JAMA Internal Medicine | Original Investigation

Efficacy of Folic Acid Therapy on the Progression of Chronic Kidney Disease
The Renal Substudy of the China Stroke Primary Prevention Trial

Xin Xu, MD, PhD; Xianhui Qin, MD, PhD; Youbao Li, MD; Danhua Sun, MD; Jun Wang, MD; Min Liang, MD; Binyan Wang, MD, PhD; Yong Huo, MD; Fan Fan Hou, MD, PhD; for the investigators of the Renal Substudy of the China Stroke Primary Prevention Trial (CSPPT)

IMPORTANCE The efficacy of folic acid therapy on renal outcomes has not been previously investigated in populations without folic acid fortification.

OBJECTIVE To test whether treatment with enalapril and folic acid is more effective in slowing renal function decline than enalapril alone across a spectrum of renal function at baseline from normal to moderate chronic kidney disease (CKD) among Chinese adults with hypertension.

DESIGN, SETTING, AND PARTICIPANTS In this substudy of eligible China Stroke Primary Prevention Trial (CSPPT), 15 104 participants with an estimated glomerular filtration rate (eGFR) 30 mL/min/1.73 m^2 or greater, including 1671 patients with CKD, were recruited from 20 communities in Jiangsu province in China.

INTERVENTIONS Participants were randomized to receive a single tablet daily containing 10 mg enalapril and 0.8 mg folic acid (n = 7545) or 10 mg enalapril alone (n = 7559).

MAIN OUTCOMES AND MEASURES The primary outcome was the progression of CKD, defined as a decrease in eGFR of 30% or more and to a level of less than 60 mL/min/1.73 m^2 if the baseline eGFR was 60 mL/min/1.73 m^2 or more, or a decrease in eGFR of 50% or more if the baseline eGFR was less than 60 mL/min/1.73 m^2; or end-stage renal disease. Secondary outcomes included a composite of the primary outcome and all-cause death, rapid decline in renal function, and rate of eGFR decline.

RESULTS Overall, 15 104 Chinese adults with a mean (range) age of 60 (45-75) years were recruited; median follow-up was 4.4 years. There were 164 and 132 primary events in the enalapril group and the enalapril–folic acid group, respectively. Compared with the enalapril group, the enalapril–folic acid group had a 21% reduction in the odds of the primary event (odds ratio [OR], 0.79; 95% CI, 0.62-1.00) and a slower rate of eGFR decline (1.28% vs 1.42% per year; P = .02). Among the participants with CKD at baseline, folic acid therapy resulted in a significant reduction in the risks for the primary event (OR, 0.44; 95% CI, 0.26-0.75), rapid decline in renal function (OR, 0.67; 95% CI, 0.47-0.96) and the composite event (OR, 0.62; 95% CI, 0.43-0.90), and a 44% slower decline in renal function (0.96% vs 1.72% per year, P < .001). Among those without CKD at baseline, there was no between-group difference in the primary end point.

CONCLUSIONS AND RELEVANCE Enalapril–folic acid therapy, compared with enalapril alone, can significantly delay the progression of CKD among patients with mild-to-moderate CKD.

TRIAL REGISTRATION clinicaltrials.gov Identifier: NCT00794885

Invited Commentary page 1451

Supplemental content at jamainternalmedicine.com

CME Quiz at jamanetworkcme.com

Author Affiliations: Author affiliations are listed at the end of this article.

Group Information: The Renal Substudy of the China Stroke Primary Prevention Trial (CSPPT) members are listed at the end of this article.

Corresponding Author: Fan Fan Hou, MD, PhD, National Clinical Research Center for Kidney Disease, State Key Laboratory for Organ Failure, Research, Renal Division, Nanfang Hospital, Southern Medical University, Guangzhou 510515, China (ffhouguangzhou@163.com).

JAMA Intern Med. 2016;176(10):1443-1450. doi:10.1001/jamainternmed.2016.4687
Published online August 22, 2016.

Chronic kidney disease (CKD) is a worldwide public health problem leading to poor outcomes and high cost;[1] CKD substantially increases the risk of progression to end-stage renal disease (ESRD) and cardiovascular disease (CVD).[2,3] It is estimated that more than 119 million Chinese people have CKD and are at high risk of developing ESRD.[4] Antihypertensive therapy, especially aimed at inhibiting the renin-angiotensin system, remains the mainstay of treatment for slowing down renal function decline.[5] However, despite treatment, the number of patients with ESRD and treated with renal replacement therapy continues to increase.[6,7] New therapeutic interventions that slow down the decline in renal function in patients with CKD are critically needed.

The high prevalence of hyperhomocysteinemia in patients with CKD has generated interest in a potential role for hyperhomocysteinemia as a risk factor for progression of CKD.[8,9] Although epidemiologic studies have shown that hyperhomocysteinemia is associated with the risk of developing albuminuria and CKD,[10,11] interventional studies using high doses of folic acid and B vitamins including cyanocobalamin, conducted in populations with folic acid fortification, have shown no benefit or harmful effects on renal outcomes.[12,13] Previous studies have suggested that folic acid fortification could be a major modifier for the efficacy of folic acid therapy on cardiovascular disease and stroke.[14-18] However, no study to date has evaluated the efficacy of folic acid therapy without cyanocobalamin on renal outcomes in a population without folic acid fortification.

The China Stroke Primary Prevention Trial (CSPPT) compared the efficacy of combination of the angiotensin-converting enzyme inhibitor enalapril and folic acid with enalapril alone in reducing the risk of first stroke in Chinese adults with hypertension.[16] This report, a prespecified renal substudy of the CSPPT, sought to examine the effects of combination of enalapril and folic acid with enalapril alone in reducing the risk of renal function decline in a hypertensive population. The unique aspects of this study are that it was conducted in a population without folic acid fortification, that it included participants across a spectrum of renal function at baseline from normal to moderate CKD, and that cyanocobalamin was not used in the therapy.

Key Points

Question Is folic acid therapy effective in delaying the renal function decline in patients with and without chronic kidney disease (CKD)?

Findings In this this randomized clinical trial of 15 104 patients (1671 with CKD), folic acid therapy reduced the risk of progression of CKD and the rate of estimated glomerular filtration rate decline. Patients with CKD benefited more from the therapy, with a 56% and 44% reduction in the risk for progression of CKD and the rate of eGFR decline, respectively.

