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Effect of folic acid supplementation on cancer risk among adults with hypertension in China: A randomized clinical trial

Xianhui Qin[1], Lin Shen[2], Rong Zhang[3], Youbao Li[1], Xiaobin Wang[4], Binyan Wang[1], Xiaodong Jiang[5], Hua Jiang[6], Yu Lei[7], Fan Fan Hou[1], Jin Gu[8] and Yong Huo[9]

[1] National Clinical Research Center for Kidney Disease; State Key Laboratory for Organ Failure Research; Renal Division, Nanfang Hospital, Southern Medical University, Guangzhou, China
[2] Key Laboratory of Carcinogenesis and Translational Research (Ministry of Education), Department of Gastrointestinal Oncology, Peking University Cancer Hospital and Institute, Beijing Cancer Hospital, Beijing, China
[3] Medical Imaging and Minimally Invasive Interventional center, Cancer Center of Sun Yat-sen University &State Key Laboratory of Oncology in Southern China, Guangzhou, China
[4] Department of Population, Family and Reproductive Health, Johns Hopkins University Bloomberg School of Public Health, Baltimore, MD
[5] Department of Oncology, First People's Hospital, Lianyungang, China
[6] Department of Oncology, Second People's Hospital, Lianyungang, China
[7] Department of Oncology, First Affiliated Hospital of Anhui Medical University, Hefei, China
[8] Key Laboratory of Carcinogenesis and Translational Research (Ministry of Education), Department of Colorectal Surgery, Peking University Cancer Hospital and Institute, Peking-Tsinghua Center for Life Sciences, Beijing, China
[9] Department of Cardiology, Peking University First Hospital, Beijing, China

The relationship of folic acid supplementation with the risk of cancer remains inconclusive. We aimed to evaluate the effects of folic acid supplementation on cancer incidence among adults with hypertension without history of stroke or myocardial infarction (MI) in the China Stroke Primary Prevention Trial (CSPPT). A total of 20,702 hypertensive adults without history of stroke or MI, stratified by *MTHFR* C677T genotypes(CC, CT and TT), were randomly assigned to receive double-blind daily treatment with a single pill containing 10 mg enalapril and 0.8 mg folic acid($n = 10,348$) or a pill containing 10 mg enalapril alone($n = 10,354$). During a median treatment duration of 4.5 years, cancer occurred in 116 participants(1.12%) in the

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Correspondence to: Yong Huo, M.D., Department of Cardiology, Peking University First Hospital, No. 8 Xishiku St, Xicheng District, Beijing 100034, China, Tel.: 86-10-83572283, Fax: 86-10-66137748, E-mail: huoyong@263.net.cn or Jin Gu, M.D., FACS Key Laboratory of Carcinogenesis and Translational Research (Ministry of Education), Department of Colorectal Surgery, Peking University Cancer Hospital and Institute, Peking-Tsinghua Center for Life Sciences, No.52 Fucheng Road, Haidian District, Beijing 100142, China, Tel.: 86-13801180717, Fax: 86 −10- 88196238, E-mail: zlgujin@126.com

enalapril-folic acid group versus 116 participants(1.12%) in the enalapril group (HR, 1.00; 95%CI, 0.77–1.29). There was also no significant difference in the HRs for specific types of cancer(esophageal, gastric, breast, lung, colorectal, head and neck, liver and gynecologic cancer or lymphoma) or cancer mortality(HR, 1.05; 95%CI, 0.69–1.58). For participants not receiving folic acid treatment (enalapril only group), *MTHFR* 677 TT genotype was an independent predictor of total cancer risk compared to CC genotype (HR, 1.86; 95%CI, 1.07–3.22). Consistently, a beneficial effect was observed in participants with *MTHFR* TT genotype and low folate levels (<9.0 ng/mL; HR, 0.47; 95%CI, 0.24–0.94). There is no evidence that 0.8 mg daily folic acid supplementation can increase the risk of cancer incidence among adults with hypertension without history of stroke or MI in China. Our data suggest a protective effect in participants with *MTHFR* TT genotype and low folate levels.

What's new?

Folic acid is celebrated for its health benefits, particularly its ability to prevent certain birth defects. But its relationship with adult cancers is complex, with supplementation potentially increasing cancer risk in populations lacking folic acid-fortified foods. In this study of Chinese patients diagnosed with hypertension but lacking history of stroke or myocardial infarction and having relatively low folic acid intake, supplementation with 0.8 mg/d folic acid had no impact on cancer risk. Analyses of folate levels and *MTHFR* C677T genotypes uncovered beneficial effects for enalapril-folic acid treatment in patients with the *MTHFR* 677 TT variant, naturally associated with low serum folate levels.

