Effect of long-term low-dose folic acid supplementation on degree of total homocysteine-lowering: major effect modifiers

Binyan Wang[1,2], Hongxu Wu[1], Youbao Li[2], Qianyun Ban[1], Xiao Huang[3], Lulu Chen[1], Jianping Li[4], Yan Zhang[4], Yimin Cui[5], Mingli He[6], Genfu Tang[1], Delu Yin[7], Junnong Li[8], Yong Huo[3], Xianhui Qin[1,2]* and Xiping Xu[1,2]*

[1]*Institute for Biomedicine, Anhui Medical University, Hefei 230032, People's Republic of China*
[2]*National Clinical Research Center for Kidney Disease, State Key Laboratory for Organ Failure Research, Key Laboratory of Organ Failure Research (Ministry of Education), Renal Division, Nanfang Hospital, Southern Medical University, Guangzhou 510515, People's Republic of China*
[3]*Department of Cardiology, Second Affiliated Hospital, Nanchang University, Nanchang 330006, People's Republic of China*
[4]*Department of Cardiology, Peking University First Hospital, Beijing 100034, People's Republic of China*
[5]*Department of Pharmacy, Peking University First Hospital, Beijing 100034, People's Republic of China*
[6]*Department of Neurology, First People's Hospital, Lianyungang 222061, People's Republic of China*
[7]*Department of Cardiology, First People's Hospital, Lianyungang 222061, People's Republic of China*
[8]*Department of Cardiology, Weinan Center Hospital, Weinan 714000, People's Republic of China*

Abstract

We sought to examine the potential modifiers in the association between long-term low-dose folic acid supplementation and the reduction of serum total homocysteine (tHcy) among hypertensive patients, using data from the China Stroke Primary Prevention Trial (CSPPT). This analysis included 16 867 participants who had complete data on tHcy measurements at both the baseline and exit visit. After a median treatment period of 4·5 years, folic acid treatment significantly reduced the tHcy levels by 1·6 µmol/l (95 % CI 1·4, 1·8). More importantly, after adjustment for baseline tHcy and other important covariates, a greater degree of tHcy reduction was observed in certain subgroups: males, the methylenetetrahydrofolate reductase *(MTHFR)* 677TT genotype, higher baseline tHcy levels (≥12·5 (median) *v.* <12·5 µmol/l), lower folate levels (<8·0 (median) *v.* ≥8·0 ng/ml), estimated glomerular filtration rate (eGFR) <60 ml/min per 1·73 m^2 (*v.* 60– <90 and ≥90 ml/min per 1·73 m^2), ever smokers and concomitant use of diuretics (*P* for all interactions <0·05). The degree of tHcy reduction associated with long-term folic acid supplementation can be significantly affected by sex, *MTHFR* C677T genotypes, baseline folate, tHcy, eGFR levels and smoking status.

Key words: Folic acid: Total homocysteine: Effect modifiers: Hypertension

Increased plasma total homocysteine (tHcy) has been reported to be associated with a variety of clinical outcomes, including hypertension, diabetes, chronic kidney diseases, CVD, cancer, psychiatric disorders and cognitive impairment[1–3]. It has been reported that hypertension and raised tHcy can synergistically increase the risk of stroke[4,5]. Furthermore, a significant positive dose–response association has been found between degree of tHcy reduction and systolic blood pressure (SBP) reduction[6], and decrease in CVD[7] or carotid intima–media thickness[8]. Consistently, a recent study found that the lowering of tHcy was significantly associated with a reduction in first stroke

risk, and may possibly serve as a useful indicator for the use of folic acid therapy in the prevention of stroke[9].

In previous randomised trials with long-term folic acid therapy, the percentage of tHcy reduction between groups ranged from 10 to 38 %[10–12]. Many of these previous trials were conducted in participants with CVD, and a high dose of folic acid (median: 2 mg) was used, often combined with vitamin B$_{12}$ and/or B$_6$[10–12]. In fact, a previous meta-analysis of randomised trials suggested that daily doses of 0·8 mg of folic acid had achieved the maximum reduction in serum tHcy levels[13]. Little further tHcy reduction is achieved by increasing the dose of

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folic acid above 0·8 mg/d[13]. Furthermore, two recent meta-analyses of randomised trials found that folic acid therapy significantly reduced the risk of stroke by more than 20 % among trials with a daily folic acid dosage of 0·8 mg or lower. Excessive doses of folic acid therapy did not lead to an additional beneficial effect[12]. More importantly, there has been a lack of randomised clinical trials that have comprehensively assessed the modifiers affecting the variation of tHcy reduction associated with long-term folic acid supplementation. The aim of the present study was to fill in these important gaps in knowledge regarding folic acid supplementation and the reduction of tHcy. A better understanding of the modifiable risk factors would be helpful for clinical and public health decision-making, for it would lead to early identification and effective risk reduction, and result in an individualised folic acid treatment regimen with the best benefit–risk ratio.

The main analyses of the China Stroke Primary Prevention Trial (CSPPT), which have been published previously, showed that folic acid therapy can reduce the risk of first stroke by 21 % among hypertensive patients[14]. The present *post hoc* analysis of the CSPPT aimed to examine the potential modifiers in the association between long-term low-dose folic acid supplementation and the reduction of tHcy among hypertensive patients without major CVD, using data from the CSPPT.

Methods

Study participants

The methods and primary results of the CSPPT have been reported elsewhere[14]. Briefly, the CSPPT was a multi-community, randomised, double-blind, controlled trial conducted from 19 May 2008 to 24 August 2013 in thirty-two communities in Jiangsu and Anhui provinces in China. Eligible participants were men and women aged 45–75 years old who had hypertension, defined as seated resting SBP ≥140 mmHg or diastolic blood pressure (DBP) ≥90 mmHg at both the screening and recruitment visit, or were on antihypertensive medication. The major exclusion criteria included history of physician-diagnosed stroke, myocardial infarction (MI), heart failure, post-coronary revascularisation or congenital heart disease.

The present study was approved by the ethics committee of the Institute of Biomedicine, Anhui Medical University, Hefei, China (FWA assurance number FWA00001263), and was registered with ClinicalTrials.gov, NCT00794885. All participants gave written informed consent before data collection.