Meaning Folic acid therapy should be considered for the clinical management of CKD, particularly in regions without folic acid fortification.

Methods

Study Design

The rationale and study design for the CSPPT has been described in detail previously.[16] Briefly, the CSPPT was a randomized, double-blind, actively controlled trial conducted from May 2008 to August 2013 in 32 communities in Anhui and Jiangsu provinces of China. The study enrolled a total of 20 702 adults with hypertension and without a history of major CVD. Participants were randomized to receive treatment with either a combination of enalapril and folic acid or enalapril alone and followed up for a median of 4.5 years.

The substudy of renal outcomes was designed to investigate the effects of folic acid on the progression of CKD in adults with hypertension. The ethics committee of the Institute of Biomedicine, Anhui Medical University and the medical ethics committee of Nanfang Hospital, Southern Medical University approved the renal substudy, and informed consent for the renal substudy was waived. For trial protocol, see Supplement 1.

Participants, Intervention, and Follow-up

Detailed inclusion and exclusion criteria for the CSPPT trial are described elsewhere.[16] The renal substudy enrolled eligible CSPPT participants from the communities in Jiangsu province, excluding those with an estimated glomerular filtration rate (eGFR) of less than 30 mL/min/1.73 m^2 or a missing eGFR at baseline. The flowchart of the participants (CONSORT diagram) is presented in eFigure 1 in Supplement 2.

Participants were randomized to receive a daily oral dose of 1 tablet containing 10 mg enalapril and 0.8 mg folic acid (single pill combination; the enalapril-folic acid group) or 1 tablet containing 10 mg enalapril only (the enalapril group). Other classes of antihypertensive medications, mostly dihydropyridine calcium channel blockers and hydrochlorothiazide, could be prescribed concomitantly if necessary.

Participants were followed up every 3 months. At each visit, the blood pressures and pulses of the participants were measured, the numbers of pills taken between visits were counted, and concomitant medications and adverse events were recorded. Before the study ended, an exit visit was conducted for collection of serum and urine samples and assessment of renal outcomes.

Laboratory Assays

Serum and spot urine samples from the participants were collected at both the baseline and the exit visit. Serum creatinine, total homocysteine, lipids, and fasting glucose were measured using automatic clinical analyzers (Beckman Coulter) at the core laboratory of the National Clinical Research Center for Kidney Disease, Guangzhou, China. Specifically, serum creatinine was measured using an enzymatic assay that has been calibrated to be isotope dilution mass spectrometry traceable. The coefficient of variation for the assay was 1.4%. Estimated GFR was calculated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation.[19] Proteinuria was determined using a dipstick test (Dirui-H100).

Outcomes

The primary outcome was progression of CKD, defined as a decrease in eGFR of 30% or more and to a level of less than 60 mL/min/1.73m^2 at the exit visit if the baseline eGFR was 60 mL/min/1.73 m^2 or more, or a decrease in eGFR of 50% or more at the exit visit if the baseline eGFR was less than 60 mL/min/1.73 m^2 or end-stage renal disease (eGFR<15 mL/min/1.73 m^2 or need for dialysis).[20]

The secondary outcomes included the following: (1) a composite outcome of the primary outcome and all-cause death; (2) rapid decline in renal function, defined as an average decline in eGFR of 5 mL/min/1.73 m^2 or more per year; and (3) annual rate of relative decline in eGFR, estimated as

$$\left(1-\sqrt[t]{\frac{\text{eGFR at exit}}{\text{eGFR at baseline}}}\right)\times 100\%$$

where t is the time length in years from baseline to the exit visit.

Other Definitions

Participants with an eGFR less than 60 mL/min/1.73 m^2 and/or proteinuria at baseline were classified as having CKD. Diabetes was defined as having a history of diabetes or a fasting glucose 7 mmol/L (to convert mmol/L to mg/dL, divide by 0.0555) or more at baseline or under glucose-lowering therapy. The treatment compliance was calculated as the percentage of days taking the study medication during the trial. Regular concomitant medication was defined as 180 or more cumulative days taking the drug of interest.

Statistical Analyses

Participants with unknown renal outcomes (n = 2187 [14%]) were excluded from the renal outcome analysis. Participants who were alive at the exit visit with unknown renal outcomes (n = 1802 [12%]) were excluded from the composite outcome analysis. There were also missing values on serum total cholesterol (n = 1), body mass index (BMI [calculated as weight in kilograms divided by height in meters squared], n = 3), smoking status (n = 6), serum total homocysteine (n = 10) and folate (n = 173), and proteinuria dipstick test (n = 554) at baseline. Multiple imputation was used to deal with missing values in the outcome analyses.

Baseline characteristics were stratified by treatment group and presented as mean (SD) for continuous variables and count (percentage) for categorical variables. Differences between the treatment groups for treatment compliance, concomitant medication use, changes in serum folate and homocysteine, and time-averaged decrease in blood pressure during the trial were compared and tested using the Wilcoxon-Mann-Whitney test or a χ^2 test. The treatment effects, expressed as odds ratios (ORs) for the dichotomous outcomes and between-group differences for the eGFR decline rate, were estimated using generalized linear regression models with adjustment for age, sex, BMI, eGFR, proteinuria, systolic blood pressure (SBP), serum glucose and total cholesterol at baseline, as well as timed-average of SBP. The strata-specific treatment effects in the participants with and without CKD at baseline were also estimated and compared under the same regression models. As additional exploratory analyses, possible modifications of the treatment effects on the primary outcome in participants with CKD at baseline were also assessed for variables including sex, *MTHFR* C677T genotype, age, eGFR, presence of diabetes, and tertiles of BMI, serum folate, and total homocysteine at baseline.

We performed analyses on the primary outcome in total, CKD, and non-CKD populations. To adjust for multiple tests, the distribution of testwise P values under the null was obtained empirically by permutation of the treatment assignment. For the primary outcome, a testwise 2-tailed P value of .027 was considered statistically significant at a studywise type I error rate of .05. All data analyses were performed using R version 3.2.0 (The R Foundation; http://www.R-project.org/).