Despite strong evidence for the many beneficial health effects of folic acid supplementation (including primary prevention of neural tube defects and stroke),[1,2] concern remains about whether it may increase the risk of cancer, especially in population without folic acid fortification.[3]

Observational studies have reported that low folate status is associated with an increased risk of cancer, while a higher intake of folate has been linked to a lower cancer risk.[4,5] However, in a combined analysis and extended follow-up of two randomized, controlled trials (Norwegian Vitamin Trial [NORVIT] and Western Norway B Vitamin Intervention Trial [WENBIT]),[3] treatment with folic acid (0.8 mg/d) plus vitamin B12 was associated with increased cancer incidence (RR, 1.21; 95% CI, 1.03–1.41) in patients with ischemic heart disease in Norway, where there is no folic acid fortification of foods. Two recent meta-analyses[6,7] of 13 randomized trials also found that the total cancer incidence associated with folic acid supplementation increased by 5–6%, although the findings did not reach the conventional definition of statistical significance (RR,1.06; 95% CI, 0.99–1.13[6]; and RR,1.05; 95% CI, 0.99–1.11,[7] respectively). Furthermore, the significantly higher total cancer incidence risk was observed among those trials with a higher percent use of lipid-lowering drugs (>60% (including the NORVIT and WENBIT), RR, 1.10; 95%CI, 1.00–1.20) or a lower dose of folic acid ($\leq$1 mg/d, RR, 1.23; 95%CI, 1.06–1.43).[7] And then, a more recently published trial[8] reported that the combined vitamin B-12 and folic acid supplementation was associated with increased incidence of cancer (HR, 1.56; 95%CI, 1.04–2.31) in The Netherlands (without folic acid fortification). Consistently, using a similar search strategy and selection criteria as a previously published meta-analysis,[7] our updated meta-analysis, of by far the largest number of randomized controlled trials (15 randomized trials),[8–22] found that folic acid supplementation was associated with marginally increased risk of cancer in trials with no or partial folic acid fortification (RR, 1.07; 95% CI: 1.00–1.15) (Fig. 1).

This report aimed to fill in the following important knowledge gaps with regards to folic acid supplementation and cancer risk.

First, all the previous randomized controlled trials were conducted in participants with adenoma[9–11] or cardiovascular disease,[12–22] and used the folic acid combined with B12 and/or B6 vitamins.[12–22] Though the possible protection against the initiation of cancer, folate may enhance the growth of existing adenomas[23] and increase the cancer risk (3 trials,[9–11] combined RR, 1.33; 95% CI, 0.98–1.80, $p = 0.07$).[6] Furthermore, the association between folic acid and cancer risk may be confounded by the concomitant vascular protective drugs and other B vitamins.[7] However, to date, there is lack of data on cancer incidence associated with folic acid treatment alone among general populations without adenoma or cardiovascular disease.

Second, there has been a particular lack of randomized clinical trials to assess cancer risk associated with folic acid supplementation in Asian countries, or other similar regions with a high cancer burden but relatively low folate intake and without folic acid fortification of foods. As a result, uncertainty remains regarding recommendations for folic acid treatment in clinical settings and population-based folic acid fortification of foods.

Third, it remains to be determined to what degree methylenetetrahydrofolate reductase (*MTHFR*) gene C677T polymorphisms and baseline folate levels could modify the effects of folic acid supplementation on the risk of cancer incidence. *MTHFR* is the main regulatory enzyme for folate metabolism.