Intervention and follow-up

Eligible participants, stratified by methylenetetrahydrofolate reductase (*MTHFR*) C677T genotypes (CC, CT or TT), were randomly assigned, in a 1:1 ratio, to one of two treatment groups: a daily oral dose of one 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 one tablet containing 10 mg of enalapril only (the enalapril group). All study investigators and participants were blinded to the randomisation procedure and the treatment assignments. During

the trial period, concomitant use of other antihypertensive drugs (mainly Ca channel blockers (CCB) or diuretics) was allowed, but not B-vitamins.

Participants were followed up every 3 months. At each visit, participant blood pressure and pulse were measured, the number of pills taken between visits was counted and the use of concomitant medications and any adverse events were recorded.

Laboratory assays

Laboratory tests were performed at the core laboratory of the National Clinical Research Center for Kidney Disease (Nanfang Hospital, Guangzhou, China). Serum tHcy, fasting lipids and glucose were measured using automatic clinical analysers (Beckman Coulter). tHcy measurement was calibrated to the National Institute of Standards and Technology standard reference material SRM1955. *MTHFR* C677T polymorphisms were detected on an ABI PRISM® 7900HT sequence detection system (Life Technologies) using the TaqMan assay. Serum folate and B_{12} at both the baseline and the exit visit were measured by a commercial laboratory using a chemiluminescent immunoassay (New Industrial).

Estimated glomerular filtration rate (eGFR) was calculated using the Chronic Kidney Disease Epidemiology Collaboration equation.

Major definitions

Hyperuricaemia was defined as a uric acid concentration ≥417 μmol/l (7 mg/dl) in men or ≥357 μmol/l (6 mg/dl) in women. A current smoker was defined as smoking at least one cigarette per d or smoking more than eighteen packs in the last year. Those who had smoked more than eighteen packs but did not smoke in the last year were defined as former smokers. We combined former smokers and current smokers into an ever-smoker group. Regular concomitant medication use was defined as taking the drug of interest for 180 or more cumulative days[15].

We defined metabolic vitamin B_{12} deficiency[16] as a serum vitamin B_{12} level of <400 pmol/l (500 pg/ml) with normal renal function (≥60 ml/min per 1·73m²), a tHcy level of ≥14 μmol/l and a folate level of 10·4 ng/ml (75 % percentile) or more.

Statistical analysis

Means (standard deviations) and proportions were calculated for population characteristics by treatment group. Differences in population characteristics were compared using two-sample t tests, signed rank tests or χ^2 tests, accordingly.

The treatment effect, expressed as the difference in tHcy level at the exit visit minus that at baseline, was estimated using generalised linear regression models with adjustment for age, sex, study centre, use of angiotensin-converting enzyme inhibitors (ACEI), *MTHFR* C677T genotypes, smoking status, BMI, tHcy, fasting glucose, total cholesterol, eGFR, folate, vitamin B_{12}, uric acid and SBP at baseline, as well as time-averaged SBP, concomitant use of CCB and concomitant use of diuretics during the trial period. In additional exploratory analyses, possible

modifications of the treatment effects on the primary outcome were assessed for the variables, including age (<60 *v.* ≥60 years), sex, *MTHFR* C677T genotypes, smoking (never *v.* ever smoking), hyperuricaemia (yes *v.* no), folate (<8·0 (median) *v.* ≥8·0 ng/ml), tHcy (<12·5 (median), ≥12·5 μmol/l), vitamin B_{12} (<380 (median) *v.* ≥380 pg/ml), eGFR (<60, 60–<90 and ≥90 ml/min per 1·73 m^2), SBP at baseline (<160 *v.* ≥160 mmHg), as well as time-averaged SBP (<140 *v.* ≥140 mmHg) and concomitant use of diuretics (yes *v.* no) during the trial period.

A two-tailed $P < 0.05$ was considered statistically significant in all analyses. R software, version 3.3.1 (http://www.R-project.org/), was used for all statistical analyses.

Results

Study participants and baseline characteristics

As illustrated in the flow chart (online Supplementary Fig. S1), a total of 20 702 participants were enrolled and randomised in the CSPPT. Of those, 10 348 participants were assigned to the enalapril–folic acid group and 10 354 participants to the enalapril group. We excluded participants who had missing data on tHcy measurements at the baseline (*n* 278) or exit visit (*n* 3557), leaving 16 867 (*n* 8418 in the enalapril–folic acid group and *n* 8449 in the enalapril group) participants in the final analysis. Participants not included in the analyses did not differ in baseline characteristics from those included in the analyses (online Supplementary Table S1).

All baseline characteristics were comparable between the enalapril and enalapril–folic acid groups (Table 1). There were 1572 (9·3 %) participants undergoing treatment with ACEI at baseline.

Blood pressure at baseline and during the treatment period

Mean SBP and DBP were highly comparable between the two groups at baseline and over the course of the trial (online Supplementary Tables S2 and S3).

Concomitant medications

The most common concomitant medications were CCB and diuretics, which were used by 82 and 53 % of the participants, respectively. There were no significant differences in concomitant medication use during the trial between the two treatment groups. The use of lipid-lowering, glucose-lowering and anti-platelet medications were very rare, all at a frequency of <2 % (online Supplementary Table S4).

Effect of folic acid therapy on change in total homocysteine levels

Mean baseline tHcy levels were comparable between the two groups (enalapril group, 14·5 (SD 8·5) μmol/l; enalapril–folic acid group, 14·4 (SD 8·1) μmol/l). After a median treatment period of 4·5 years, mean tHcy was 14·6 (SD 8·0) μmol/l in the enalapril group compared with 12·9 (SD 6·3) μmol/l in the

enalapril–folic acid group (group difference: −1·6 μmol/l; 95 % CI −1·8, −1·4 μmol/l).

As expected, a greater group difference was found in participants with higher baseline tHcy levels (≥12·5 (median): −2·4; 95 % CI −2·9, −2·0; *v.* <12·5 μmol/l: −0·7; 95 % CI −0·9, −0·6 μmol/l) (Table 2).

Furthermore, although the group differences were all significant in participants with lower (<12·5 μmol/l) or higher (≥12·5 μmol/l) baseline tHcy levels, tHcy increased in those with baseline tHcy <12·5 μmol/l, whereas it decreased in those with baseline tHcy ≥12·5 μmol/l in both treatment groups.