Results

Study Participants and the Baseline Characteristics

The renal substudy included a total of 15 104 patients with hypertension from the CSPPT, including 1671 (11.1%) with CKD at baseline. Of the participants, 12 917 (85.5%) completed renal outcome assessment, and 385 (2.5%) passed away before the end of the CSPPT with unknown renal outcomes (eFigure 1 in Supplement 2). The participants with unknown outcomes did not differ from those with complete outcome data in baseline characteristics (eTable 1 in Supplement 2). The baseline characteristics of the participants, stratified by the presence of CKD, were comparable among the 2 treatment groups (**Table 1**). The age of the participants ranged from 45 to 75 years with a mean of 60 years. The participants with CKD had higher blood pressure, serum total homocysteine, and fasting glucose than those without CKD. The prevalence of elevated serum total homocysteine (>15 µmol/L) and diabetes was 42% and 24%, respectively, in the CKD group, as compared with 26% and 12%, respectively, in the non-CKD group.

Treatment Adherence and Blood Pressure

The length of follow-up ranged from 4.0 to 4.8 years with a median of 4.4 years. The mean treatment compliance, defined as the percentage of the study medicine actually taken during the trial, was 76% in both treatment groups.

In the total study population, the time-averaged blood pressure during the trial was 140/84 mm Hg, representing an average decrease of 28/12 mm Hg from the baseline. The drop in blood pressure was slightly greater in the participants with CKD group than in those without. There was no significant difference in the blood pressure during the entire period of the trial between the 2 treatment groups (eFigure 2 in Supplement 2).

Changes in Serum Homocysteine and Folate

The baseline serum levels of folic acid and homocysteine were comparable between the 2 treatment groups in the total population as well as in the CKD subgroup (**Table 2**). At the exit visit, serum folate levels increased 5.1 and 15.4 ng/mL (to convert ng/mL to nmol/L, multiply by 2.266), respectively, from the baseline in the enalapril and the enalapril-folic acid group. As expected, the enalapril-folic acid group had a much greater drop in serum homocysteine than did the enalapril group (1.9 vs 0.2 µmol/L [to convert µmol/L to mg/L, divide 7.397];

Table 1. Baseline Characteristics of the Study Participants by Treatment and Strata of CKD[a]

Variables[b]	All Participants		Participants With CKD		Participants Without CKD	
	Enalapril (n = 7559)	Enalapril-FA (n = 7545)	Enalapril (n = 804)	Enalapril-FA (n = 867)	Enalapril (n = 6755)	Enalapril-FA (n = 6678)
Age, mean (SD), y	59.4 (7.6)	59.5 (7.5)	60.1 (8.1)	60.3 (8.3)	59.4 (7.6)	59.4 (7.4)
Male, No. (%)	2983 (39.5)	2919 (38.7)	348 (43.3)	374 (43.1)	2635 (39.0)	2545 (38.1)
Self-reported, No. (%)						
Diabetes	292 (3.9)	272 (3.6)	52 (6.5)	58 (6.7)	240 (3.6)	214 (3.2)
Hyperlipidemia	223 (3.0)	222 (2.9)	21 (2.6)	31 (3.6)	202 (3.0)	191 (2.9)
Blood pressure, mean (SD), mm Hg						
Systolic	168.5 (21.1)	168.1 (20.8)	173.0 (24.8)	172.5 (25.4)	168.0 (20.5)	167.5 (20.1)
Diastolic	95.3 (12.1)	95.4 (11.8)	97.7 (14.6)	97.8 (13.5)	95.0 (11.7)	95.1 (11.5)
BMI, mean (SD)	25.6 (3.6)	25.7 (3.6)	25.8 (3.7)	26.0 (3.8)	25.6 (3.5)	25.6 (3.5)
Current smoking, No. (%)	1710 (22.6)	1707 (22.6)	181 (22.5)	222 (25.6)	1529 (22.6)	1485 (22.3)
MTHFR C677T, No. (%)						
CC	1769 (23.4)	1768 (23.4)	196 (24.4)	182 (21.0)	1573 (23.3)	1586 (23.7)
CT	3773 (49.9)	3779 (50.1)	379 (47.1)	452 (52.1)	3394 (50.2)	3327 (49.8)
TT	2017 (26.7)	1998 (26.5)	229 (28.5)	233 (26.9)	1788 (26.5)	1765 (26.4)
Laboratory results, mean (SD)						
Glucose, mmol/L	6.1 (1.8)	6.0 (1.8)	6.7 (2.6)	6.7 (2.4)	6.0 (1.7)	6.0 (1.7)
TC, mmol/L	5.7 (1.2)	5.7 (1.2)	5.8 (1.5)	5.7 (1.3)	5.7 (1.1)	5.7 (1.2)
TG, mmol/L	1.7 (1.0)	1.7 (1.5)	1.8 (1.1)	1.8 (1.2)	1.7 (1.0)	1.7 (1.5)
HDL-C, mmol/L	1.3 (0.4)	1.3 (0.4)	1.3 (0.4)	1.3 (0.4)	1.3 (0.4)	1.3 (0.4)
eGFR, mL/min/1.73 m^2	94.3 (12.7)	94.0 (12.9)	87.0 (20.2)	85.7 (20.8)	95.1 (11.2)	95.1 (11.1)
Proteinuria, No. (%)	721 (9.9)	758 (10.4)	721 (90.1)	758 (87.9)	...	...
Medication use, No. (%)						
RAAS inhibitors	710 (9.4)	696 (9.2)	95 (11.8)	92 (10.6)	615 (9.1)	604 (9.0)
CCB	525 (6.9)	543 (7.2)	74 (9.2)	80 (9.2)	451 (6.7)	463 (6.9)
Diuretics	201 (2.7)	197 (2.6)	20 (2.7)	19 (2.2)	181 (2.7)	178 (2.7)
Glucose-lowering drugs	129 (1.7)	146 (1.9)	22 (2.7)	29 (3.3)	107 (1.6)	117 (1.8)
Lipid-lowering drugs	62 (0.8)	66 (0.9)	6 (0.7)	8 (0.9)	56 (0.8)	58 (0.9)
Antiplatelet drugs	312 (4.1)	273 (3.6)	43 (5.3)	34 (3.9)	269 (4.0)	239 (3.6)

Abbreviations: BMI, body mass index (calculated as weight in kilograms divided by height in meters squared); CCB, calcium channel blocker; CKD, chronic kidney disease; eGFR, estimated glomerular filtration rate; FA, folic acid; HDL-C, high-density lipoprotein cholesterol; *MTHFR*, methylenetetrahydrofolate reductase; RAAS, renin-angiotensin-aldosterone system; TC, total cholesterol; ellipses, not applicable or no data available.