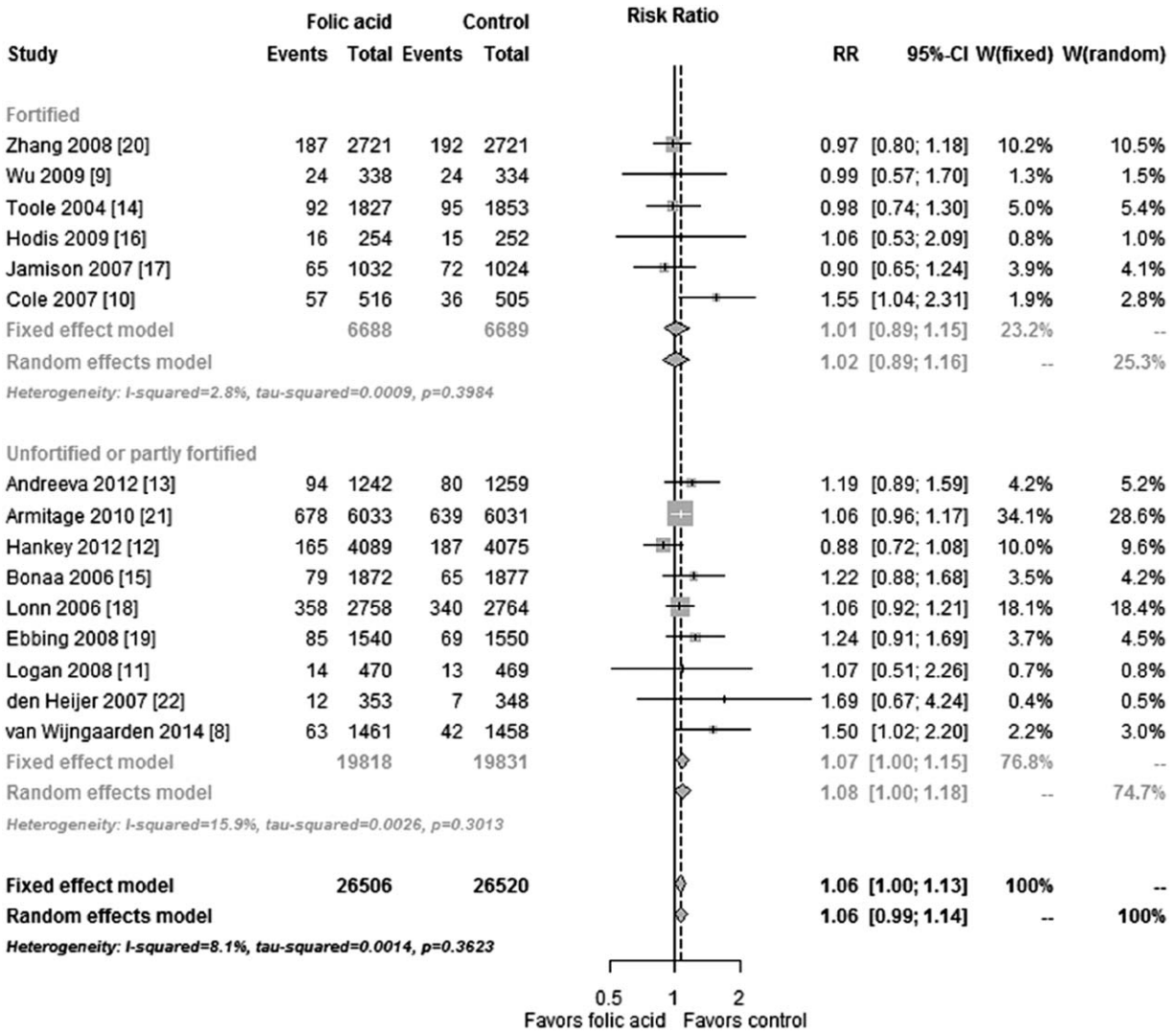

Figure 1. Forest plot of relative risk (RR) and 95% confidence interval (95% CI) of cancer incidence for folic acid treatment versus control in individual trials and pooled data before the CSPPT.

Polymorphisms of *MTHFR 677 C → T* have been shown to lead to a decrease in the enzyme activity, resulting in decreased levels of serum folate, an increased concentration of plasma homocysteine, and genomic DNA hypomethylation, particularly in those with low folate intake.[24] A previous case-control study reported that the increased cancer risk was found for MTHFR 677 TT genotype (*vs.* CC; OR, 1.52; 95% CI, 1.31–1.77), especially in trials with low folate levels (OR, 2.05; 95% CI, 1.13–3.72).[25] However, a post-trial observational follow-up study[3] showed that the HRs of cancer incidence or mortality for folic acid versus non-folic acid treatment groups were higher among individuals with the *MTHFR* 677 TT genotype than among those with CC or CT genotypes. To date, the potential modifying effects of *MTHFR* genotypes and baseline folate levels on cancer risk associated with folic acid treatment have not yet been evaluated in randomized trials.

Hypertension is recognized as a major and modifiable risk factor of stroke. Furthermore, hypertension and elevated homocysteine concentrations have shown a multiplicative effect on cardiovascular disease risk.[26,27] We speculated that hypertensive adulsts may particularly benefit from homocysteine-lowering therapy along with anti-hypertension therapy. Therefore, the China Stroke Primary Prevention Trial (CSPPT), a large scale randomized control trial, aimed to evaluate whether enalapril-folic acid therapy is more effective in reducing first stroke than enalapril alone in a hypertensive Chinese population. For this report, we conducted a detailed analysis of cancer endpoints (a prespecified outcome) in the CSPPT[2] to evaluate the effect of folic acid supplementation on cancer incidence among adults with hypertension without a history of stroke or myocardial infarction (MI) in China. A unique strength of the study was our ability to assess the modifying effects of individual MTHFR genotypes and baseline folate levels.

Subjects and Methods

Participants

The methods and primary results of the CSPPT trial have been reported elsewhere.[2] This study was approved by the Ethics Committee of the Institute of Biomedicine, Anhui Medical University, Hefei, China (FWA assurance number: FWA00001263) and registered with ClinicalTrials.gov, NCT00794885. All participants gave written informed consent.

Briefly, the CSPPT was a multi-community, randomized, double-blind, controlled trial conducted from May 19, 2008 to August 24, 2013 in 32 communities in China. Eligible participants were men and women aged 45–75 years old who had hypertension, defined as seated resting systolic blood pressure (SBP) $\geq$140 mmHg or diastolic blood pressure (DBP) $\geq$90 mmHg at both the screening and recruitment visit, or were on an anti-hypertensive medication. The major exclusion criteria included history of physician-diagnosed stroke, MI, heart failure, post-coronary revascularization, congenital heart disease, severe angiotensin converting enzyme inhibitors related adverse effects, severe mental disorders, pregnant or breastfeeding women, or individuals with long-term use of folic acid, vitamin B12 or vitamin B6.

Procedures

Eligible participants, stratified by *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 enalapril and 0.8 mg folic acid (single pill combination, the enalapril-folic acid group); or a daily oral dose of one tablet containing 10 mg enalapril only (the enalapril group). Both types of tablets were concealed in a single-capsule formulation and were identical in appearance, size, color and taste. All study investigators and participants were blinded to the randomization procedure and the treatment assignments.

Participants were scheduled for follow-up every three months. At each follow-up visit, vital signs, study drug compliance, concomitant medication use, adverse events and possible endpoint events were documented by trained research staff and physicians. During the trial period, concomitant use of other antihypertensive drugs (mainly calcium channel blockers or diuretics) was allowed, but not B-vitamins. Participants were scheduled for follow-up every three months.