Stratified analyses by potential effect modifiers

Stratified analyses were performed to assess the treatment effect on the primary outcome in various subgroups (Fig. 1). After adjustment for baseline tHcy and other important covariates (age, sex, study centre, use of ACEI, *MTHFR* C677T genotypes, smoking status, BMI, fasting glucose, total cholesterol, eGFR, folate, vitamin B_{12}, uric acid and SBP at baseline, as well as time-averaged SBP, concomitant use of CCB and concomitant use of diuretics during the trial period), a greater degree of tHcy reduction was observed in certain subgroups: males, the *MTHFR* 677TT genotype, higher baseline tHcy levels, lower folate levels, eGFR <60 ml/min per 1·73 m^2, concomitant use of diuretics and ever smokers (*P* for all interactions <0·05). However, SBP either at baseline or over the treatment period, baseline use of ACEI and concomitant use of CCB had no significant effect on the efficacy of folic acid therapy in lowering tHcy levels (Fig. 1 and online Supplementary Table S5).

When split into two groups according to baseline tHcy, similar trends for the same modifiers were found in participants with higher (≥12·5 μmol/l) (Fig. 2) or lower (<12·5 μmol/l) (Fig. 3) tHcy levels.

The prevalence of metabolic B_{12} deficiency was about 4·0 %. The tHcy-lowering effect of folic acid therapy was attenuated and became insignificant in participants with metabolic B_{12} deficiency (online Supplementary Table S6).

Discussion

Our present analysis is the first study to comprehensively evaluate the effect modifiers of long-term daily 0·8-mg folic acid supplementation in lowering tHcy levels among adults with hypertension without stroke and MI. As expected, long-term daily 0·8-mg folic acid supplementation significantly lowered tHcy levels in this population. More importantly, male, *MTHFR* 677TT genotype, higher baseline tHcy levels, lower folate levels, eGFR <60 ml/min per 1·73 m^2, ever smokers and concomitant use of diuretics were major modifiers for the efficacy of folic acid supplementation in lowering tHcy levels. All these factors should be taken into account in the identification of participants who may be most likely to benefit from tHcy-lowering therapy.

Serum tHcy concentration is determined by genetic and a series of environmental factors. *MTHFR* is a critical enzyme for folate and tHcy metabolism. *MTHFR* converts 5,10-

Table 1. Baseline characteristics of the China Stroke Primary Prevention Trial study participants*
(Numbers and percentages; mean values and standard deviations; medians and 25th–75th percentiles)

Variables	Total		Enalapril		Enalapril–folic acid	
	n	%	n	%	n	%
n	16 867		8449		8418	
Male	6794	40·3	3408	40·3	3386	40·2
Age (years)						
Mean	60·0		60·0		60·1	
SD	7·4		7·4		7·4	
BMI (kg/m^2)						
Mean	25·1		25·0		25·1	
SD	3·7		3·7		3·7	
Systolic blood pressure (mmHg)						
Mean	167·3		167·3		167·2	
SD	20·5		20·6		20·4	
Diastolic blood pressure (mmHg)						
Mean	94·2		94·1		94·3	
SD	11·9		12·1		11·7	
MTHFR C677T polymorphisms						
CC	4560	27·0	2275	26·9	2285	27·1
CT	8279	49·1	4148	49·1	4131	49·1
TT	4028	23·9	2026	24·0	2002	23·8
Smoking status						
Never	11 725	69·5	5875	69·5	5850	69·5
Former	1273	7·6	655	7·8	618	7·3
Current	3862	22·9	1918	22·7	1944	23·1
Laboratory results (mmol/l)						
Total cholesterol						
Mean	5·5		5·5		5·5	
SD	1·2		1·2		1·2	
TAG						
Mean	1·7		1·7		1·7	
SD	1·2		0·9		1·4	
HDL-cholesterol						
Mean	1·3		1·3		1·3	
SD	0·4		0·4		0·4	
Fasting glucose						
Mean	5·8		5·8		5·8	
SD	1·7		1·7		1·6	
Vitamin B$_{12}$ (pg/ml)						
Median	378		379		378	
25th–75th percentile	315–473		315–475		314–472	
Folate (ng/ml)						
Median	8·0		8·0		8·0	
25th–75th percentile	5·5–10·4		5·6–10·4		5·5–10·4	
eGFR (ml/min per 1·73 m^2)						
Mean	93·6		93·7		93·5	
SD	13·0		12·8		13·1	
Medication use						
Antihypertensive drugs	7903	46·9	3980	47·1	3923	46·6
ACE inhibitors	1572	9·3	783	9·3	789	9·4
Angiotensin II type I receptor blockers	15	0·1	7	0·1	8	0·1
Ca channel blockers	1674	9·9	830	9·8	844	10·0
Diuretics	362	2·1	184	2·2	178	2·1
β-Blockers	147	0·9	78	0·9	69	0·8
Lipid-lowering drugs	142	0·8	73	0·9	69	0·8
Glucose-lowering drugs	261	1·5	117	1·4	144	1·7

MTHFR, methylenetetrahydrofolate reductase; eGFR, estimated glomerular filtration rate; ACE, angiotensin-converting enzyme.
* For continuous variables, values are presented as means and standard deviations.

methylenetetrahydrofolate into 5-methyltetrahydrofolate, which is required for the remethylation of tHcy. The polymorphism of MTHFR 677C → T leads to a reduction in enzyme activity, resulting in increased concentrations of tHcy and lower levels of serum folate[2]. Serum tHcy increases with age, and men usually have higher levels than women with the same age. The sex-related tHcy difference may be partly explained by the effect of oestrogen in women[17]. Furthermore, patients with chronic kidney diseases have lower levels of folate owing to inadequate intake, increased loss or demand, and usually have higher tHcy levels. At the same time, tobacco smoke exposure is associated with higher tHcy levels owing to decreased folate intake[18,19] and increased folate turnover[20,21]. Accordingly, our study also suggests that participants with the MTHFR 677 TT genotype,

Table 2. Mean total homocysteine levels (µmol/l) at baseline and exit visit by treatment group
(Mean values and standard deviations; mean values and 95 % confidence intervals)