[a] CKD was defined as eGFR less than 60 mL/min/1.73 m^2 or presence of proteinuria at baseline.

[b] For continuous variables, values are presented as mean (SD).

Table 2. Serum Homocysteine and Folate Levels at Baseline and After Treatment

Variables	All Participants			Participants With CKD			Participants Without CKD		
	Enalapril (n = 7559)	Enalapril-FA (n = 7545)	Group Difference, Mean (95% CI)	Enalapril (n = 804)	Enalapril-FA (n = 867)	Group Difference, Mean (95% CI)	Enalapril (n = 6755)	Enalapril-FA (n = 6678)	Group Difference, Mean (95% CI)
Total homocysteine, μmol/L									
Baseline, mean (SD)	14.7 (9.1)	14.7 (8.9)	−0.0 (−0.3 to 0.3)	16.8 (10.7)	17.1 (11.3)	0.3 (−0.7 to 1.4)	14.5 (8.9)	14.4 (8.5)	−0.1 (−0.4 to 0.2)
Exit, mean (SD)	14.5 (8.1)	12.7 (6.2)	−1.8 (−2.0 to −1.5)	16.2 (11.2)	14.0 (7.2)	−2.2 (−3.1 to −1.2)	14.3 (7.7)	12.5 (6.0)	−1.7 (−2.0 to −1.5)
Change, mean[a] (SD)	−0.2 (7.2)	−1.9 (7.9)	−1.7 (−2.0 to −1.5)	−0.1 (9.8)	−2.9 (9.9)	−2.9 (−3.9 to −1.8)	−0.2 (6.8)	−1.8 (7.6)	−1.6 (−1.9 to −1.3)
Folate, ng/mL[b]									
Baseline, mean (SD)	7.7 (3.2)	7.6 (3.2)	−0.0 (−0.1 to 0.1)	7.5 (3.1)	7.4 (3.1)	−0.2 (−0.5 to 0.2)	7.7 (3.3)	7.7 (3.2)	−0.0 (−0.1 to 0.1)
Exit, mean (SD)	12.7 (5.9)	23.1 (17.1)	10.3 (9.9 to 10.8)	13.0 (9.9)	25.0 (19.9)	12.0 (10.3 to 13.6)	12.7 (5.3)	22.8 (16.7)	10.1 (9.7 to 10.6)
Change, mean[a] (SD)	5.1 (5.9)	15.4 (17.2)	10.3 (9.9 to 10.8)	5.5 (10.1)	17.7 (20.3)	12.3 (10.5 to 14.0)	5.0 (5.2)	15.1 (16.8)	10.1 (9.6 to 10.6)

Abbreviations: CKD, chronic kidney disease; FA, folic acid.

[a] Change was defined as the exit value minus the baseline value.

[b] To convert to μmol/L to mg/L, divide 7.397; to convert ng/mL to nmol/L, multiply by 2.266.

Table 3. Treatment Effects on the Primary and Secondary Outcomes in All Participants

Outcomes	Participants, No.	Enalapril[a]	Enalapril-FA[a]	Unadjusted[b]	Adjusted[b,c]	*P* Value[c]
Primary outcome						
Progression of CKD	12 917	164 (2.5)	132 (2.1)	0.81 (0.64 to 1.02)	0.79 (0.62 to 1.00)	.05
Secondary outcomes						
Rapid decline in eGFR	12 916	426 (6.6)	390 (6.1)	0.92 (0.80 to 1.06)	0.91 (0.79 to 1.05)	.21
Composite events	13 302	360 (5.4)	321 (4.8)	0.89 (0.77 to 1.04)	0.89 (0.76 to 1.04)	.15
Decline in eGFR, % per y	12 916	1.42 (3.45)[d]	1.28 (3.33)[d]	−0.14 (−0.25 to −0.02)[e]	−0.13 (−0.24 to −0.02)[e]	.02

Abbreviations: BMI, body mass index (calculated as weight in kilograms divided by height in meters squared); CKD, chronic kidney disease; eGFR, estimated glomerular filtration rate; FA, folic acid; SBP, systolic blood pressure.

[a] Unless otherwise indicated, values are the number of events (percentage).

[b] Unless otherwise indicated, values are odds ratios (95% CI).

[c] Adjusted for age, sex, eGFR, proteinuria, serum glucose, total cholesterol, SBP, BMI at baseline, and time-averaged SBP during treatment.

[d] Mean (SD).

[e] Group difference (95% CI).

Table 4. Presence of CKD Modifies the Treatment Effects on the Primary and Secondary Outcomes

Outcomes	Participants, No.	Enalapril[a]	Enalapril-FA[a]	Unadjusted[b]	Adjusted[b,c]	*P* Value[c]	*P* Value for Interaction[c]
Primary outcome							.02
Non-CKD	11 513	118 (2.0)	108 (1.9)	0.93 (0.71 to 1.21)	0.94 (0.72 to 1.23)	.65	
CKD	1404	46 (6.8)	24 (3.3)	0.47 (0.29 to 0.78)	0.45 (0.27 to 0.76)	.003	
Secondary outcomes							
Rapid decline in eGFR							.06
Non-CKD	11 513	341 (5.9)	325 (5.7)	0.97 (0.83 to 1.13)	0.97 (0.83 to 1.14)	.74	
CKD	1403	85 (12.5)	65 (9.0)	0.69 (0.49 to 0.97)	0.67 (0.47 to 0.96)	.03	
Composite events							.06
Non-CKD	11 827	279 (4.7)	261 (4.4)	0.95 (0.80 to 1.13)	0.97 (0.81 to 1.16)	.73	
CKD	1475	81 (11.3)	60 (7.9)	0.67 (0.47 to 0.95)	0.65 (0.45 to 0.94)	.02	
Decline in eGFR, % per y							.002
Non-CKD	11 513	1.38 (2.99)[d]	1.32 (2.86)[d]	−0.06 (−0.19 to 0.06)[e]	−0.06 (−0.17 to 0.06)[e]	.35	
CKD	1403	1.72 (6.08)[d]	0.96 (5.81)[d]	−0.76 (−1.11 to −0.40)[e]	−0.62 (−0.95 to −0.29)[e]	<.001	

Abbreviations: CKD, chronic kidney disease; eGFR, estimated glomerular filtration rate; FA, folic acid; SBP, systolic blood pressure.

