	147.4 (119.9)	146.9 (82.0)
HDL-C, mean (SD), mg/dL	52.0 (14.0)	51.8 (13.9)
Fasting glucose, mean (SD), mg/dL	104.5 (30.6)	104.5 (30.6)
Creatinine, mean (SD), mg/dL	0.7 (0.2)	0.7 (0.2)
Homocysteine, median (IQR), μmol/L[b]	12.5 (10.5-15.5)	12.5 (10.5-15.5)
Vitamin B_{12}, median (IQR), pg/mL[b]	379.6 (314.3-475.2)	379.8 (315.7-478.2)
Medication use, No. (%)		
Antihypertensive drugs	4721 (45.6)	4815 (46.5)
Angiotensin-converting enzyme inhibitors	938 (9.1)	955 (9.2)
Angiotensin II receptor blockers	10 (0.1)	8 (0.1)
Calcium channel blockers	1034 (10.0)	1035 (10.0)
Diuretics	218 (2.1)	217 (2.1)
β-Blockers	84 (0.8)	91 (0.9)
Lipid-lowering drugs	81 (0.8)	85 (0.8)
Glucose-lowering drugs	166 (1.6)	151 (1.5)
Antiplatelet drugs	285 (2.8)	322 (3.1)

Abbreviations: HDL-C, high-density lipoprotein cholesterol; IQR, interquartile range; *MTHFR*, methylenetetrahydrofolate reductase.

SI conversions: To convert total cholesterol, triglycerides, and HDL-C to mmol/L, multiply by 0.0259. To convert glucose to mmol/L, multiply by 0.0555.

[a] Calculated as weight in kilograms divided by height in meters squared.

[b] Wilcoxon signed rank test was used.

pants (0.3%) in the enalapril group were lost to follow-up before completion of the study. All participants who were lost to follow-up were included in the final analysis, with data censored at the time of the last follow-up visit.

Effects of Folic Acid Therapy on Serum Folate Levels

Serum folate levels were measured for the majority of participants at the baseline and exit visits. Baseline folate levels were comparable between the enalapril-folic acid and enalapril groups within each genotype strata. After treatment, folate levels increased by a median of 11.2 ng/mL in the enalapril-folic acid group compared with 4.4 ng/mL in the enalapril group, and the median increase in folate levels after treatment did not differ by *MTHFR* C677T genotypes (**Table 2** and eFigure 1 in Supplement 2).

Blood Pressure at Baseline and During the Treatment Period

Mean systolic and diastolic blood pressures were highly comparable between the 2 groups at baseline and over the course of the trial (Table 2 and eFigure 2 in Supplement 2). Mean blood pressure levels during the trial period were 139.7/83.0 mm Hg in the enalapril-folic acid group and 139.8/83.1 mm Hg in the enalapril group and were comparable across all genotypes. During the trial, on average, 57.1% of participants used other antihypertensive drugs concomitantly, among whom 41.2% used 1 additional drug and 15.9% used 2 additional drugs. The major classes of concomitant antihypertensive agents used during the trial were calcium channel blockers (48.8% in the enalapril-folic acid group and 48.9% in the enalapril group) and diuretics (24.0% in the enalapril-folic acid group and 24.2% in the enalapril group).

Table 2. Serum Folate Level and Blood Pressure at Baseline and After Treatment by Treatment Group for the Overall Sample and by *MTHFR* Genotype