	Enalapril		Enalapril–folic acid		
	Mean	SD	Mean	SD	P
All participants					
n	8449		8418		
At baseline	14·5	8·5	14·4	8·1	0·633
At exit	14·6	8·0	12·9	6·3	<0·001
Change†	0·1	7·1	−1·5*	7·7	<0·001
Change by group			−1·6		
95 % CI			−1·8, −1·4		
Baseline total homocysteine					
<12·5 µmol/l (median)					
n	4260		4189		
At baseline	10·2	1·5	10·2	1·5	0·699
At exit	11·6	3·0	10·8	3·5	<0·001
Change†	1·3*	2·8	0·6*	3·4	<0·001
Change by group			−0·7		
95 % CI			−0·9, −0·6		
≥12·5 µmol/l					
n	4189		4229		
At baseline	18·7	10·4	18·5	9·7	0·314
At exit	17·6	10·1	15·0	7·7	<0·001
Change†	−1·1*	9·5	−3·5*	9·8	<0·001
Change by group			−2·4		
95 % CI			−2·9, −2·0		

* Significantly different from baseline ($P < 0.05$).
† Change = total homocysteine level at exit − total homocysteine level at baseline.

lower folate or eGFR levels and those who are males and ever smokers seemed to have higher tHcy levels at baseline (Fig. 1). However, it is still unknown whether these factors modify the effect of long-term folic acid supplementation in reducing tHcy.

Consistent with previous studies[13,22], baseline tHcy concentration was the major determinant of tHcy-lowering after folic acid treatment in the present study. A greater tHcy reduction was found in participants with higher baseline tHcy levels. More importantly, although the group difference was also significant among participants with lower baseline tHcy levels, tHcy levels significantly increased even among those in the folic acid treatment group over the 4·5-year treatment period. These results suggest that, beyond folic acid therapy, more safe and effective strategies need to be developed for the further control of tHcy levels.

After the adjustment of baseline tHcy, *MTHFR* C677T did not significantly affect the tHcy-lowering effect of folic acid therapy in our previous study (*n* 445)[23], nor in the study by Ho *et al.*[24] (*n* 443). However, the study by Fohr *et al.*[25] suggested that, although the three genotype groups did not differ significantly in tHcy concentration at baseline, subjects with the TT genotype still responded with a larger decrease in tHcy levels than did those without the mutation. The present study, which included a total of 16 644 participants and is by far the largest study of its kind, concluded that even with the adjustment of baseline tHcy, folate and other important covariates, those participants with the TT genotype had a stronger tHcy reduction than those with either the CC or TT genotypes. However, the modifying effect of the *MTHFR* C677T genotype was weaker and became insignificant in participants with lower baseline tHcy levels. Furthermore, a lower degree of tHcy increase in

participants with lower baseline tHcy (Fig. 3) and a greater degree of tHcy reduction in participants with higher baseline tHcy (Fig. 2) was also found in those with lower folate levels, eGFR <60 ml/min per 1·73 m^2, males and ever smokers, independent of baseline tHcy levels. However, more studies are needed to confirm our results, and to further detect the underlying mechanisms involved in these modifying effects.

In folate-replete populations, the main determinant of elevated tHcy is metabolic vitamin B_{12} deficiency[16,26]. This is very common and quite often missed because physicians tend to assume incorrectly that a serum B_{12} in the reference range represents adequacy of B_{12} function[27]. In the present study, the tHcy-lowering effect of folic acid therapy was very attenuated and became insignificant in those with metabolic B_{12} deficiency. These results highlight the importance of using vitamin B_{12} in addition to folic acid to lower tHcy. Moreover, methylcobalamin should be used in place of cyanocobalamin[28], particularly in persons with impaired renal function (which includes the elderly)[29].

The mean tHcy reduction (−1·6 µmol/l) in the CSPPT is relatively small compared with other published trials; we offer the following explanations. First, the CSPPT supplemented with folic acid alone, whereas most other studies combined folate, B_6 and/or B_{12}[10–12]. The addition of other B vitamins may have helped to further lower the tHcy levels in these studies (about 7 % more than folic acid supplementation alone)[13]. Second, there are important differences in characteristics between the participants of the CSPPT and those of previously published trials, including younger age, lower percentage of males and relatively higher eGFR levels[10–12]. We have found that these factors can significantly modify the effect of folic acid

Subgroup	Level	Size	Baseline tHcy Mean (SD)	Enalapril Mean (SD)	Enalapril–FA Mean (SD)	β (95 % CI)		Interaction P value
Age (years)								0·011
	<60	8484	13·4 (7·8)	−0·2 (6·5)	−1·6 (6·7)	−1·4 (−1·6, −1·2)		
	≥60	8383	15·4 (8·7)	0·5 (7·6)	−1·4 (8·6)	−1·9 (−2·2, −1·6)		
Sex								<0·001
	Female	6794	12·6 (5·2)	0·4 (4·8)	−0·6 (5·6)	−1·0 (−1·2, −0·9)		
	Male	10 073	17·1 (10·9)	−0·2 (9·4)	−2·7 (9·9)	−2·6 (−3·0, −2·3)		
MTHFR C677T								<0·001
	CC	4560	12·4 (4·2)	0·9 (4·0)	−0·1 (4·9)	−1·0 (−1·2, −0·7)		
	CT	8279	13·1 (5·1)	0·8 (5·0)	−0·5 (4·5)	−1·3 (−1·4, −1·1)		
	TT	4028	19·5 (13·5)	−2·0 (11·6)	−5·1 (12·7)	−3·4 (−4·0, −2·9)		
Smoking status								<0·001
	Never	11 725	13·3 (6·7)	0·3 (6·0)	−1·0 (7·0)	−1·3 (−1·5, −1·1)		
	Ever	5135	17·0 (10·8)	−0·2 (9·0)	−2·7 (8·9)	−2·6 (−3·0, −2·3)		
Hyperuricaemia								0·011
	Yes	1940	14·2 (8·1)	0·1 (6·7)	−1·4 (7·6)	−1·6 (−1·8, −1·4)		
	No	14 925	16·3 (9·4)	0·1 (9·1)	−2·1 (8·4)	−2·3 (−2·9, −1·6)		
Folate (ng/ml)								<0·001
	<8·0	8366	16·3 (10·5)	−0·8 (7·9)	−3·0 (9·0)	−2·3 (−2·5, −2·0)		
	≥8·0	8358	12·5 (4·6)	1·0 (6·0)	0·0 (5·7)	−1·0 (−1·3, −0·8)		
Total homocysteine (μmol/l)								<0·001
	<12·5	8449	10·2 (1·5)	1·3 (2·8)	0·6 (3·4)	−0·7 (−0·9, −0·6)		
	≥12·5	8418	18·6 (10·1)	−1·1 (9·5)	−3·5 (9·8)	−2·5 (−2·9, −2·1)		
Vitamin B_{12} (pg/ml)								<0·001
	<380	8459	15·8 (9·9)	−0·2 (8·1)	−2·1 (9·2)	−2·0 (−2·3, −1·7)		
	≥380	8266	13·0 (6·1)	0·5 (5·8)	−0·8 (5·6)	−1·4 (−1·6, −1·2)		
eGFR (ml/min per 1·73 m^2)								<0·001
	<60	323	22·3 (12·6)	−1·1 (16·6)	−5·3 (11·6)	−4·7 (−7·5, −1·9)		
	60−<90	4922	16·7 (9·8)	−0·1 (8·1)	−2·3 (8·8)	−2·2 (−2·5, −1·8)		
	≥90	11 519	13·2 (7·1)	0·3 (6·1)	−1·0 (6·9)	−1·4 (−1·6, −1·2)		
Baseline SBP (mmHg)								0·784
	<160	6281	14·2 (8·2)	0·1 (7·1)	−1·5 (6·7)	−1·7 (−2·0, −1·4)		
	≥160	10 586	14·6 (8·4)	0·2 (7·0)	−1·5 (8·2)	−1·7 (−1·9, −1·4)		
Time-averaged SBP (mmHg)								0·966
	<140	9575	14·2 (8·3)	0·0 (6·7)	−1·6 (7·4)	−1·7 (−1·9, −1·4)		
	≥140	7292	14·7 (8·4)	0·3 (7·5)	−1·3 (8·0)	−1·7 (−2·0, −1·4)		
Concomitant use of diuretics during the trial period								0·018
	Yes	8898	14·9 (9·2)	15·0 (9·3)	14·9 (9·1)	−1·9 (−2·1, −1·6)		
	No	7969	13·8 (7·1)	13·9 (7·5)	13·8 (6·8)	−1·4 (−1·7, −1·2)		