[a] Unless otherwise indicated, values are the number of events (percentage).

[b] Unless otherwise indicated, values are odds ratios (95% CI).

[c] Adjusted for age, sex, eGFR, proteinuria, serum glucose, total cholesterol, SBP, and BMI at baseline, and time-averaged SBP during treatment.

[d] Mean (SD).

[e] Group difference (95% CI).

P < .001). In the enalapril-folic acid group, both the size of the increase in serum folate and the drop in homocysteine were greater in the participants with CKD than in those without CKD. In particular, the greatest drop in serum homocysteine was in TT homozygotes of *MTHFR* C677T polymorphism, while the magnitude of the declines in those with CC/CT genotypes was relatively small (eFigure 3 in Supplement 2).

Treatment Effects on the Primary and Secondary Outcomes

In the total population, the primary event occurred in 164 (2.5%) and 132 (2.1%) participants, respectively, in the enalapril group and the enalapril-folic acid group (**Table 3**). Compared with the enalapril group, the enalapril-folic acid group had a 21% reduction in the adjusted risk of the primary event (OR, 0.79; 95% CI, 0.62-1.00), a significantly slower rate of eGFR decline (1.28% vs 1.42% per year; *P* = .02), and a 9% and 11% reduction in the risk of rapid renal function decline and the composite outcome, respectively, although the trend was not statistically significant.

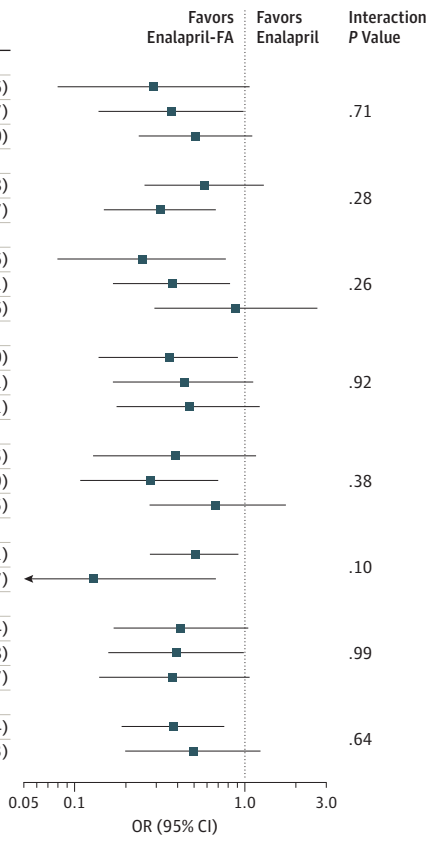
We were particularly interested in evaluating the effects of folic acid in the participants with CKD who were at a higher risk of renal dysfunction. Among those with CKD, the primary event occurred in 46 (6.8%) and 24 (3.3%) participants, respectively, in the enalapril group and the enalapril-folic acid group (**Table 4**). Compared with the enalapril group, the enalapril-folic acid group had a significant reduction in the adjusted odds of the primary event (OR, 0.44; 95% CI, 0.26-0.75), rapid decline in renal function (OR, 0.67; 95% CI, 0.47-0.96), and the composite event (OR, 0.62; 95% CI, 0.43-0.90). Treatment with enalapril-folic acid also resulted in a 44% slower rate of renal function decline (0.96% vs 1.72% per year; *P* < .001). In contrast, among the participants without CKD there was little difference between the 2 treatment groups in the effects on both the primary and the secondary outcomes. The interaction tests indicated that the presence of CKD at baseline was a significant modifier for the treatment effect.

Concomitant Medications

The most common concomitant medications were dihydropyridine and hydrochlorothiazide, which were used in 78% and 57% of the participants, respectively (eTable 2 in Supplement 2). There were no significant differences in concomitant medications during the trial between the 2 treatment groups. Use of other classes of antihypertensive drugs, lipid-lowering,

Figure. Primary Outcome in Subgroups of Patients With Chronic Kidney Disease

Source	Patients, No.	Enalapril Event No., %	Enalapril-FA Event No., %	OR (95% CI)	Interaction *P* Value
Age, y					
45-55	404	9 (4.7)	4 (1.9)	0.29 (0.08-1.06)	
55-65	554	15 (5.4)	7 (2.5)	0.37 (0.14-0.97)	.71
65-75	446	22 (10.4)	13 (5.5)	0.51 (0.24-1.10)	
Sex					
Male	585	18 (6.3)	12 (4.0)	0.58 (0.26-1.28)	.28
Female	819	28 (7.1)	12 (2.8)	0.32 (0.15-0.67)	
MTHFR C677T					
CC	320	16 (9.5)	5 (3.3)	0.25 (0.08-0.76)	
CT	697	22 (6.9)	12 (3.2)	0.38 (0.17-0.81)	.26
TT	387	8 (4.1)	7 (3.6)	0.89 (0.30-2.66)	
Folate, ng/mL					
≤5.54	465	16 (7.6)	8 (3.1)	0.36 (0.14-0.90)	
5.54-8.47	475	14 (6.0)	9 (3.7)	0.44 (0.17-1.11)	.92
≥8.48	464	16 (6.8)	7 (3.1)	0.47 (0.18-1.21)	
Total homocysteine, μmol/L					
≤12	479	11 (4.9)	6 (2.4)	0.39 (0.13-1.15)	
12-16.4	476	23 (9.5)	8 (3.4)	0.28 (0.11-0.69)	.38
≥16.5	449	12 (5.6)	10 (4.3)	0.70 (0.28-1.75)	
eGFR, mL/min/1.73 m^2					
≥60	1165	37 (6.5)	22 (3.7)	0.51 (0.28-0.91)	.10
30-60	239	9 (8.3)	2 (1.5)	0.13 (0.02-0.67)	
BMI					
≤24.2	461	17 (7.4)	8 (3.5)	0.42 (0.17-1.04)	
24.2-27.3	470	16 (7.4)	9 (3.5)	0.40 (0.16-0.98)	.99
≥27.4	473	13 (5.5)	7 (2.9)	0.38 (0.14-1.07)	
Diabetes					
Yes	1070	30 (5.8)	15 (2.7)	0.38 (0.19-0.74)	.64
No	334	16 (9.9)	9 (5.2)	0.50 (0.20-1.23)	



Diabetes was defined as having a history of diabetes or a fasting glucose level of 7 mmol/L or more (to convert mmol/L to mg/dL, divide by 0.0555) at baseline or under glucose-lowering therapy. BMI indicates body mass index (calculated as weight in kilograms divided by height in meters squared); eGFR, estimated glomerular filtration rate.

glucose-lowering, and antiplatelet medications were very rare, all at a frequency of less than 2%.