Measurements	Overall Sample		*MTHFR* CC Genotype		*MTHFR* CT Genotype		*MTHFR* TT Genotype	
	Enalapril-Folic Acid	Enalapril	Enalapril-Folic Acid	Enalapril	Enalapril-Folic Acid	Enalapril	Enalapril-Folic Acid	Enalapril
Folate, median (IQR), ng/mL								
At baseline	8.1 (5.6-10.4)	8.1 (5.6-10.5)	9.0 (6.5-11.5)	9.0 (6.6-11.6)	8.1 (5.7-10.5)	8.2 (5.7-10.5)	6.5 (4.8-9.1)	6.5 (4.8-9.1)
No. of participants with available data	10 243	10 256	2791	2810	5043	5027	2409	2419
At exit visit	19.9 (14.7-23.3)	13.0 (9.7-16.0)	20.4 (15.4-23.6)	14.1 (10.7-16.8)	19.8 (14.7-23.4)	13.0 (9.9-16.0)	19.2 (13.8-22.9)	11.7 (8.1-14.9)
No. of participants with available data	8426	8418	2291	2260	4140	4138	1995	2020
Change[a]	11.2 (5.8-16.8)	4.4 (1.6-7.3)	10.9 (5.6-16.3)	4.2 (1.6-72)	11.1 (5.8-16.8)	4.4 (1.6-7.4)	11.8 (5.8-17.0)	4.5 (1.7-7.4)
No. of participants with available data	8341	8340	2265	2244	4097	4093	1979	2003
Systolic blood pressure, mean (SD), mm Hg								
At baseline	166.8 (20.4)	166.9 (20.4)	166.1 (20.0)	166.9 (20.1)	167.1 (20.4)	166.8 (20.4)	167.2 (20.6)	167.2 (20.9)
No. of participants with available data	10 348	10 354	2821	2831	5095	5081	2432	2442
Over treatment period	139.7 (11.1)	139.8 (11.3)	139.4 (10.8)	139.8 (10.9)	139.8 (11.1)	139.9 (11.5)	139.7 (11.5)	139.7 (11.3)
No. of participants with available data	10 348	10 351	2821	2830	5095	5079	2432	2442
Diastolic blood pressure, mean (SD), mm Hg								
At baseline	94.2 (11.8)	94.0 (12.0)	93.5 (11.7)	93.5 (12.2)	94.2 (12.0)	94.0 (11.9)	94.9 (11.4)	94.7 (12.1)
No. of participants with available data	10 348	10 354	2821	2831	5095	5081	2432	2442
Over treatment period	83.0 (7.5)	83.1 (7.6)	82.6 (7.5)	82.7 (7.7)	83.0 (7.4)	83.1 (7.6)	83.6 (7.4)	83.6 (7.5)
No. of participants with available data	10 348	10 351	2821	2830	5095	5079	2432	2442

Abbreviations: IQR, interquartile range; *MTHFR*, methylenetetrahydrofolate reductase.

[a] Change in folate level = exit folate level − baseline folate level.

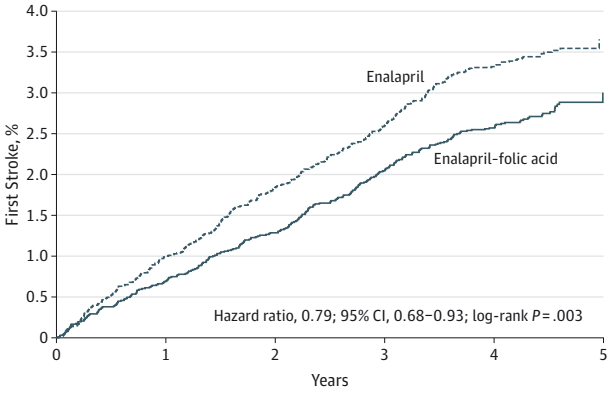
Efficacy of Folic Acid Therapy for the Primary and Secondary Outcomes

In June 2013, after a median of 48 months of treatment and 590 primary end-point events, the DSMB performed the fourth interim analysis and observed a significant efficacy difference ($P = .003$ by log-rank test) between the 2 treatment groups. The difference exceeded the boundary of the prespecified stopping rule, with a z score of 2.77, corresponding to a nominal α level of approximately .0056. As such, the DSMB recommended early termination of the trial. After evaluating the DSMB's recommendation, the steering committee terminated the trial, and all participants were invited back for a final visit during a 3-month period.

Using the ITT set, the Kaplan-Meier curves of the cumulative event rate of first stroke in the 2 treatment groups are shown in **Figure 2**. During a median treatment duration of 4.5 years (interquartile range, 4.2-4.7 years), first stroke occurred in 282 participants (2.7%) in the enalapril-folic acid group compared with 355 participants (3.4%) in the enalapril group, representing an absolute risk reduction of 0.7% and a relative risk reduction of 21% (HR, 0.79 [95% CI, 0.68-0.93]; $P = .003$; number needed to treat [4.5 years] = 141 [95% CI, 85-426]) (**Table 3**). Analyses of a composite outcome consisting of the primary outcome and all-cause death yielded consistent results (5.4% in the enalapril-folic acid group vs 6.2% in the enalapril group; HR, 0.86; 95% CI, 0.77-0.97; $P = .01$). Analyses of the primary outcome using the per-protocol set (No. of events/No. of participants: 152/7159 in the enalapril-folic acid group and 199/7152 in the enalapril group) yielded a similar effect (HR, 0.76; 95% CI, 0.62-0.94; $P = .01$).