−5 −4 −3 −2 −1 0 1

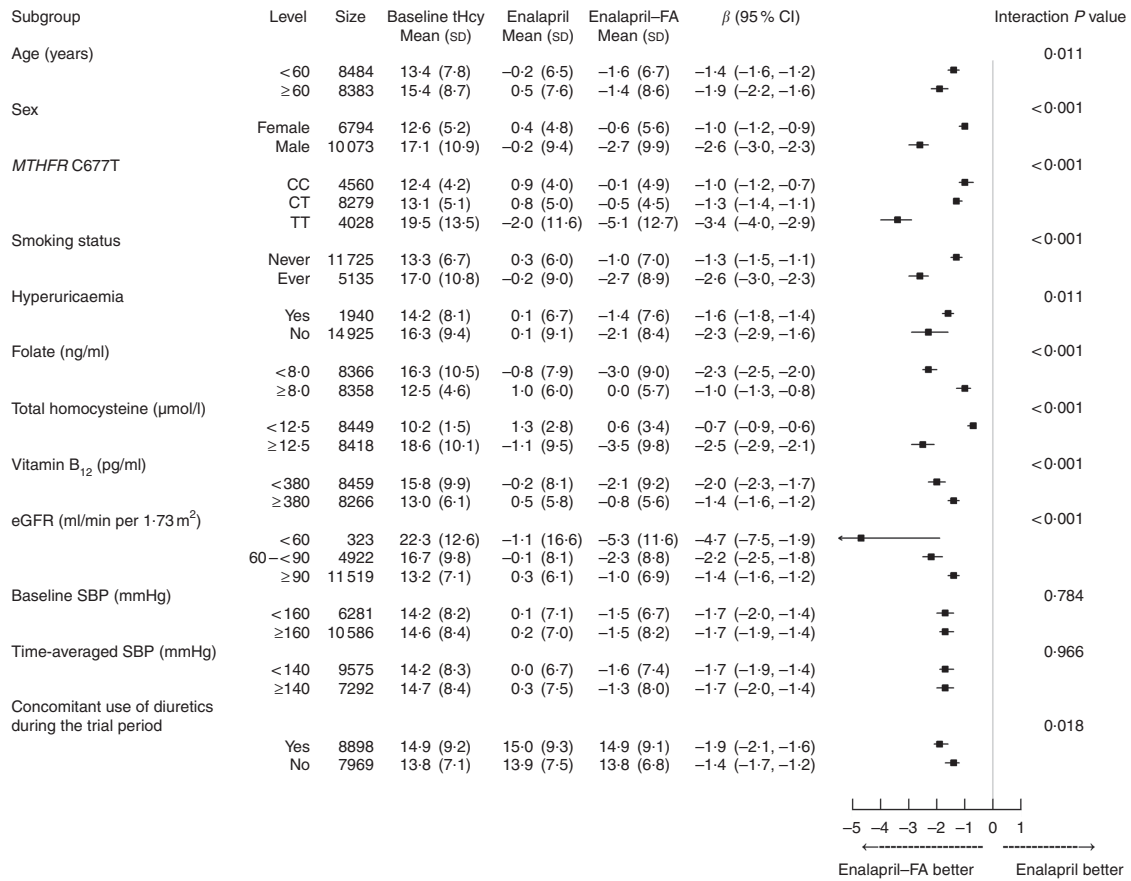←———————————— Enalapril–FA better   Enalapril better ————————————→

Fig. 1. Stratified analyses by potential effect modifiers. Adjusted, if not stratified, for age, sex, study centre, use of angiotensin-converting enzyme inhibitors, methylenetetrahydrofolate reductase (MTHFR) C677T genotypes, smoking status, BMI, total homocysteine (tHcy), fasting glucose, total cholesterol, estimated glomerular filtration rate (eGFR), folate, vitamin B_{12}, uric acid and systolic blood pressure (SBP) at baseline, as well as time-averaged SBP, concomitant use of calcium channel blockers and concomitant use of diuretics during the trial period. Hyperuricaemia was defined as a uric acid concentration ≥417 μmol/l (7 mg/dl) in men or ≥357 μmol/l (6 mg/dl) in women. FA, folic acid.

supplementation in lowering tHcy. Last, in the CSPPT, there was a 54 % increase in folate level in the control group during the course of the trial[14]. The cause of this increase is unclear. Over the course of the study, subjects received nutritional health education, which may have led to improved dietary choices[29,30]. Regardless of the cause, this change diminished the contrast between the two treatment groups and probably attenuated the effect estimation. Therefore, the potential true effect of folic acid supplementation on tHcy reduction could be underestimated in this setting.