Exploratory Subgroup Analyses

We further performed exploratory subgroup analysis to assess the treatment effect on the primary outcome in various subgroups among participants with CKD (**Figure**). A larger reduction in the risk of the primary event was observed in the subgroup of baseline eGFR less than 60 mL/min/1.73 m^2 compared with that of baseline eGFR 60 mL/min/1.73 m^2 or more, though the difference did not reach statistical significance (P = .10). Other variables, including levels of serum folate and total homocysteine, did not appear to modify the treatment effect. It should be noted that at the existing sample size, the power for detecting a moderate interaction in the test is quite limited, such that a negative finding in the test would not necessarily confirm an absence of interaction.

Discussion

We found that treatment with enalapril-folic acid, as compared with enalapril alone, reduced the risk of progression of CKD by 21% and the rate of eGFR decline by 10% in hypertensive patients. More importantly, the presence of CKD at baseline was a significant modifier of the treatment effect (P for interaction = .02). Patients with CKD benefited most from the folic acid therapy, with a 56% and 44% reduction in the risk for progression of CKD and the rate of eGFR decline, respectively. In contrast, the renal protective effect in those without CKD was nominal.

Blood pressure control is essential to slow down the progression of CKD.[21,22] In our study, blood pressures, treatment compliance, and the use of other conventional antihypertensive drugs remained comparable between the 2 treatment groups across all stages of the trial. We also adjusted for baseline SBP, timed-average of SBP, and other possible confounders in our outcome analysis. Therefore, the beneficial effect of the enalapril-folic acid therapy that we observed could not be attributed to differences in blood pressure control throughout the study. Moreover, because of the low vascular disease burden and low frequency of vascular protective medication use in the study population, our results are unlikely to be affected by possible drug interactions.[23,24]

Our study is the first to show significant renal protection from folic acid therapy in a population without folic acid for-

tification. In contrast, previous trials have reported a null or harmful effect of supplementation with folic acid and B vitamins including cyanocobalamin.[12,13] The HOST study[13] reported that treatment with high doses of folic acid (40 mg/d) and other B vitamins (vitamin B_6, 100 mg/d; vitamin B_{12}, 2 mg/d) did not improve survival or delay the time to initiating dialysis in patients with advanced CKD or ESRD.[13] The DIVINe trial[12] showed that, compared with placebo, treatment with folic acid (2.5 mg/d) and B vitamins (vitamin B_6, 25 mg/d; cyanocobalamin, 1 mg/d) resulted in a greater decrease in GFR and an increase in vascular events in 238 patients with diabetic nephropathy. This discrepancy may be partly explained by differences in patient characteristics and treatment schemes among these studies. First, baseline folic acid levels may have an impact on the effectiveness of the folic acid therapy. Our study was conducted in a population without grain fortification of folic acid while the other 2 studies were undertaken in regions with folic acid fortification. The mean baseline serum folate was 7.7 ng/mL in our study, much lower than the means of 15 to approximately 16.5 ng/mL in the HOST and the DIVINe studies. The CSPPT study has previously shown that folic acid therapy can reduce the risk of stroke with a significantly larger effect size in those with lower serum folate at baseline.[16] Second, our study participants were those with mild-to-moderate CKD, while the HOST study enrolled patients with advanced CKD or ESRD. It is possible that the underlying burden of disease in the HOST study was too great to produce a measurable benefit from a folic acid supplementation; the beneficial effect of folic acid therapy may be most evident in patients with mild-to-moderate CKD. Third, unlike the other 2 studies, our study used a low dose of folic acid (0.8 mg/d) without combination with other B vitamins. After treatment, mean serum folate rose to 23 ng/mL in our study as compared with more than 2000 ng/mL in the HOST study.[13] At such a high level, much of the folate in the blood would be unmetabolized folic acid, of which the pharmacological effects on renal and vascular events have not yet been characterized.[25] Moreover, the renal protective effect might be offset by the toxic effects associated with pharmacological doses of B vitamins. Our findings support the hypothesis that a high dose of cyanocobalamin, which was administered together with folic acid, might be the culprit of the renal adverse outcomes observed in the DIVINe study.[12,26,27]

Inadequate folate intake is prevalent in most countries without mandatory folic acid fortification, including China and other developing countries. In our exploratory analysis, we found that baseline folate did not modify the effect of folic acid on progression of CKD. However, the highest tertile of serum folate level (>8.47 ng/mL) was still very low. In addition, the sample size of our study was not large enough to detect a moderate interaction effect. Whether the renal protective effect of folic acid that we observed could be extrapolated to populations with higher folate levels will require further verification.

Several limitations are worth mentioning. This was a substudy of the CSPPT trial. The renal function of the study participants was only assessed at baseline and the exit visit. More frequent renal function assays would allow for a more accurate assessment of change in renal function over time. In addition, 12% of the participants had incomplete outcome data and were excluded from the analysis. However, the baseline characteristics were fairly balanced among the 2 treatment groups and those who dropped out, which should have alleviated the bias. Furthermore, we performed a sensitivity analysis using a composite outcome that included both the primary outcome and all-cause death and confirmed a significant renal protection effect from the folic acid therapy. Owing to the study limitations, confirmation of our findings in an independent population without folic acid fortification is badly needed.