Stroke cases were further classified into ischemic or hemorrhagic stroke based on computed tomographic (n = 577) or magnetic resonance imaging (n = 168) findings. Among 110 participants who had both computed tomographic and magnetic resonance imaging scans, the concordance rate of stroke outcomes was 100%. If imaging data were not available (n = 2),

Figure 2. Kaplan-Meier Curves of Cumulative Hazards of First Stroke by Treatment Group



No. at risk

Enalapril-folic acid	10348	10200	10059	9911	8976	812
Enalapril	10354	10170	10008	9864	8892	818

Hazard ratio estimated using the Cox proportional hazards model.

Table 3. Hazard Ratios for Primary and Secondary Outcomes

Outcomes	No. (%) With Outcome		Hazard Ratio (95% CI)[a]	*P* Value[b]
	Enalapril-Folic Acid (n = 10 348)	Enalapril (n = 10 354)		
First stroke (primary outcome)[c]	282 (2.7)	355 (3.4)	0.79 (0.68-0.93)[d]	.003
Secondary outcomes				
Ischemic stroke	223 (2.2)	292 (2.8)	0.76 (0.64-0.91)	.002
Hemorrhagic stroke	58 (0.56)	62 (0.60)	0.93 (0.65-1.34)	.71
Composite of stroke, myocardial infarction, or death due to cardiovascular causes	324 (3.1)	405 (3.9)	0.80 (0.69-0.92)	.002
Myocardial infarction[e]	25 (0.24)	24 (0.23)	1.04 (0.60-1.82)	.89
Death due to cardiovascular causes[f]	43 (0.4)	43 (0.4)	1.00 (0.66-1.53)	>.99
All-cause death	302 (2.9)	320 (3.1)	0.94 (0.81-1.10)	.47

[a] Estimated using the Cox proportional hazards model.

[b] Derived from the log-rank test.

[c] Two cases with uncertain type of stroke were included in the primary outcome. A total of 28 cases (23 cases with hemorrhagic stroke, 4 cases with ischemic stroke, and 1 case with uncertain type of stroke) were fatal stroke (18 in the enalapril-folic acid group and 10 in the enalapril group).

[d] Adjustment for age, sex, *MTHFR* C677T polymorphism, systolic and diastolic blood pressure at baseline, mean systolic and diastolic blood pressure over the treatment period, body mass index, study center, baseline vitamin B_{12}, folate, homocysteine, creatinine, total cholesterol, triglycerides, high-density lipoprotein cholesterol, fasting glucose, smoking, and alcohol consumption did not substantially change the results (hazard ratio, 0.80; 95% CI, 0.68-0.93; *P* = .005).

[e] A total of 9 cases (5 in the enalapril-folic acid group and 4 in the enalapril group) were fatal myocardial infarctions.

[f] A total of 49 cases (20 in the enalapril-folic acid group and 29 in the enalapril group) were fatal other cardiovascular events.

a stroke was defined clinically. Analyses of secondary outcomes showed significant reductions among participants in the enalapril-folic acid group in the risk of ischemic stroke (2.2% in the enalapril-folic acid group vs 2.8% in the enalapril group; HR, 0.76; 95% CI, 0.64-0.91; *P* = .002) and composite cardiovascular events (3.1% in the enalapril-folic acid group vs 3.9% in the enalapril group; HR, 0.80; 95% CI, 0.69-0.92; *P* = .002) (Table 3 and eFigure 3 in Supplement 2). However, there was no significant difference between groups in the risk of hemorrhagic stroke (0.56% in the enalapril-folic acid group vs 0.60% in the enalapril group; HR, 0.93; 95% CI, 0.65-1.34; *P* = .71), MI (0.24% in the enalapril-folic acid group vs 0.23% in the enalapril group; HR, 1.04; 95% CI, 0.60-1.82; *P* = .89), or all-cause deaths (2.9% in the enalapril-folic acid group vs 3.1% in the enalapril group; HR, 0.94; 95% CI, 0.81-1.10; *P* = .47) (Table 3).