Laboratory assays

MTHFR C677T (rs1801133) polymorphisms were detected on an ABI PRISM® 7900HT sequence detection system (Life Technologies, CA) using the TaqMan assay. The concordance rate for duplicates was 99.4%. Serum folate and B12 at both the baseline and the exit visit were measured by a commercial lab using a chemiluminescent immunoassay (New Industrial, Shenzhen, China). Serum homocysteine, fasting lipids and glucose at both the baseline and the exit visit were measured using automatic clinical analyzers (Beckman Coulter, CA) at the core lab of the National Clinical Research Center for Kidney Disease (Nanfang Hospital, Guangzhou, China).

Outcomes

Cancer incidence, a pre-specified endpoint of the CSPPT, was the main outcome in this analysis. Cancer could be diagnosed based on either positive pathologic findings or specific clinical manifestations. Acceptable evidence for pathologic findings included original or photocopied pathological reports; and original or photocopied medical records from hospitals in which pathological results were cited. When pathological data were not available, cases were independently reviewed by two oncologists. Cancer was diagnosed only when both physicians made the same clinical diagnosis based on clinical manifestations and examinations.

All cancer events were reviewed and adjudicated by an independent Endpoint Adjudication Committee, whose members were unaware of study-group assignments.

Statistical analysis

We assumed an annual cancer incidence rate of 0.3% in the enalapril group.[28] It was estimated that a sample size of 20,000 would provide about 80% power to detect a treatment effect with a HR of 0.70 during the 5-year follow up, and with a Type-I error rate of 5%.

The efficacy analyses were conducted according to the intention-to-treat principle. 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 efficacy index for an outcome was the time from randomization to the first event of the outcome of interest. The cumulative event rates of cancer incidence in the enalapril-folic acid and the enalapril alone group, respectively, were estimated using the Kaplan–Meier method. The crude and adjusted HRs and their 95% confidence intervals (CI) were estimated by the Cox proportional hazard regression model. Tests for effect modification by pre-specified subgroups used interaction terms between subgroup indicators and randomized assignment by the Wald test. A two-tailed $p < 0.05$ was considered to be statistically significant in all analyses. R software, version 2.15.1 (http://www.R-project.org/) was used for all statistical analyses.

Results

Study participants and baseline characteristics

The flow of participants was presented in the Supporting Information Appendix Figure 1. A total of 10,348 and 10,354 participants were assigned to the enalapril-folic acid and enalapril group, respectively. None of the participants had physician-diagnosed cancer at baseline. The majority of

Table 1. Estimates of hazard ratios for total cancer incidence, major cancer subtypes and cancer mortality

Outcomes	no. (%) Enalapril ($N = 10,354$)	no. (%) Enalapril-folic acid ($N = 10,348$)	Hazard Ratio[1] (95% CI)	*p* Value
Cancer incidence				
Overall	116(1.12)	116(1.12)	1.00(0.77–1.29)	>0.99
Major Cancer Subtypes				
Esophageal cancer	25(0.24)	29(0.28)	1.16(0.68–1.98)	0.586
Gastric cancer	20(0.19)	23(0.22)	1.15(0.63–2.10)	0.646
Breast cancer	16(0.15)	11(0.11)	0.69(0.32–1.48)	0.339
Lung cancer	10(0.10)	16(0.15)	1.60(0.73–3.53)	0.243
Colorectal cancer	6(0.06)	13(0.13)	2.17(0.82–5.70)	0.117
Head and neck cancer	6(0.06)	7(0.07)	1.17(0.39–3.47)	0.782
Lymphoma	7(0.07)	2(0.02)	0.29(0.06–1.38)	0.118
Liver cancer	5(0.05)	4(0.04)	0.80(0.21–2.98)	0.740
Bladder cancer	8(0.08)	0(0.00)	–	–
Gynecologic cancer	5(0.05)	3(0.03)	0.60(0.14–2.51)	0.484
Other cancer	8(0.08)	8(0.08)	1.00(0.38–2.66)	>0.99
Cancer Mortality	44(0.42)	46(0.44)	1.05(0.69–1.58)	0.833

[1]Hazard ratios and 95% confidence intervals (CIs) were estimated using the Cox proportional hazard model.