Conclusions

Enalapril-folic acid therapy compared with enalapril alone significantly slowed down the progression of CKD in patients with hypertension with mild-to-moderate CKD. The number needed to treat was 29. Given the magnitude of renal protection suggested by this study as well as the safety and the low cost, the potential role of folic acid therapy in the clinical management of patients with CKD in regions without folic acid fortification should be vigorously examined.

ARTICLE INFORMATION

Published Online: August 22, 2016. doi:10.1001/jamainternmed.2016.4687

Author Affiliations: National Clinical Research Center for Kidney Disease, Nanfang Hospital, Southern Medical University, Guangzhou, China (Xu, Qin, Li, Sun, J. Wang, Liang, B. Wang, Hou); State Key Laboratory of Organ Failure Research, Nanfang Hospital, Southern Medical University, Guangzhou, China (Xu, Qin, Li, Sun, J. Wang, Liang, B. Wang, Hou); Guangdong Provincial Institute of Nephrology, Nanfang Hospital, Southern Medical University, Guangzhou, China (Xu, Qin, Li, Sun, J. Wang, Liang, B. Wang, Hou); Division of Nephrology, Nanfang Hospital, Southern Medical University, Guangzhou, China (Xu, Qin, Li, Sun, J. Wang, Liang, B. Wang, Hou); Department of Cardiology, Peking University First Hospital, Beijing, China (Huo).

Author Contributions: Dr Hou had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. Drs Xu and Qin contributed equally to this article.
Study concept and design: Xu, Qin, Li, Sun, Liang, Wang, Y. Huo, F. Hou.
Acquisition, analysis, or interpretation of data: Xu, Qin, Li, Sun, Wang, Wang, Y. Huo, F. Hou.
Drafting of the manuscript: Xu, Qin, Sun, Liang, Y. Huo, F. Hou.
Critical revision of the manuscript for important intellectual content: Xu, Qin, Li, Wang, Liang, Wang, Y. Huo, F. Hou.
Statistical analysis: Xu, Qin, Li, Sun, F. Hou.
Obtained funding: Xu, Liang, Wang, F. Hou.
Administrative, technical, or material support: Wang, Liang, Wang, F. Hou.
Study supervision: Liang, Y. Huo, F. Hou.
Other: Sun, Wang.

Conflict of Interest Disclosures: Dr Xu reports grants from the Major Scientific and Technological Planning Project of Guangzhou, the Science, Technology and Innovation Committee of Shenzhen, and personal fees from AUSA Research Institute, Shenzhen AUSA. Dr Qin reports grants from the National Science Foundation and consulting fees from AUSA Research Institute, Shenzhen AUSA. Dr B. Wang reports grants from the National Science Foundation, the Special Project on the Integration of Industry, Education and Research of Guangdong Province, and consulting fees from AUSA Research Institute, Shenzhen AUSA. Dr Y. Huo reports grants from the National Major Scientific and Technological Special Project and nonfinancial support from Shenzhen AUSA. Dr F. Hou reports grants from the Major State Basic Research Development Program of

China, Ministry of Science and Technology of the People's Republic of China, the National Key Technology Support Program of China, the Major Scientific and Technological Planning Project of Guangzhou, the National Natural Science Foundation of China, the Guangzhou Clinical Research Center for Chronic Kidney Disease Program, and State Key Laboratory for Organ Failure Research, Guangzhou, China. No other conflicts were reported.

Funding/Support: This study was supported by the Major State Basic Research Development Program of China (973 Program 2012CB517703 to Dr F. Hou), the National Key Technology Support Program of China (grant No. 2013BAI09B06; grant No. 2015BAI2B07 to Dr F. Hou), the Major Scientific and Technological Planning Project of Guangzhou (grant No. 201607020004 to Dr Xu; grant No. 15020010 to Dr F. Hou), the Science, Technology and Innovation Committee of Shenzhen (grant No. JCYL20130401162636527 to Dr Xu), the National Natural Science Foundation of China (Key program) (grant No. 81430016 to Dr F. Hou), the Special Project on the Integration of Industry, Education and Research of Guangdong Province (grant No. 2011A091000031 to Dr B. Wang), and the Guangzhou Clinical Research Center for Chronic Kidney Disease Program (grant No. 7415695988305 to Dr F. Hou).

Role of the Funder/Sponsor: The funders/sponsors had no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; preparation, review, or approval of the manuscript; and decision to submit the manuscript for publication.

Group Information for the Renal Substudy of the China Stroke Primary Prevention Trial (CSPPT): *Writing Group:* Xin Xu, Xianhui Qin, Jun Wang, Ming Liang, Binyan Wang, Yong Huo, Fan Fan Hou. *Steering Committee:* Kejiang Cao; Luyuan Chen; Xiaoshu Cheng; Yimin Cui; Qiang Dong; Junbo Ge; Pingjin Gao; Runlin Gao; Dayi Hu; Fan Fan Hou; Yong Huo; Xunming Ji; Jianping Li; Nanfang Li; Xiaoying Li; Lisheng Liu; Changsheng Ma; Ningling Sun; Jian'an Wang; Wen Wang; Xian Wang, Chuanshi Xiao; Xinchun Yang; Dingliang Zhu; Gang Zhao; Lianyou Zhao. *Statistical Group:* Xin Xu, Youbao Li. *Clinical Sites (Coordinators):* Anfeng Township Central Hospital, Donghai (Yong Li); Baita Township Hospital, Donghai (Xiangming Li); Banzhuang Township Central Hospital, Ganyu (YanLuan Wan); Chengtou Township Hospital, Ganyu (Jingzhi Tan); Ganma Township Hospital, Ganyu (Shuhong Dong); Haitou Township Central Hospital, Ganyu (Hongtuan Xu); Henggou Rural Hospital, Donghai (Jiahong Liu); Huandun Township Hospital, Ganyu (Jian Xu); Huangchuan Township Central Hospital, Donghai (Daogang Li); Linian Rural Hospital, Donghai (Changyin Liu); Lizhuang Township Hospital, Ganyu (Jinbo Xu); Qinghu Township Central Hospital, Donghai (Chunlei Liu); Shahe Township Central Hospital, Ganyu (Libo Xu); Shanzuokou Rural Hospital, Donghai (Zhong Zhou); Shilianghe Rural Hospital, Donghai (Zhenchao Zhou); Shiliu Township Hospital, Donghai (Shuyong Liu); Shuangdian Township Hospital, Donghai (Chungen Tang); Taolin Township Central Hospital, Donghai (Zongpan Xu); Tashan Township Hospital, Ganyu (Zhenggen Xiong); Tuofeng Township Hospital, Donghai (Xinyan Zhu).