Stratified Analyses by Important Covariables

Stratified analyses were performed by *MTHFR* C677T genotype (CC, CT, and TT); quartiles of homocysteine, folate, and vitamin B_{12} levels; age by decade; sex; and cigarette smoking status. There were no significant interactions in any of the subgroups (*P* > .05 for all comparisons), including folate level (*P* = .16) and *MTHFR* C677T genotype (*P* = .16); however, the beneficial effect appeared to be more pronounced in participants with lower baseline folate levels (eFigure 4 in Supplement 2).

Exploratory Analysis by Baseline Folate Levels and *MTHFR* C677T Genotypes

eFigure 5 in Supplement 2 presents the rates of first stroke among the enalapril-folic acid group vs the enalapril group

stratified by *MTHFR* C677T genotype and baseline folate level quartile. In the enalapril group, among participants with the CC genotype (normal homozygous), there was an inverse relationship between baseline folate level and risk of stroke (P = .01 for linear trend). A similar pattern, to a lesser degree, was observed among participants with the CT genotype (heterozygous) (P = .01 for linear trend). In contrast, participants with the TT genotype (homozygous variant) had a persistently high risk of stroke across all folate quartiles (P = .65 for linear trend). Furthermore, in those with the CC and CT genotypes, the greatest risk reduction was in the lowest quartile.

eTable 2 in Supplement 2 further estimates the efficacy of folic acid therapy on first stroke within each of the genotype and baseline folate subgroups. Among participants with the CC genotype, folic acid therapy significantly reduced stroke risk in those with folate levels below the median (absolute risk reduction, 2.1%; HR, 0.45; 95% CI, 0.29-0.72; P = .001). A similar pattern was observed to a lesser degree among those with the CT genotype, with the greatest benefit in the lowest quartile (absolute risk reduction, 1.4%; HR, 0.68; 95% CI, 0.44-1.07; P = .10). In contrast, among those with the TT genotype, the preventive effect of folic acid therapy on stroke was mainly observed in the highest folate quartile (absolute reduction, 2.8%; HR, 0.24; 95% CI, 0.10-0.58; P = .001).

Adverse Events

There were no significant differences between the 2 treatment groups in terms of the frequencies of any adverse events (excluding the study outcomes) reported, as defined by the Medical Dictionary for Regulatory Activities for primary system organ classification, and any drug-related adverse events (eTables 3 and 4 in Supplement 2). There were no statistical differences between the treatment groups for other safety outcomes, including any serious adverse events, adverse events leading to drug withdrawal, and abnormal laboratory test results with clinical significance between the treatment groups.

Discussion

The effectiveness of folic acid supplementation in stroke prevention is not well established.[3] The CSPPT, a large randomized trial among adults with hypertension in China without a history of stroke or MI, found that enalapril-folic acid therapy, compared with enalapril alone, significantly reduced the relative risk of first stroke by 21%. Further adjustment for important covariables, including baseline homocysteine levels, did not substantially change the results (Table 3).

Clarke et al[18] reported a meta-analysis based on 7 trials and found no significant benefit of folic acid supplementation on stroke risk (n = 35603; rate ratio, 0.96; 95% CI, 0.87-1.06). The latest and the most comprehensive meta-analysis by Huo et al,[14] which included all the trials reported in the meta-analysis by Clarke et al, found that folic acid supplementation significantly reduced the risk of stroke (15 randomized trials; n = 55 764; relative risk, 0.92; 95% CI, 0.86-1.00; P = .04); in particular, among trials in regions with no or partial folic acid fortification (n = 43 426; relative risk, 0.89; 95% CI, 0.82-0.97; P = .01) and among trials with a lower percentage use of statins (relative risk, 0.77; 95% CI, 0.64-0.92; P = .005).