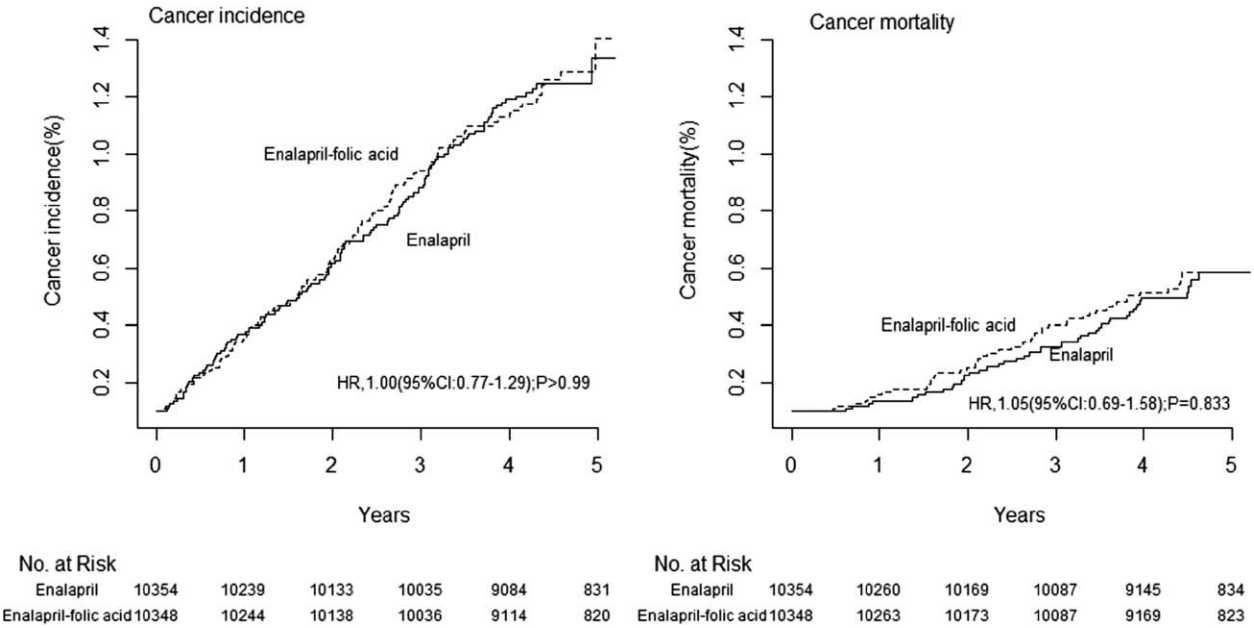

Figure 2. Kaplan–Meier curves of cumulative hazard ratios for cancer incidence and mortality.

participants [7,190 (69.4%) in the enalapril group and 7,191 (69.5%) in the enalapril-folic acid group] took at least 70% of their study medication throughout the trial and had no major protocol violations. A total of 72 (0.7%) participants in the enalapril-folic acid group and 69 participants (0.7%) 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.

The genotype frequency of *MTHFR C677T* polymorphisms was 27.3% ($n = 5,652$), 49.2% ($n = 10,176$) and 23.5% ($n = 4,874$), respectively, for CC, CT and TT genotypes. There was no significant difference in baseline characteristics between the enalapril and enalapril-folic acid groups within

each genotype stratum (all p values > 0.05) (Supporting Information Appendix Table 1).

Efficacy of folic acid supplementation on cancer incidence

The Kaplan–Meier curve of the cumulative event rate of cancer incidence for each of the two treatment groups is shown in Figure 2. During a median treatment duration of 4.5 (IQR: 4.2–4.7) years, cancer occurred in 116 participants (1.12%) in the enalapril-folic acid group as compared to 116 participants (1.12%) in the enalapril group (HR, 1.00; 95% CI, 0.77–1.29; $p > 0.99$). Analyses of the cancer incidence using the per-protocol set (no. of events/no. of participants: 68/7,190 in the enalapril group, and 73/7,191 in the enalapril-folic acid group) yielded a similar effect (HR, 1.07; 95% CI, 0.77–1.49; $p = 0.675$).

There was also no significant difference in the HR for any cancer subtype (esophageal, gastric, breast, lung, colorectal, head and neck, liver and gynecologic cancer or lymphoma) or cancer mortality (HR, 1.05; 95% CI: 0.69–1.58; $p = 0.833$) (Table 1).

Stratified analyses by important covariables

In the stratified analyses, there were no significant interactions between treatment for subgroups by *MTHFR C677T* genotypes (CC, CT and TT; p for interaction= 0.390); folate levels [<9.0 (median concentration in the participants with CC genotype), ≥9.0 ng/mL; p for interaction = 0.286]; sex (p for interaction = 0.476); age by decades (<55, 55–<65, ≥65 years; p for interaction = 0.871); cigarette smoking status [never, ever (former or current); p for interaction = 0.296]; alcohol drinking status [never, ever (former or current); p for interaction = 0.302]; quartiles of homocysteine (p for interaction = 0.644) and vitamin B12 (p for interaction = 0.680) levels; body mass index (BMI, <23, 23–<27, ≥27 kg/m^2; p for interaction= 0.666); total cholesterol (TC,<5.2, 5.2–<6.2, ≥6.2 mmol/L; p for interaction = 0.608); fasting glucose (<5.6, 5.6–<7.0, ≥7.0 mmol/L or diabetes; p for interaction = 0.920); baseline blood pressure (BP, <160/100, 160–<180/100–<110, ≥180/110 mmHg; p for interaction = 0.375); mean SBP over the treatment period (<140, ≥140 mmHg; p for interaction = 0.756); and use of antihypertensive drugs at baseline (yes, no; p for interaction = 0.411) (Tables 2 and 3).

Exploratory analysis by baseline folate levels and MTHFR C677T genotypes

For participants not receiving folic acid treatment (enalapril only group), *MTHFR* 677 TT genotype was an independent predictor of total cancer risk compared to CC genotype (HR, 1.86; 95% CI, 1.07–3.22) (Table 3).

However, folic acid supplementation did not have apparent effects on cancer incidence specific to participants with MTHFR 677 CC (HR, 1.32; 95% CI, 0.80–2.17; $p = 0.282$), CT (HR, 0.97; 95% CI, 0.66–1.42; $p = 0.876$) and TT (HR, 0.78; 95% CI, 0.47–1.32; $p = 0.359$) genotypes; or with different folate levels (<9.0 ng/mL: HR, 0.89; 95% CI, 0.63–1.26; $p = 0.512$; ≥9.0 ng/mL: HR, 1.15; 95% CI, 0.78–1.70; $p = 0.471$). Nevertheless, in the analysis of the combined modifying effect of MTHFR C677T genotypes and baseline folate levels, a significant beneficial effect of enalapril-folic acid treatment on cancer incidence was seen in participants with lower baseline folate levels (<9.0 ng/mL) and TT genotype (HR,0.47; 95% CI, 0.24–0.94; $p = 0.033$) (Table 3).