The CSPPT found that the combined use of enalapril and folic acid, compared with enalapril alone, significantly reduced the risk of first stroke by 21 % (hazard ratio (HR) 0·79; 95 % CI 0·68, 0·93) among hypertensive patients[14]. Our previous *post hoc* analysis of the CSPPT demonstrated that percent decline in tHcy was significantly associated with a reduction in stroke risk in hypertensive patients[12]. When percentage decline in tHcy was assessed as tertiles, a significantly lower stroke risk was found in those in tertiles 2 and 3 (HR 0·79; 95 % CI 0·64, 0·97) compared with participants in tertile 1[12]. Accordingly, tHcy reduction can be considered a reliable biomarker that indicates the beneficial effect of folic acid therapy in the prevention of stroke. However, in the CSPPT, the group difference in tHcy

was only 1·6 μmol/l (about an 11 % reduction); therefore, we speculate that tHcy reduction may only partially explain the stroke risk reduction associated with folic acid therapy. We cannot ignore the direct antioxidant and antithrombotic effects of folate[31]. More studies and analyses are needed to further illuminate the benefits of folate, independent of tHcy lowering, on the risk of stroke.

Our study had several strengths. First, the CSPPT included hypertensive participants without pre-existing stroke or MI. The low vascular disease burden and the low frequency of cardiac and vascular protective drug use made our results less likely to be confounded by these drugs. Second, the CSPPT, which had a large sample size and obtained individual data on MTHFR C677T genotypes, folate, vitamin B_{12}, tHcy levels and other important factors at baseline, offers an exceptional opportunity to comprehensively evaluate potential effect modifiers for the tHcy-lowering effect of folic acid supplementation. Third, the CSPPT measured tHcy levels for 98·7 % of participants at baseline and 81·5 % of participants at the exit visit[14]. Most relevant trials, however, only measured tHcy in a fraction of the total participants[32–34]. Therefore, the tHcy levels reported by these trials might not represent the whole spectrum of folate and tHcy levels in the total participants.
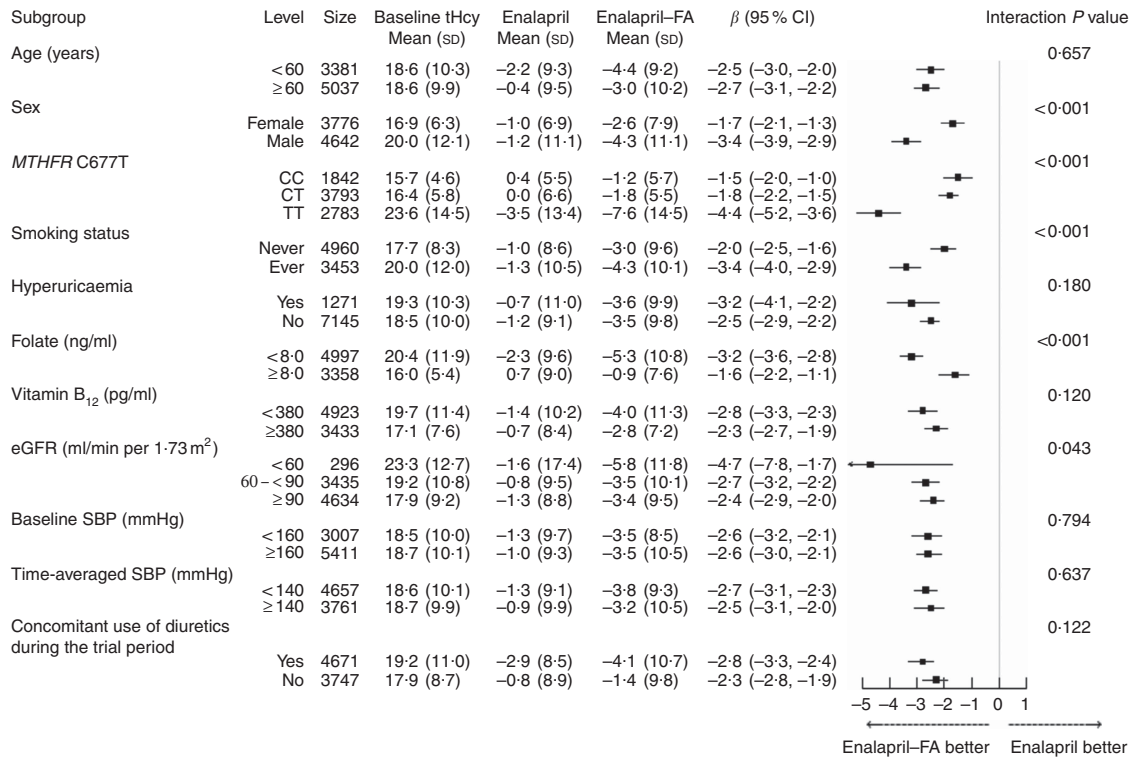
Subgroup	Level	Size	Baseline tHcy Mean (SD)	Enalapril Mean (SD)	Enalapril–FA Mean (SD)	β (95 % CI)	Interaction P value
Age (years)							0·657
	<60	3381	18·6 (10·3)	−2·2 (9·3)	−4·4 (9·2)	−2·5 (−3·0, −2·0)	
	≥60	5037	18·6 (9·9)	−0·4 (9·5)	−3·0 (10·2)	−2·7 (−3·1, −2·2)	
Sex							<0·001
	Female	3776	16·9 (6·3)	−1·0 (6·9)	−2·6 (7·9)	−1·7 (−2·1, −1·3)	
	Male	4642	20·0 (12·1)	−1·2 (11·1)	−4·3 (11·1)	−3·4 (−3·9, −2·9)	
MTHFR C677T							<0·001
	CC	1842	15·7 (4·6)	0·4 (5·5)	−1·2 (5·7)	−1·5 (−2·0, −1·0)	
	CT	3793	16·4 (5·8)	0·0 (6·6)	−1·8 (5·5)	−1·8 (−2·2, −1·5)	
	TT	2783	23·6 (14·5)	−3·5 (13·4)	−7·6 (14·5)	−4·4 (−5·2, −3·6)	
Smoking status							<0·001
	Never	4960	17·7 (8·3)	−1·0 (8·6)	−3·0 (9·6)	−2·0 (−2·5, −1·6)	
	Ever	3453	20·0 (12·0)	−1·3 (10·5)	−4·3 (10·1)	−3·4 (−4·0, −2·9)	
Hyperuricaemia							0·180
	Yes	1271	19·3 (10·3)	−0·7 (11·0)	−3·6 (9·9)	−3·2 (−4·1, −2·2)	
	No	7145	18·5 (10·0)	−1·2 (9·1)	−3·5 (9·8)	−2·5 (−2·9, −2·2)	
Folate (ng/ml)							<0·001
	<8·0	4997	20·4 (11·9)	−2·3 (9·6)	−5·3 (10·8)	−3·2 (−3·6, −2·8)	
	≥8·0	3358	16·0 (5·4)	0·7 (9·0)	−0·9 (7·6)	−1·6 (−2·2, −1·1)	
Vitamin B₁₂ (pg/ml)							0·120
	<380	4923	19·7 (11·4)	−1·4 (10·2)	−4·0 (11·3)	−2·8 (−3·3, −2·3)	
	≥380	3433	17·1 (7·6)	−0·7 (8·4)	−2·8 (7·2)	−2·3 (−2·7, −1·9)	
eGFR (ml/min per 1·73 m²)							0·043
	<60	296	23·3 (12·7)	−1·6 (17·4)	−5·8 (11·8)	−4·7 (−7·8, −1·7)	
	60 – <90	3435	19·2 (10·8)	−0·8 (9·5)	−3·5 (10·1)	−2·7 (−3·2, −2·2)	
	≥90	4634	17·9 (9·2)	−1·3 (8·8)	−3·4 (9·5)	−2·4 (−2·9, −2·0)	
Baseline SBP (mmHg)							0·794
	<160	3007	18·5 (10·0)	−1·3 (9·7)	−3·5 (8·5)	−2·6 (−3·2, −2·1)	
	≥160	5411	18·7 (10·1)	−1·0 (9·3)	−3·5 (10·5)	−2·6 (−3·0, −2·1)	
Time-averaged SBP (mmHg)							0·637
	<140	4657	18·6 (10·1)	−1·3 (9·1)	−3·8 (9·3)	−2·7 (−3·1, −2·3)	
	≥140	3761	18·7 (9·9)	−0·9 (9·9)	−3·2 (10·5)	−2·5 (−3·1, −2·0)	
Concomitant use of diuretics during the trial period							0·122
	Yes	4671	19·2 (11·0)	−2·9 (8·5)	−4·1 (10·7)	−2·8 (−3·3, −2·4)	
	No	3747	17·9 (8·7)	−0·8 (8·9)	−1·4 (9·8)	−2·3 (−2·8, −1·9)	