The variable strength of the association between folic acid supplementation and stroke risk across the trials may be due to important differences in study design and study participant characteristics. Prior to the CSPPT, there had been a particular lack of adequately powered randomized clinical trials on the primary prevention of stroke. Four trials of folic acid supplementation were published that had more than 200 stroke events: SEARCH (534 events; HR, 1.02; 95% CI, 0.86-1.21),[4] VITATOPS (748 events; HR, 0.92; 95% CI,0.81-1.06),[5] HOPE-2 (258 events; HR, 0.75; 95% CI, 0.59-0.97),[6] and VISP (300 events; HR, 1.04; 95% CI, 0.84-1.29).[7] All 4 studies were conducted among patient populations with preexisting cardiovascular disease and none had stroke as the primary outcome. The CSPPT, with 637 stroke events in a sample size of 20 702, is by far the largest among the trials of primary prevention of stroke and is second only to VITATOPS[5] (mainly stroke recurrence) among all trials of stroke prevention.

The CSPPT, with data on individual baseline folate levels and *MTHFR* genotypes, has provided convincing evidence that baseline folate level is an important determinant of efficacy of folic acid therapy in stroke prevention. Although previous meta-analyses of randomized trials showed a greater beneficial effect of folic acid therapy in the prevention of stroke in low folate settings,[13-15] these data were ecologic in nature. The CSPPT is the first large-scale randomized trial to test the hypothesis using individual measures of baseline folate levels. In this population without folic acid fortification, we observed considerable individual variation in plasma folate levels and clearly showed that the beneficial effect appeared to be more pronounced in participants with lower folate levels. In comparison, the VISP study was conducted in the United States, a region with folic acid fortification.[7] Mandatory folic acid fortification in North America has had a significant positive effect on the population's plasma folate levels.[19] The mean folate levels at baseline in the VISP study was about 28 nmol/L (12.4 ng/mL), which was about 50% higher than that in the CSPPT trial. Therefore, it is not surprising that previous folic acid trials conducted in high folate regions generally yielded null results, which were likely due to the "ceiling effect" of folic acid supplementation.[14]

The effect of *MTHFR* genotype on stroke needs to be assessed in the context of baseline folate levels, as indicated by a large meta-analysis of genetic studies and clinical trials by Holmes et al.[15] The authors showed that the effect of *MTHFR* genotype on stroke risk is subject to modification by population dietary folate levels (based on ecological data). They speculated that there would be a larger effect of folic acid intervention (relative risk, 0.78; 95% CI, 0.68-0.90) in a low folate region (Asia). To our knowledge, the CSPPT is the first large-scale randomized trial to test the hypothesis using individual measures of *MTHFR* genotype and baseline folate level. Such a design allows for (1) controlling for genetic confounding by stratified randomization based on *MTHFR* C677T genotype in the main analyses and (2) exploring the joint effect of baseline folate level and *MTHFR* genotype on the efficacy of folic acid therapy. The results from the joint analyses of *MTHFR*

genotype and baseline folate level showed that among participants with the CC or CT genotypes, the highest risk of stroke and the greatest benefit of folic acid therapy were in those with the lowest baseline folate levels. In addition, our data suggest that individuals with the TT genotype may require a higher dosage of folic acid supplementation to overcome biologically insufficient levels (as reflected in the relatively greater folate requirement with the TT genotype).

Another unique aspect of the CSPPT was the low percentage of concomitant use of lipid-lowering drugs and antiplatelet agents among the participants. The low vascular disease burden and the low frequency of use of cardiac and vascular protective drugs made our results less likely to be affected by these drugs and possible drug interactions.[20,21] In the HOPE-2 trial,[22] participants who did not take lipid-lowering drugs or antiplatelet agents experienced a larger treatment benefit from folic acid supplementation. Meanwhile, in the SEARCH trial,[4] which failed to observe a treatment benefit, all participants took a daily dose of 20 mg or 80 mg of simvastatin.

Hypertension is the primary risk factor for stroke.[3] However, none of the previously reported trials compared blood pressure control over the treatment period. Our trial attempted to ensure the comparability of blood pressure levels between the treatment groups both at baseline and throughout follow-up, during which blood pressure control was achieved using a standard protocol of enalapril, 10 mg/d, plus other antihypertensive agents as needed. As such, the CSPPT lends further support that folic acid therapy can lead to an additional 21% risk reduction of first stroke compared with antihypertension treatment alone. A synergy of enalapril (an angiotensin-converting enzyme inhibitor) with folic acid is possible based on the findings of a subanalysis in the WAFACS trial.[11]