There were no significant differences between the two treatment groups in terms of the frequencies of any adverse events reported, as defined by the Medical Dictionary for Regulatory Activities for primary system organ classification; and any drug related adverse events (Supporting Information Appendix Tables A3 and A4).

Discussion

With the exception of half of the participants from the VITATOPS trial[12] who were recruited from Asia (low folate regions), the rest of the participants who were included in relevant trials[8–11,13–22] were all from regions with a high intake of folic acid or with established policies of folic acid fortification. The CSPPT was the first randomized controlled trial exclusively conducted in Asia (a regions with a high burden of cancer and low folate) to test the potential benefits and risks of folic acid supplementation in relation to cancer risk.

In the CSPPT, folic acid supplementation provided neither beneficial nor harmful effects on the risk of total cancer incidence in adults with hypertension and without history of stroke or MI in China. Furthermore, though our previous meta-analysis[7] reported the inverse dose response trend ($p = 0.056$) between percent baseline hypertension and log-RR for total cancer incidence associated with folic acid supplementation among the included randomized trials, in the CSPPT, neither blood pressure levels at baseline nor those over the treatment period significantly modified the association between folic acid supplementation and cancer risk. These results suggest that the effect of folic acid supplementation on cancer risk is not dependent on blood pressure levels, and it is likely that our results may apply to normotensive individuals.

In humans, folate plays the fundamental role of providing methyl groups for *de novo* deoxynucleotide synthesis and for intracellular methylation reactions. Low folate status has been associated with DNA strand breaks, impaired DNA repair, increased mutations and aberrant DNA methylation.[29] *MTHFR* is the main regulatory enzyme for folate metabolism. The availability of individual data on *MTHFR* genotypes and measurements of baseline folate levels in the CSPPT offers an exceptional opportunity to examine whether these factors modify the relationship of folic acid supplementation with cancer risk among adults with hypertension. Findings from such analyses will provide critically needed evidence to inform clinical and public health practice, and lay a foundation for personalized prevention of cancer. In our current analysis, neither the *MTHFR C677T* genotypes (p for interaction = 0.356 for CT *vs.* CC, and 0.176 for TT *vs.* CC) nor the baseline folate levels (p for interaction = 0.286 for ≥9.0 *vs.* <9.0 ng/mL) alone significantly modified the therapeutic

Table 2. Subgroup analysis of total cancer incidence

	No. of events/no. of participants (%)						
	Enalapril ($N = 10,354$)	Enalapril-folic acid ($N = 10,348$)	Hazard ratio (95% CI)	Adjusted hazard ratio (95% CI)[1]	p Value[1]	p for interaction[1,2]	p for interaction[1,3]
Sex						0.476	0.476
Male	59/4,252(1.39)	63/4,245(1.48)	1.07(0.75–1.52)	1.11(0.78–1.60)	0.558		
Female	57/6,102(0.93)	53/6,103(0.87)	0.93(0.64–1.35)	0.92(0.63–1.33)	0.648		
Age, years							0.871
<55	21/2,888(0.73)	19/2,868(0.66)	0.91(0.49–1.70)	0.90(0.48–1.72)	0.758	Ref	
55–<65	55/4,584(1.20)	54/4,545(1.19)	0.99(0.68–1.44)	1.00(0.69–1.46)	0.998	0.733	
≥65	40/2,882(1.39)	43/2,935(1.47)	1.06(0.69–1.63)	1.07(0.69–1.66)	0.750	0.600	
Smoking							0.296
Never	66/7,135(0.93)	60/7,119(0.84)	0.91(0.64–1.29)	0.90(0.63–1.27)	0.539	Ref	
Ever smoker	50/3,217(1.55)	56/3,222(1.74)	1.12(0.77–1.64)	1.20(0.81–1.77)	0.362	0.296	
Alcohol drinking							0.302
Never	76/7,113(1.07)	68/7,158(0.95)	0.89(0.64–1.23)	0.91(0.65–1.26)	0.554	Ref	
Ever drinker	40/3,238(1.24)	48/3,181(1.51)	1.23(0.81–1.86)	1.22(0.79–1.86)	0.368	0.302	
Homocysteine, μmol/L							0.644
<10.5	29/2,597(1.12)	23/2,572(0.89)	0.80(0.46–1.38)	0.74(0.42–1.30)	0.294	Ref	
10.5–<12.5	27/2,506(1.08)	29/2,487(1.17)	1.08(0.64–1.82)	1.06(0.62–1.80)	0.841	0.324	
12.5–<15.5	31/2,563(1.21)	32/2,595(1.23)	1.02(0.62–1.67)	1.01(0.62–1.66)	0.958	0.426	
≥15.5	29/2,548(1.14)	32/2,556(1.25)	1.10(0.67–1.82)	1.21(0.72–2.02)	0.467	0.221	
Vitamin B12, pg/mL							0.680
<315	24/2,542(0.94)	28/2,586(1.08)	1.15(0.67–1.98)	1.12(0.65–1.93)	0.692	Ref	
315–<380	35/2,595(1.35)	28/2,553(1.10)	0.81(0.49–1.34)	0.78(0.47–1.28)	0.320	0.349	
380–<477	28/2,537(1.10)	28/2,569(1.09)	0.99(0.58–1.66)	1.01(0.60–1.70)	0.975	0.737	
≥477	26/2,583(1.01)	31/2,536(1.22)	1.21(0.72–2.04)	1.22(0.72–2.06)	0.453	0.890	
BMI, kg/m^2							0.666
<23	43/3,243(1.33)	50/3,199(1.56)	1.18(0.79–1.77)	1.17(0.77–1.76)	0.462	Ref	
23–<27	48/4,258(1.13)	43/4,290(1.00)	0.89(0.59–1.34)	0.88(0.58–1.34)	0.556	0.382	
≥27	25/2,851(0.88)	23/2,853(0.81)	0.92(0.52–1.62)	0.94(0.52–1.69)	0.833	0.583	
TC, mmol/L							0.608
<5.2	57/4,221(1.35)	48/4,147(1.16)	0.86(0.59–1.26)	0.87(0.59–1.28)	0.486	Ref	
5.2–<6.2	35/3,364(1.04)	40/3,381(1.18)	1.14(0.72–1.79)	1.10(0.70–1.75)	0.678	0.394	
≥6.2	24/2,582(0.93)	28/2,633(1.06)	1.14(0.66–1.97)	1.11(0.64–1.93)	0.700	0.427	