Axis: −5 −4 −3 −2 −1 0 1   Enalapril–FA better   Enalapril better

Fig. 2. Stratified analyses by potential effect modifiers in patients with higher baseline total homocysteine (tHcy) levels (≥12·5 μmol/l). Adjusted, if not stratified, for age, sex, study centre, use of angiotensin-converting enzyme inhibitors, methylenetetrahydrofolate reductase (*MTHFR*) C677T genotypes, smoking status, BMI, tHcy, fasting glucose, total cholesterol, estimated glomerular filtration rate (eGFR), folate, vitamin B₁₂, uric acid and systolic blood pressure (SBP) at baseline, as well as time-averaged SBP, concomitant use of calcium channel blockers and concomitant use of diuretics during the trial period. Hyperuricaemia was defined as a uric acid concentration ≥417 μmol/l (7 mg/dl) in men or ≥357 μmol/l (6 mg/dl) in women. FA, folic acid.

Subgroup	Level	Size	Baseline tHcy Mean (SD)	Enalapril Mean (SD)	Enalapril–FA Mean (SD)	β (95 % CI)	Interaction P value
Age (years)							0·960
	<60	5103	10·0 (1·5)	1·1 (2·7)	0·4 (2·8)	−0·7 (−0·9, −0·6)	
	≥60	3346	10·5 (1·4)	1·7 (2·9)	1·0 (4·2)	−0·7 (−1·0, −0·5)	
Sex							0·047
	Female	6297	10·0 (1·5)	1·2 (2·6)	0·5 (2·8)	−0·7 (−0·8, −0·5)	
	Male	2152	10·8 (1·3)	1·9 (3·4)	0·9 (4·8)	−1·0 (−1·3, −0·7)	
MTHFR C677T							0·172
	CC	2718	10·2 (1·5)	1·2 (2·4)	0·7 (4·1)	−0·6 (−0·9, −0·4)	
	CT	4486	10·2 (1·5)	1·4 (2·9)	0·6 (3·1)	−0·7 (−0·9, −0·6)	
	TT	1245	10·4 (1·5)	1·4 (3·3)	0·4 (2·8)	−1·0 (−1·4, −0·7)	
Smoking status							0·042
	Never	6765	10·1 (1·5)	1·2 (2·6)	0·6 (3·5)	−0·7 (−0·8, −0·5)	
	Ever	1682	10·7 (1·3)	1·9 (3·5)	0·8 (2·8)	−1·0 (−1·3, −0·7)	
Hyperuricaemia							0·766
	Yes	669	10·5 (1·5)	1·6 (2·8)	0·7 (2·9)	−0·8 (−1·2, −0·4)	
	No	7780	10·2 (1·5)	1·3 (2·8)	0·6 (3·5)	−0·7 (−0·9, −0·6)	
Folate (ng/ml)							0·005
	<8·0	3369	10·3 (1·5)	1·4 (2·9)	0·5 (2·6)	−1·0 (−1·1, −0·8)	
	≥8·0	5000	10·2 (1·5)	1·3 (2·8)	0·7 (3·9)	−0·6 (−0·8, −0·4)	
Vitamin B₁₂ (pg/ml)							0·499
	<380	3536	10·4 (1·5)	1·4 (3·1)	0·6 (3·3)	−0·8 (−1·0, −0·6)	
	≥380	4833	10·1 (1·5)	1·3 (2·6)	0·6 (3·5)	−0·7 (−0·9, −0·5)	
eGFR (ml/min per 1·73 m²)							0·110
	<60	27	11·1 (1·3)	3·0 (3·4)	1·8 (2·9)	−3·1 (−7·2, 0·9)	
	60 – <90	1487	10·8 (1·2)	1·5 (2·9)	0·5 (2·7)	−1·0 (−1·3, −0·7)	
	≥90	6885	10·1 (1·5)	1·3 (2·8)	0·6 (3·6)	−0·7 (−0·8, −0·5)	
Baseline SBP (mmHg)							0·371
	<160	3274	10·2 (1·5)	1·3 (2·9)	0·5 (3·2)	−0·8 (−1·0, −0·6)	
	≥160	5175	10·2 (1·5)	1·4 (2·8)	0·7 (3·6)	−0·7 (−0·9, −0·5)	
Time-averaged SBP (mmHg)							0·623
	<140	4966	10·2 (1·5)	1·3 (2·8)	0·6 (3·8)	−0·7 (−0·9, −0·5)	
	≥140	3483	10·3 (1·5)	1·5 (2·8)	0·7 (2·7)	−0·8 (−1·0, −0·6)	
Concomitant use of diuretics during the trial period							0·478
	Yes	4227	10·2 (1·5)	1·4 (2·7)	0·6 (4·0)	−0·8 (−1·0, −0·6)	
	No	4222	10·2 (1·5)	1·2 (2·9)	0·7 (2·7)	−0·7 (−0·9, −0·5)	