Inadequate folate intake is prevalent in most countries without mandatory folic acid fortification, including in Asia and other continents. The *MTHFR* 677 TT variant, which leads to a 60% reduction in the enzyme function, is present in all populations but with variable frequency (usually 2%-25%).[23] Based on recently published US National Health and Nutrition Examination Survey folate data[24,25] and our unpublished folate data from the Boston Birth Cohort, there is substantial variability in blood folate levels within the US population and across racial/ethnic groups. We speculate that even in countries with folic acid fortification and widespread use of folic acid supplements such as in the United States and Canada, there may still be room to further reduce stroke incidence using more targeted folic acid therapy—in particular, among those with the TT genotype and low or moderate folate levels.

Several potential concerns or limitations are worth mentioning. This study focused on primary prevention of stroke in adults with hypertension; the generalizability of our findings to secondary prevention of stroke or adults without hypertension remains to be determined. In addition, the CSPPT was designed to have adequate power for analyzing the primary outcome but was underpowered for assessing some secondary outcomes, particularly hemorrhagic stroke, MI, and total mortality. The mechanisms underlying effect modification by *MTHFR* C677T polymorphisms and baseline folate levels remain to be investigated. This trial used a fixed dosage of folic acid (0.8 mg/d); the optimal dosage for a given *MTHFR* genotype and baseline folate level remains to be established.

Conclusions

Among adults with hypertension in China without a history of stroke or MI, the combined use of enalapril and folic acid, compared with enalapril alone, significantly reduced the risk of first stroke. This finding is consistent with a benefit from folate use among adults with hypertension and low baseline folate levels.

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REFERENCES

1. Lozano R, Naghavi M, Foreman K, et al. Global and regional mortality from 235 causes of death for 20 age groups in 1990 and 2010: a systematic analysis for the Global Burden of Disease Study 2010. *Lancet*. 2012;380(9859):2095-2128.

2. Lloyd-Jones D, Adams RJ, Brown TM, et al; American Heart Association Statistics Committee and Stroke Statistics Subcommittee. Heart disease and stroke statistics—2010 update: a report from the American Heart Association. *Circulation*. 2010; 121(7):e46-e215.

3. Meschia JF, Bushnell C, Boden-Albala B, et al; American Heart Association Stroke Council; Council on Cardiovascular and Stroke Nursing; Council on Clinical Cardiology; Council on Functional Genomics and Translational Biology; Council on Hypertension. Guidelines for the primary prevention of stroke: a statement for healthcare professionals from the American Heart Association/American Stroke Association. *Stroke*. 2014;45(12):3754-3832.

4. Armitage JM, Bowman L, Clarke RJ, et al; Study of the Effectiveness of Additional Reductions in Cholesterol and Homocysteine Collaborative Group. Effects of homocysteine-lowering with folic acid plus vitamin B12 vs placebo on mortality and major morbidity in myocardial infarction survivors: a randomized trial. *JAMA*. 2010;303(24):2486-2494.

5. VITATOPS Trial Study Group. B vitamins in patients with recent transient ischaemic attack or stroke in the Vitamins to Prevent Stroke (VITATOPS) trial: a randomised, double-blind, parallel, placebo-controlled trial. *Lancet Neurol*. 2010;9(9):855-865.

6. Lonn E, Yusuf S, Arnold MJ, et al; Heart Outcomes Prevention Evaluation 2 Investigators. Homocysteine lowering with folic acid and B vitamins in vascular disease. *N Engl J Med*. 2006; 354(15):1567-1577.

7. Toole JF, Malinow MR, Chambless LE, et al. Lowering homocysteine in patients with ischemic stroke to prevent recurrent stroke, myocardial infarction, and death: the Vitamin Intervention for Stroke Prevention (VISP) randomized controlled trial. *JAMA*. 2004;291(5):565-575.

8. Galan P, Kesse-Guyot E, Czernichow S, Briancon S, Blacher J, Hercberg S; SU.FOL.OM3 Collaborative

Group. Effects of B vitamins and omega 3 fatty acids on cardiovascular diseases: a randomised placebo controlled trial. *BMJ*. 2010;341:c6273.

9. Bønaa KH, Njølstad I, Ueland PM, et al; NORVIT Trial Investigators. Homocysteine lowering and cardiovascular events after acute myocardial infarction. *N Engl J Med*. 2006;354(15):1578-1588.