Table 2. Subgroup analysis of total cancer incidence (Continued)

	No. of events/no. of participants (%)						
	Enalapril ($N = 10,354$)	Enalapril-folic acid ($N = 10,348$)	Hazard ratio (95% CI)	Adjusted hazard ratio (95% CI)[1]	p Value[1]	p for interaction[1,2]	p for interaction[1,3]
Fasting glucose, mmol/L							0.929
<5.6	76/5,727(1.33)	77/5,779(1.33)	1.00(0.73–1.38)	0.97(0.71–1.34)	0.871	Ref	
5.6–<7.0	27/3,290(0.82)	27/3,257(0.83)	1.01(0.59–1.72)	1.11(0.64–1.91)	0.717	0.716	
≥7.0 or diabetes[4]	13/1,158(1.12)	12/1,130(1.06)	0.94(0.43–2.07)	0.98(0.44–2.18)	0.965	0.960	
Blood pressure, mmHg							0.375
<160/100	49/3,257(1.50)	38/3,232(1.18)	0.78(0.51–1.19)	0.79(0.52–1.21)	0.285	Ref	
160–<180/100–<110	43/4,250(1.01)	50/4,260(1.17)	1.16(0.77–1.74)	1.17(0.77–1.77)	0.465	0.204	
≥180/110	24/2,847(0.84)	28/2,856(0.98)	1.16(0.67–2.00)	1.18(0.68–2.04)	0.556	0.280	
Mean SBP over the treatment period, mmHg							0.756
<140	59/5,805(1.02)	62/5,869(1.06)	1.04(0.73–1.49)	1.04(0.72–1.49)	0.839	Ref	
≥140	57/4,548(1.25)	54/4,479(1.21)	0.96(0.66–1.39)	0.96(0.66–1.40)	0.848	0.756	
Anti-hypertensive drugs							0.411
No	59/5,539(1.07)	66/5,627(1.17)	1.10(0.77–1.56)	1.13(0.79–1.61)	0.510	Ref	
Yes	57/4,815(1.18)	50/4,721(1.06)	0.89(0.61–1.31)	0.90(0.61–1.31)	0.577	0.411	

[1]Adjusted, if not stratified, for age, sex, MTHFR C677T polymorphism, systolic blood pressure (SBP) and diastolic blood pressure (DBP) at baseline, mean SBP and DBP during treatment period, body mass index (BMI), study centers, folate, homocysteine, vitamin B12, creatinine, total cholesterol, triglycerides, high density lipoprotein-cholesterol, fasting glucose levels, use of antihypertensive drugs, smoking and alcohol drinking status.
[2]p values were estimated based on the Wald test.
[3]p values were estimated based on the Likelihood ratio test.
[4]Diabetes was defined as self-reported diabetes or use of glucose-lowering drugs.

Table 3. Joint effect of *MTHFR C677T* polymorphisms and baseline folate levels on total cancer incidence

	No. of events/no. of participants (%)						
	Enalapril ($N = 10,354$)	Enalapril-folic acid ($N = 10,348$)	Hazard ratio (95% CI)	Adjusted hazard ratio (95% CI)[1]	p Value[1]	p for interaction[1,2]	p for interaction[1,3]
Folate, ng/mL							0.286
<9.0	66/6,169(1.07)	59/6,199(0.95)	0.89(0.63–1.26)	0.89(0.63–1.26)	0.512	Ref	
≥9.0	47/4,087(1.15)	56/4,044(1.38)	1.20(0.82–1.77)	1.15(0.78–1.70)	0.471	0.286	
MTHFR C677T polymorphism							0.390
CC	27/2,831(0.95)	36/2,821(1.28)	1.34(0.81–2.20)	1.32(0.80–2.17)	0.282	Ref	
CT	55/5,081(1.08)	54/5,095(1.06)	0.98(0.67–1.43)	0.97(0.66–1.42)	0.876	0.356	
TT	34/2,442(1.39)	26/2,432(1.07)	0.76(0.46–1.27)	0.78(0.47–1.32)	0.359	0.176	
Folate <9.0 ng/mL							0.077
CC	13/1,386(0.94)	18/1,394(1.29)	1.38(0.67–2.81)	1.40(0.68–2.88)	0.357	Ref	
CT	28/2,985(0.94)	29/3,033(0.96)	1.02(0.61–1.72)	1.01(0.60–1.70)	0.966	0.491	
TT	25/1,798(1.39)	12/1,772(0.68)	0.49(0.24–0.97)	0.47(0.24–0.94)	0.033	0.035	
Folate ≥9.0 ng/mL							0.378
CC	14/1,424(0.98)	18/1,397(1.29)	1.30(0.65–2.62)	1.23(0.60–2.50)	0.571	Ref	
CT	26/2,042(1.27)	24/2,010(1.19)	0.94(0.54–1.63)	0.91(0.52–1.59)	0.746	0.532	
TT	7/621(1.13)	14/637(2.20)	1.96(0.79–4.85)	1.90(0.75–4.81)	0.176	0.437	