Axis: −3 −2 −1 0 1   Enalapril–FA better   Enalapril better

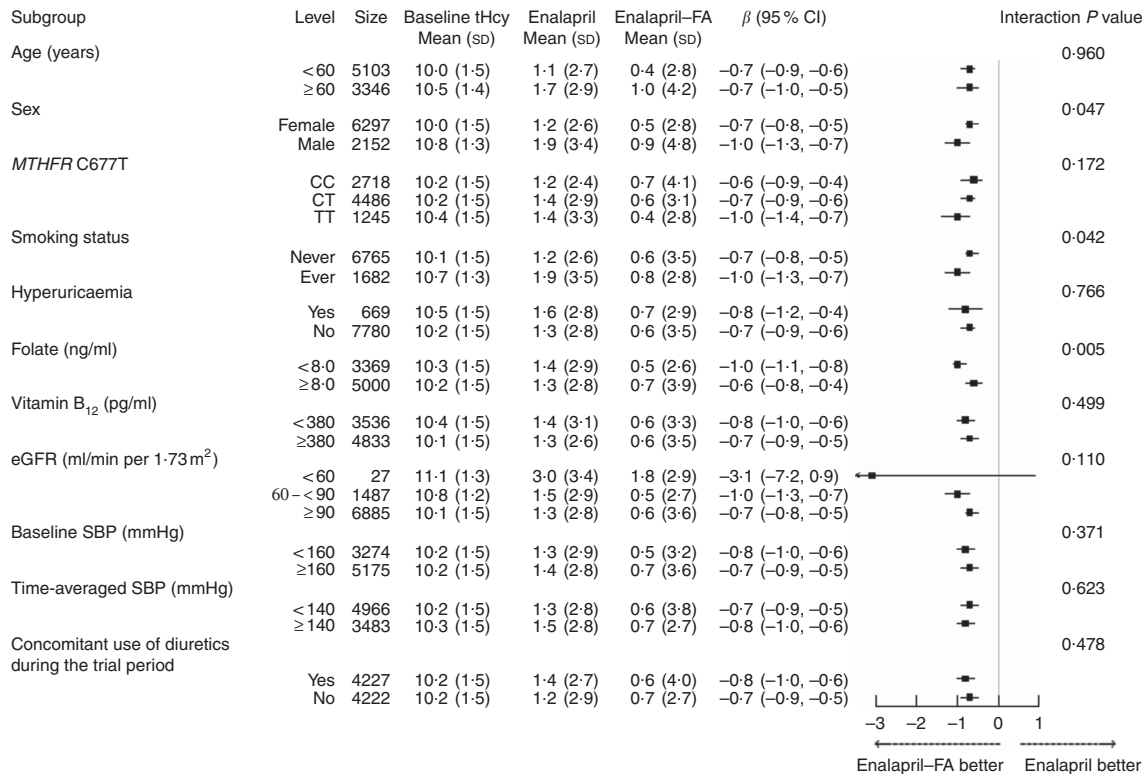**Fig. 3.** Stratified analyses by potential effect modifiers in patients with lower baseline total homocysteine (tHcy) levels (<12·5 μmol/l). Adjusted, if not stratified, for age, sex, study centre, use of angiotensin-converting enzyme inhibitors, methylenetetrahydrofolate reductase (*MTHFR*) C677T genotypes, smoking status, BMI, tHcy, fasting glucose, total cholesterol, estimated glomerular filtration rate (eGFR), folate, vitamin B₁₂, uric acid and systolic blood pressure (SBP) at baseline, as well as time-averaged SBP, concomitant use of calcium channel blockers and concomitant use of diuretics during the trial period. Hyperuricaemia was defined as a uric acid concentration ≥417 μmol/l (7 mg/dl) in men or ≥357 μmol/l (6 mg/dl) in women. FA, folic acid.

The present study also had some limitations. First, this study focused on adults with hypertension; the generalisability of our results to adults without hypertension remains to be determined. However, SBP both at baseline and over the treatment period did not significantly affect the tHcy-lowering effect of folic acid supplementation. Second, we did not measure other polymorphisms in the folate pathway, such as the *MTHFR* A1298C variant. Overall, our results still need to be investigated and confirmed in future studies.

In conclusion, long-term daily 0·8 mg folic acid supplementation can significantly lower tHcy levels in a population of Chinese hypertensive patients with no history of CVD. More importantly, the degree of tHcy reduction is highly variable, and can be significantly affected by sex, *MTHFR* C677T genotypes, baseline folate, eGFR and smoking status. All these factors should be taken into account in the identification of participants who may be most likely to benefit from therapy to lower tHcy. The present study, therefore, provides useful data to inform the debate on folic acid fortification for public health reasons and folic acid supplementation for the prevention of a variety of clinical outcomes.

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Supplementary material

For supplementary material/s referred to in this article, please visit https://doi.org/10.1017/S0007114518002477

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