REFERENCES

1. Collins AJ, Foley RN, Chavers B, et al. US Renal Data System 2013 Annual Data Report. *Am J Kidney Dis*. 2014;63(1)(suppl):A7.

2. Peralta CA, Shlipak MG, Judd S, et al. Detection of chronic kidney disease with creatinine, cystatin C, and urine albumin-to-creatinine ratio and association with progression to end-stage renal disease and mortality. *JAMA*. 2011;305(15):1545-1552.

3. Sarnak MJ, Levey AS, Schoolwerth AC, et al; American Heart Association Councils on Kidney in Cardiovascular Disease, High Blood Pressure Research, Clinical Cardiology, and Epidemiology and Prevention. Kidney disease as a risk factor for development of cardiovascular disease: a statement from the American Heart Association Councils on Kidney in Cardiovascular Disease, High Blood Pressure Research, Clinical Cardiology, and Epidemiology and Prevention. *Hypertension*. 2003;42(5):1050-1065.

4. Zhang L, Wang F, Wang L, et al. Prevalence of chronic kidney disease in China: a cross-sectional survey. *Lancet*. 2012;379(9818):815-822.

5. Drawz PE, Rosenberg ME. Slowing progression of chronic kidney disease. *Kidney Int Suppl (2011)*. 2013;3(4):372-376.

6. Meguid El Nahas A, Bello AK. Chronic kidney disease: the global challenge. *Lancet*. 2005;365(9456):331-340.

7. Mills KT, Xu Y, Zhang W, et al. A systematic analysis of worldwide population-based data on the global burden of chronic kidney disease in 2010. *Kidney Int*. 2015;88(5):950-957.

8. Marti F, Vollenweider P, Marques-Vidal PM, et al. Hyperhomocysteinemia is independently associated with albuminuria in the population-based CoLaus study. *BMC Public Health*. 2011;11:733.

9. Ponte B, Pruijm M, Marques-Vidal P, et al. Determinants and burden of chronic kidney disease in the population-based CoLaus study: a cross-sectional analysis. *Nephrol Dial Transplant*. 2013;28(9):2329-2339.

10. Jager A, Kostense PJ, Nijpels G, et al. Serum homocysteine levels are associated with the development of (micro)albuminuria: the Hoorn study. *Arterioscler Thromb Vasc Biol*. 2001;21(1):74-81.

11. Ninomiya T, Kiyohara Y, Kubo M, et al. Hyperhomocysteinemia and the development of chronic kidney disease in a general population: the Hisayama study. *Am J Kidney Dis*. 2004;44(3):437-445.

12. House AA, Eliasziw M, Cattran DC, et al. Effect of B-vitamin therapy on progression of diabetic nephropathy: a randomized controlled trial. *JAMA*. 2010;303(16):1603-1609.

13. 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.

14. Qin X, Huo Y, Langman CB, et al. Folic acid therapy and cardiovascular disease in ESRD or advanced chronic kidney disease: a meta-analysis. *Clin J Am Soc Nephrol*. 2011;6(3):482-488.

15. Qin X, Huo Y, Xie D, Hou F, Xu X, Wang X. Homocysteine-lowering therapy with folic acid is effective in cardiovascular disease prevention in patients with kidney disease: a meta-analysis of randomized controlled trials. *Clin Nutr*. 2013;32(5):722-727.

16. Huo Y, Li J, Qin X, et al; CSPPT Investigators. Efficacy of folic acid therapy in primary prevention of stroke among adults with hypertension in China: the CSPPT randomized clinical trial. *JAMA*. 2015;313(13):1325-1335.

17. Jardine MJ, Kang A, Zoungas S, et al. The effect of folic acid based homocysteine lowering on cardiovascular events in people with kidney disease: systematic review and meta-analysis. *BMJ*. 2012;344:e3533.

18. Myung SK, Ju W, Cho B, et al; Korean Meta-Analysis Study Group. Efficacy of vitamin and antioxidant supplements in prevention of cardiovascular disease: systematic review and meta-analysis of randomised controlled trials. *BMJ*. 2013;346:f10.

19. Levey AS, Stevens LA, Schmid CH, et al; CKD-EPI (Chronic Kidney Disease Epidemiology Collaboration). A new equation to estimate glomerular filtration rate. *Ann Intern Med*. 2009;150(9):604-612.

20. Systolic Blood Pressure Intervention Trial (SPRINT) protocol. November 1, 2012 (https://www.sprinttrial.org/public/Protocol_Current.pdf).

21. Lv J, Ehteshami P, Sarnak MJ, et al. Effects of intensive blood pressure lowering on the progression of chronic kidney disease: a systematic review and meta-analysis. *CMAJ*. 2013;185(11):949-957.

22. Sarnak MJ, Greene T, Wang X, et al. The effect of a lower target blood pressure on the progression of kidney disease: long-term follow-up of the modification of diet in renal disease study. *Ann Intern Med*. 2005;142(5):342-351.

23. Hou W, Lv J, Perkovic V, et al. Effect of statin therapy on cardiovascular and renal outcomes in patients with chronic kidney disease: a systematic review and meta-analysis. *Eur Heart J*. 2013;34(24):1807-1817.

24. Shurraw S, Hemmelgarn B, Lin M, et al; Alberta Kidney Disease Network. Association between glycemic control and adverse outcomes in people with diabetes mellitus and chronic kidney disease: a population-based cohort study. *Arch Intern Med*. 2011;171(21):1920-1927.

25. Pfeiffer CM, Sternberg MR, Fazili Z, et al. Unmetabolized folic acid is detected in nearly all serum samples from US children, adolescents, and adults. *J Nutr*. 2015;145(3):520-531.

26. Spence JD, Eliasziw M, House AA. B-vitamin therapy for diabetic Nephropathy [Reply]. *JAMA*. 2010;304:636-637.

27. Spence JD. Metabolic B12 deficiency: a missed opportunity to prevent dementia and stroke. *Nutr Res*. 2016;36(2):109-116. doi:10.1016/j.nutres.2015.10.003.