[1]Adjusted, if not stratified, for age, sex, MTHFR C677T polymorphism, SBP and DBP at baseline, mean SBP and DBP during treatment period, body mass index, study centers, homocysteine, vitamin B12, creatinine, total cholesterol, triglycerides, HDL cholesterol, fasting glucose levels, use of antihypertensive drugs, smoking and alcohol drinking.
[2]p values were estimated based on the Wald test.
[3]p values were estimated based on the Likelihood ratio test.

effects of enalapril-folic acid in relation to cancer incidence. However, in our previous results from the CSPPT,[2] a higher stroke risk and a greater beneficial effect of folic acid supplementation for stroke primary prevention were consistently observed in those with insufficient baseline folate levels (CC genotype < 9.0 ng/mL and CT genotype < 5.7 ng/mL), or biologically insufficient folate levels (as reflected in the relatively greater folate requirement for the TT genotype). Notably, in our current analysis, a higher cancer risk was observed in those with both insufficient baseline folate levels (<9.0 ng/mL) and biologically insufficient folate levels (TT genotype), and, accordingly, the major beneficial effect of enalapril-folic acid treatment was seen in this subpopulation. These results suggest that the effects of folate on risk of outcomes appear at lower levels in cancer compared with stroke, then this might speculatively support an involvement via mechanisms common to both outcomes but to different degrees. However, these results were from an un-defined subgroup analysis. To fully understand the findings we would need larger numbers of cancers to study subtypes. Further studies are warranted to evaluate and confirm our results.

The *MTHFR 677* TT variant is present in all populations with variable frequency (usually 2–25%).[30] Inadequate folate intake is prevalent in most countries without mandatory folic acid fortification, including Asia and other continents. Furthermore, there is substantial variability in blood folate levels within the U.S. population and across racial/ethnic groups.[31] We speculate that even in countries with folic acid fortification, there may still be a room to further reduce cancer incidence, using more targeted folic acid supplementation, particularly among those with TT genotype and low folate levels.

Our study had several strengths and novel features. First, the CSPPT was conducted in China, a country with no mandatory fortification of foods with folic acid or widespread use of folic acid supplements. Second, the CSPPT focused on hypertensive participants without pre-existing stroke or MI. The low vascular disease burden and the low frequency use of cardiac and vascular protective drugs made our results less likely to be affected by these drugs and possible drug interactions. Third, we used folic acid alone (0.8 mg/d) at physiological doses (<1 mg/d), rather than in combination with high dose vitamin B6 or B12 used in previous trials. In fact, the possible increased risk of cancer associated with high vitamins B12 or B6 intake had been reported in recent studies.[32,33] The total folic acid intake in the U.S. even after the introduction of folic acid fortification of food is only about 250–400 μg/d in women and 300–420 μg/d in men.[34] Our results are therefore relevant to the public health debate about the safety of mandatory folic acid fortification for protection against neural tube defects in general populations.

Our study also had some limitations. First, van Wijngaarden *et al.*[8] reported that the increased cancer risk associated with folic acid supplementation was more pronounced in participants aged >80 years. However, we only included participants aged 45–75 years. The relationship of folic acid supplementation with cancer risk in a very elderly population should be further examined in future studies. Second, the CSPPT was underpowered for assessing the risk of different cancer subtypes or cancer risk in different *MTHFR* C677T genotypes, and may not have had a long enough follow-up duration. The continued follow-up of the study participants for a longer duration may provide a more definitive answer regarding the risk of different cancer subtypes or cancer risk in different *MTHFR* C677T genotypes in relation to folic acid supplementation. Third, the CSPPT was conducted in adults with hypertension in China. It is not reasonable to use folic acid or placebo alone in participants with known hypertension. So we could not use a full factorial design. Furthermore, the generalizability of our findings to non-hypertensive adults or populations in other countries remains to be determined.

Conclusions

In summary, there is no evidence that 0.8 mg daily folic acid supplementation can increase the risk of cancer incidence and mortality among adults with hypertension without history of stroke or MI in China. Furthermore, our data suggest a protective effect in participants with MTHFR TT genotype and low folate levels. These findings are applicable to individuals exposed to folic acid supplementation at a dosage <1 mg/d for preventive purposes, and are also relevant to the public health debate about the safety of mandatory folate fortification for protecting against neural tube defects in general populations. Our results have important clinical and public health implications.

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