10. Jamison RL, Hartigan P, Kaufman JS, et al; Veterans Affairs Site Investigators. Effect of homocysteine lowering on mortality and vascular disease in advanced chronic kidney disease and end-stage renal disease: a randomized controlled trial. *JAMA*. 2007;298(10):1163-1170.

11. Albert CM, Cook NR, Gaziano JM, et al. Effect of folic acid and B vitamins on risk of cardiovascular events and total mortality among women at high risk for cardiovascular disease: a randomized trial. *JAMA*. 2008;299(17):2027-2036.

12. Ebbing M, Bleie Ø, Ueland PM, et al. Mortality and cardiovascular events in patients treated with homocysteine-lowering B vitamins after coronary angiography: a randomized controlled trial. *JAMA*. 2008;300(7):795-804.

13. Wang X, Qin X, Demirtas H, et al. Efficacy of folic acid supplementation in stroke prevention: a meta-analysis. *Lancet*. 2007;369(9576):1876-1882.

14. Huo Y, Qin X, Wang J, et al. Efficacy of folic acid supplementation in stroke prevention: new insight from a meta-analysis. *Int J Clin Pract*. 2012;66(6):544-551.

15. Holmes MV, Newcombe P, Hubacek JA, et al. Effect modification by population dietary folate on the association between *MTHFR* genotype, homocysteine, and stroke risk: a meta-analysis of genetic studies and randomised trials. *Lancet*. 2011;378(9791):584-594.

16. Fang XH, Zhang XH, Yang QD, et al. Subtype hypertension and risk of stroke in middle-aged and older Chinese: a 10-year follow-up study. *Stroke*. 2006;37(1):38-43.

17. O'Brien PC, Fleming TR. A multiple testing procedure for clinical trials. *Biometrics*. 1979;35(3):549-556.

18. Clarke R, Halsey J, Lewington S, et al; B-Vitamin Treatment Trialists' Collaboration. Effects of lowering homocysteine levels with B vitamins on cardiovascular disease, cancer, and cause-specific mortality: meta-analysis of 8 randomized trials involving 37 485 individuals. *Arch Intern Med*. 2010;170(18):1622-1631.

19. Jacques PF, Selhub J, Bostom AG, Wilson PW, Rosenberg IH. The effect of folic acid fortification on plasma folate and total homocysteine concentrations. *N Engl J Med*. 1999;340(19):1449-1454.

20. Wald DS, Morris JK, Wald NJ. Reconciling the evidence on serum homocysteine and ischaemic heart disease: a meta-analysis. *PLoS One*. 2011;6(2):e16473.

21. Hankey GJ, Eikelboom JW, Yi Q, et al; VITATOPS Trial Study Group. Antiplatelet therapy and the effects of B vitamins in patients with previous stroke or transient ischaemic attack: a post-hoc subanalysis of VITATOPS, a randomised, placebo-controlled trial. *Lancet Neurol*. 2012;11(6):512-520.

22. Saposnik G, Ray JG, Sheridan P, McQueen M, Lonn E; Heart Outcomes Prevention Evaluation 2 Investigators. Homocysteine-lowering therapy and stroke risk, severity, and disability: additional findings from the HOPE 2 trial. *Stroke*. 2009;40(4):1365-1372.

23. Wilcken B, Bamforth F, Li Z, et al. Geographical and ethnic variation of the 677C>T allele of 5,10 methylenetetrahydrofolate reductase (*MTHFR*): findings from over 7000 newborns from 16 areas world wide. *J Med Genet*. 2003;40(8):619-625.

24. Yang Q, Cogswell ME, Hamner HC, et al. Folic acid source, usual intake, and folate and vitamin B-12 status in US adults: National Health and Nutrition Examination Survey (NHANES) 2003-2006 [published correction appears in *Am J Clin Nutr*. 2010;92(4):1001]. *Am J Clin Nutr*. 2010;91(1):64-72.

25. Marchetta CM, Hamner HC. Blood folate concentrations among women of childbearing age by race/ethnicity and acculturation, NHANES 2001-2010 [published online June 17, 2014]. *Matern Child Nutr*. doi:10.1111/mcn.12134.